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Synergy of lignocelluloses pretreatment by sodium carbonate and bacterium to enhance enzymatic hydrolysis of rice straw

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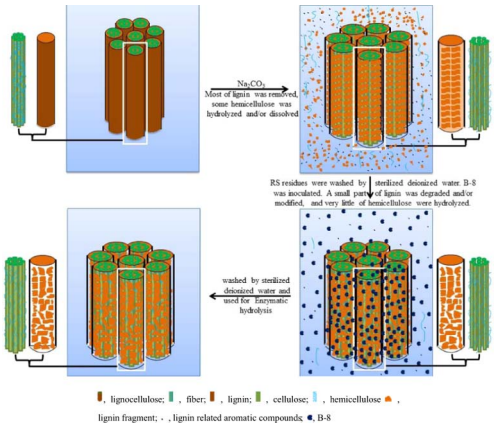
GRAPHICAL ABSTRACT



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ABSTRACT

We studied a new strategy for pretreatment of rice straw (RS) to enhance enzymatic hydrolysis under mild condition. This approach uses the synergy of sodium carbonate (Na_2CO_3) and the bacterial strain Cupriavidus basilensis B-8 (hereafter B-8). After synergistic Na_2CO_3 and B-8 pretreatment (SNBP), the reducing sugar yield varied from 335.3 mg/g to 799.6 mg/g under different conditions. This increased by 13–31% over Na_2CO_3 pretreatment (284.2–719.2 mg/g) and 3.42–8.15 times over the untreated RS (98 mg/g). Moreover, the composition of RS was changed significantly through decreases in lignin and hemicellulose. We confirmed this change by compositional analysis and physicochemical characterization of the structure of RS before and after pretreatment. We also elaborated a mechanism for SNBP to better explain RS changes and bacterial effects on enzymatic hydrolysis.

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1. Introduction

Continued reductions in the availability of oil resources and the continued increase in global energy demand have contributed to the development of renewable biofuel resources. Lignocellulosic feedstocks, such as straw, corn stover and energy crops, are sustainable sources of biofuels and bioproducts (Dyk and Pletschke, 2012; Himmel et al., 2007). The biological conversion platform for the production of biofuels from biomass involves three main stages: (1) pretreatment to reduce the recalcitrance of the lignocellulose structure, (2) enzymatic hydrolysis of cellulose or hemicellulose to sugar and (3) microbial fermentation of sugar to ethanol or other fuels (Alvira et al., 2010; Huang et al., 2011). For this platform, the recalcitrant structure of biomass against the hydrolysis step is the limiting factor hampering commercial usage. The main factors that make lignocellulose resistant to enzymatic conversion include lignin content, cellulose crystallinity, and substrate accessible surface area (Yu et al., 2010; Zhao et al., 2012). It is desirable to remove or modify lignin by a pretreatment strategy to reduce the crystallinity of cellulose and increase the accessible surface area for enzymatic digestibility (Hendriks and Zeeman, 2009; Zhu et al., 2010). Many techniques have been developed for the pretreatment of lignocellulose. Typical physical and chemical pretreatments such as dilute acids, alkalis, microwaves, ionizing radiation, steam explosion, and oxidation, or varied combinations, require special equipment and consume large amounts of energy (Ravindran and Jaiswal, 2016). In addition, these pretreatments typically produce some inhibitors that affect subsequent enzymatic hydrolysis and microbial fermentation, as well as acidic or alkaline wastewater, which requires subsequent processing to ensure environmental safety (Brodeur et al., 2011; Keller et al., 2003).

Basidiomycetes evolved two direct (biological and chemical) mechanisms for attacking wood: white-rot fungi completely removed lignin from plant cell wall via extracellular hemi-dependent peroxidases, copper-dependent laccases and other oxidases (Martinez et al., 2009), while brown-rot fungi removed the hemicellulose and cellulose with only modification to the lignin via Fenton reaction (Geib et al., 2008). Inspired by these understandings, scientists have been encouraged to perform various pretreatments assisted by fungi, which were designed to reduce consumption of energy and reagent (Yu et al., 2009). However, there are few limitations in applying the fungi-assisted strategy for pilot-scale processes, however, and the major hindrances include the long incubation time (> 10 d) required for effective delignification as well as limited knowledge on their mechanism (Ravindran and Jaiswal, 2016; Saha et al., 2016; Xu et al., 2010; Zhong et al., 2011). Although bacteria are not considered to directly decompose lignin in the nature, they grow faster (< 7 d) and harbor enzyme systems for degradation of modified lignin, i.e., Kraft lignin (Chen et al., 2012; Shi et al., 2013a; Shi et al., 2013b; Shi et al., 2017). Accordingly, a significant attention deserves to be focused on lignocellulose pretreatment assisted by bacteria. However, reports on bacteria-assisted pretreatment of lignocellulose are limited.

In our previous work, *Cupriavidus Basilensis B-8* (hereafter B-8) has been confirmed as a potential bacterium for lignin degradation. Moreover, the degradation steps and intermediates of lignin-derived aromatic compounds were identified through genomic sequencing and systematic analysis in the bacterial-mediated Kraft lignin degradation (Shi et al., 2013a, 2017). Therefore, B-8 is a promising substitute for fungi in assisting lignocellulose pretreatment. Herein, rice straw (RS) was used as a model lignocellulosic biomass. The influences of bacteria on the sodium carbonate (Na_2CO_3) pretreatment of RS and the followed enzymatic hydrolysis were investigated. We also study the physicochemical characteristics of the pretreated RS to provide new insights into lignocellulose pretreatment. This novel synergy pretreatment presents more promising for biological pretreatments in terms of digestibility and time-consuming in industrial application.

2. Materials and methods

2.1. Bacterial strain and RS preparation

The bacterial strain *Cupriavidus Basilensis B-8* (here after B-8) isolated from the steeping fluid of eroding bamboo slips of Kingdom Wu during the Three-Kingdoms Dynasty has been deposited in the China General Microbiological Culture Collection Center (CGMCC) with the accession number of CGMCC 4240. Cells grown in the Luria-Bertani (LB) broth medium with a shaking speed of 125 rpm at 30 °C were used as seed culture for B-8 pretreatment. Rice straw (RS) was obtained from Jining (Shandong, China). The air-dried RS was ground and sieved to 60 mesh by biomass grinder (YB-2500A, Yunbang Co., Ltd, Zhejiang, China). The solid residues were washed with deionized water and dried at 50 °C before using it as a feedstock for pretreatment. All the chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd., of China.

2.2. Pretreatment

Sodium carbonate at concentration of 2%, 4%, 6%, 8% and 10% (w/v) was used to pretreat the RS samples. The RS was initially mixed in a conical flask with 100 mL Na_2CO_3 solution to obtain a solid loading 10% (w/v). The pretreatment was conducted at 80 °C for 180 min or 121 °C for 60 min in a water bath. After Na_2CO_3 pretreatment, the obtained slurry was filtered. The solid residues were washed to neutral pH with deionized water and air-dried under 50 °C for further analysis.

B-8 pretreatment was carried out as follows: bacterial cells were expanded in LB medium, and cells were harvested until the optical density at 600 nm of inoculums reached 1.0; 10 mL of the inoculum was centrifuged and washed three times with sterile water and then inoculated into 100 mL mineral salt medium (Shi et al., 2013a) with 1g of RS sample. The biological pretreatment was carried out in a rotary shaker at 30 °C with a speed of 125 rpm. The bacteria-treated RS was collected after 2 d of incubation. And the obtained residues were washed by deionized water and air-dried under 50 °C for further analysis. All of the experiments were carried out in triplicate to yield average results and standard deviations presented in all figures.

2.3. Determination of enzymatic hydrolysis and chemical composition

The enzymatic hydrolysis of untreated and pretreated RS was performed by Cellulase Cellic (CTec2, Novozymes), and reducing sugar yield analysis measured by the DNS assay as described previously (Yan et al., 2017). The composition of RS was determined based on a laboratory analytical procedure provided by previous study (Teramoto et al., 2008).

2.4. Analytical methods

Samples before and after pretreatments were coated with gold and observed under a scanning electron microscope (SEM, JSM-IT300LA, JEOL, Tokyo, Japan). Atomic force microscopy (AFM) imaging was conducted in tapping mode on a NanoMan™ VS + MultiMode V scanning probe microscope (Veeco Company, Plainview, NY, USA). An X-ray diffractometer (XRD, TTR III, Rigaku, Japan) was used to measure the crystallinity of cellulose in treated and untreated RS (Chai et al., 2014; Yan et al., 2014). Crystallinity (%) was calculated using the following equation:

$$\text{Crystallinity index (CrI)} = [(I_{002} - I_{amp})/I_{002}]$$

where

I_{002}: The intensity of the (0 0 2) peak is near 2θ = 22.5°,
I_{amp}: The intensity of the background at about 2θ = 16.3°.