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Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull

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ABSTRACT

Two novel two-step pretreatments for enzymatic hydrolysis of rice hull (RH) were proposed to lower the severity requirement of fungal pretreatment time. They consisted of a mild physical or chemical step (ultrasonic and H_2O_2) and a subsequent biological treatment (*Pleurotus ostreatus*). The combined pretreatments led to significant increases of the lignin degradation than those of one step pretreatments. After enzymatic hydrolysis of the pretreated RH, the net yields of total soluble sugar (TS) and glucose (G) increased greatly. The combined pretreatment of H_2O_2 (2%, 48 h) and *P. ostreatus* (18 d) was more effective than sole pretreatment of *P. ostreatus* for 60 d. It could remarkably shorten the residence time and reduce the losses of carbohydrates. Ligninase analyses and SEM observations indicated that the enhancing of the efficiency could possibly attribute to the structure disruption of the RH during the first pretreatment step. So, the combined pretreatment could be recommended to different lignocellulosic materials for enzyme based conversions.

1. Introduction

Rice hull (RH) is the main byproducts of paddy process and accounts for 20–25% of the whole weight. More than 113 million metric tons of RHs are generated each year through out the world. It is a low bulk density lignocellulosic material with approximate 20–25% lignin, 35–40% cellulose and 15–20% hemicellulose. Because of its widespread availability and relative low cost, it has the potential to serve as the feedstock for production of fuel ethanol (Saha et al., 2005).

During the process of the enzyme-based biological conversions from the lignocellulosic materials to ethanol, pretreatment is a restrictive and the most costly step because of their recalcitrant structures. The main goal of pretreatment is to break lignin seal and disrupt crystalline structure of cellulose (Mosier et al., 2005; Wyman, 1999, 2007). Typically pretreatments, such as steam explosion, organic solvent, sulfuric acid, hot alkali and combination of steam with acid or alkali, require special instrument and consume a lot of energy (Playne, 1984; Sawada et al., 1987; Silverstein et al., 2007; Datar et al., 2007). Moreover, they often lead to the losses of carbohydrates and generate some inhibitors to the subsequent enzymatic hydrolysis during the severity of the operational condition. It makes the process uneconomical and environmental unfriendliness. Thus, effective pretreatments are still needed to reduce costs, improve cellulose digestibility, simplify the upstream and downstream operations and provide additional revenues from byproducts.

Biological pretreatment, as a safe and environmental friendly method for lignin removal from lignocellulose, is attracting extensive interests (Akin et al., 1993; Keller et al., 2003; Taniguchi et al., 2005; Zhang et al., 2007). White-rot fungi are the most promising microorganisms used for biological pretreatment because of their abilities to selectively degradation of lignin. Presently, lots of studies on pretreatments with various white-rot fungi have been reported. Many white-rot fungi were applied to pretreatment of wheat straw for enzymatic hydrolysis, and found that about 30% of cellulose was converted to glucose by *Pleurotus florida* in 60 d (Muller and Trosch, 1986). Hattaka (1983) studied 19 white-rot fungi and the results showed that 35% of the wheat straw cellulose could be converted to sugar by enzymatic hydrolysis after *P. ostreatus* pretreatment for 35 d. A similar conversion rate was obtained when rice straw was pretreated by *P. ostreatus* for 60 d (Taniguchi et al., 2005). Pretreatment with *Coriolus versicolor* for enzymatic hydrolysis of bamboo residues was investigated, and 37% of saccharification rate was achieved (Zhang et al., 2007). These reports showed a great increase in conversion rate by biological pretreatment. However, relative low efficiency, considerable loss of carbohydrates and long residence time are the three major disadvantages for the fungal pretreatment. New strategies should be used to overcome these feeble sides.

Ultrasonic has been employed to extract lignin and hemicellulose from lignocellulosic materials (Sun and Tomkinson, 2002a,b).

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When materials in a liquid suspension are treated by ultrasonic, particles are subjected to either surface erosion (via cavitational collapse in the surrounding liquid), or size reduction (due to fission through interparticle collision or the collapse of cavitation bubbles formed on the surface). Some studies suggested that pretreatment of lignocellulose substrates with ultrasonic could be recommended for the intensification of bioconversion both in nature and under production condition (Kadimaliev et al., 2003).

Hydrogen peroxide may enhance enzymatic conversion from lignocellulose to sugar through oxidative dilignification and reduction of cellulose crystallinity (Gould, 1985). Increased lignin solubilization and cellulose availability were observed during the H_2O_2 pretreatment of wheat straw, oak and Douglas fir (Martel and Gould, 1990; Kim et al., 2001; Yang et al., 2002).

To enhance the efficiency of the biological pretreatment and lower the severity requirements of the residence time, we employed a two-step pretreatment. The RH was pretreated with ultrasonic or H_2O_2, and then dealt with by white-rot fungus *P. ostreatus*. To evaluate the effects of the combined pretreatments, changes in the components of the RH were investigated. We also tested the net sugar yields of enzymatic hydrolysis of the pretreated RH. To gain insight into the mechanism of the combined pretreatments, activities of ligninases produced by *P. ostreatus* were also determined. And scanning electron microscopy (SEM) was used to observe the structural change of the RH after the pretreatments.

2. Methods

2.1. Biomass, chemicals, media and microorganism

RH was obtained from Wuhan City, Hubei Province. It was about 5 mm in length, 2.5–5 mm in width and 0.5 mm in thickness. The main composition of the RH was as follow (on the basis of dry mater): cellulose 40 ± 0.1%; hemicellulose 19 ± 0.2%; lignin 25 ± 0.4%; others (mainly ash) 16 ± 0.2%. The raw material was washed thoroughly with tap water until clean and colorless, and then dried in air for further treatment.

The cellulase was a commercial enzyme from *Trichoderma reesei* (Shanghai DongFeng Chemical Industry). The carboxymethyl cellulose activity in international unit was 30 ± 0.2 IU/mg, measured by the method of Mandels and Weber (1969). The filter-paper activity was 15 ± 0.1 FPU/mg, determined following the standard procedure recommended by the Commission on Biotechnology of the International Union of Pure and Applied Chemistry (IUPAC) (Ghose, 1987). And its cellobiase activity in cellobiase units (CBU) was 1.18 ± 0.03 CBU/mg, assayed according to Vlasenko et al. (1997). All other chemicals were analytical grade.

P. ostreatus BP-035 was stored in our laboratory. Mycelial mats which have been grown on potato dextrose agar slant in test tubes, were suspended in a solution of 0.1% Tween 80, and then inoculated to 50 mL basic medium (g/L: glucose 10, KH_2PO_4 0.8, NH_4NO_3 2, Na_2HPO_4 0.4, $MgSO_4{\cdot}7H_2O$ 0.5, yeast extract 2) containing 5 mL of microelement solution (g/L: 0.001 g of $ZnSO_4 \cdot 7H_2O$, 0.005 g of $FeSO_4 \cdot 7H_2O$, 0.06 g of $CaCl_2 \cdot 2H_2O$, 0.005 g of $CuSO_4 \cdot 7H_2O$ and 0.005 g of $MnSO_4 \cdot H_2O$). The fungus was cultured on a rotary shaker at 180 rpm. Mycelial pellets were harvested after 5 d and then mixed with a laboratory blender (WH-2 mini vortexer, 2000 rpm) three times for 20 s with 30 s intervals. This suspension would act as inoculum.

2.2. Pretreatment

2.2.1. One step pretreatment

2.2.1.1. Ultrasonic pretreatment and H_2O_2 pretreatment. Five grams of RH was pretreated at a solid loading of 15% (W/V). For ultrasonic pretreatment, the samples were treated with ultrasonic at 25 °C for 10, 20, 30, 40, 50 and 60 min in different glass beakers, using the ultrasonic system (250 W, 40 kHz) (KQ-250B; Jiang Yi Instrument Co., Ltd., Shanghai, China). For H_2O_2 pretreatment, it was used at the concentrations of 1%, 2%, 3%, 4% and 5% (W/V) for 48 h. All these pretreatments were performed at 25 °C under normal pressure. The pretreated RH was washed with distilled water thoroughly and then dried at 50 °C for further analyses.

2.2.1.2. Fungal pretreatment. In biological pretreatment, the prepared inoculum of *P. ostreatus* was added to solid medium of 5 g of treated or untreated RH, and supplied with nutrient solutions (low nitrogen Kirk medium without glucose (per liter): ammonium tartrate 0.8 g, KH_2PO_4 0.2 g, vitamin A 1.5 mM, acetate buffer (pH 4.4) 20 mM, $MgSO_4 \cdot 7H_2O$ 0.5 g, vitamin B 1 mg, $CaCl_2$ 0.1 g) to adjust the moisture to 65% in a 250 mL Erlenmeyer flask (Taniguchi et al., 2005). The culture was incubated statically in 28 °C for 6–60 d. Periodic sampling was done for every 3 or 6 d.

2.2.2. Combined pretreatment

Some samples of the RH after ultrasonic pretreatment or H_2O_2 pretreatment were chosen for the subsequent fungal pretreatment. The selection was based on a compromise of having the lower percentage of lignin and the higher net yields of sugar, and using the lower concentration of chemical or the shorter pretreatment time. The pretreated RH was washed with distilled water thoroughly and then dried at 50 °C for the biological pretreatment. The prepared inoculum of *P. ostreatus* was inoculated on 5 g of the pretreated RH, and added with the nutrient solution (low nitrogen Kirk medium without glucose) to adjust moisture to 65% in a 250 mL Erlenmeyer flask (Taniguchi et al., 2005). The culture was then incubated statically in 28 °C for 18 d.

2.3. Analysis method

The components (cellulose, hemicellulose and lignin) of pretreated and unpretreated RH were analyzed according to the procedures of Goering and Van Soest (1971). The degradation rates of compositions were calculated on the basis of the amounts of the compositions in the untreated RH. The amounts of total soluble sugar (TS) and glucose (G) of the enzymatic hydrolysates were tested according to the methods of phenol–sulfuric acid (Dubois et al., 1956) and glucose oxidase-peroxidase (Glucose detection kit, Rongsheng Biotch Co., Ltd., Shanghai, China), respectively. All data were corrected to 100% dry matter (DM) basis.

Enzyme assays: The biological pretreated sample was suspended with distilled water and stirred for 20 min, followed by filtration and centrifugation, the supernatant was used for ligninase activity assays. Lip (lignin peroxidase) activity was measured with Azure B (Frederick, 1992). The reaction mixture contained 0.1 M sodium tartrate buffer (pH 3.0), 32 μM Azure B, and 1.65 mL of culture filtrate in a total volume of 3 mL. The reaction was started by adding H_2O_2 to a final concentration of 0.2 mM, and the decrease of A_{651} was monitored. MnP (manganese peroxidase) activity was measured by the method described by Pick and Keisare (1980) with some modifications, with 0.01% phenol red as a substrate in 100 mM tartrate buffer (pH 5.0) containing 0.1 mM $MnSO_4$ and 0.1 mM H_2O_2, the reaction was stopped by adding 50 μL of 4 M NaOH per mL of the reaction mixture and the absorbance at 610 nm was measured. Control assays of phenol red oxidation in the absence of Mn^{2+} were carried out by omitting $MnSO_4$ from the reaction mixture. MnP activity was calculated by subtracting the value of phenol red-oxidizing activity in the absence of Mn^{2+} from the value of phenol red-oxidizing activity in the presence of manganese. Activity was