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Pretreatment by microwave/alkali of rice straw and its enzymic hydrolysis

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Abstract

Combination pretreatment of rice straw using microwave and alkali and its enzymic hydrolysis were investigated and compared with the alkali-alone pretreated process. First, the effect of microwave power and pretreatment time on the weight loss and composition of rice straw was examined. The results show that higher microwave power with shorter pretreatment time and the lower microwave power with longer pretreatment time had almost the same effect on the weight loss and composition at the same energy consumption. The comparison was then made between the effect of the microwave/alkali pretreatment and the alkali-alone one on the weight loss and composition of rice straw. The rice straw had a weight loss of 44.6% and composition cellulose 69.2%, lignin 4.9% and hemicellulose 10.2% after 30-min microwave/alkali pretreatment at 700 W while it only had a weight loss of 41.5% and composition cellulose 65.4%, lignin 6.0% and hemicellulose 14.3% after 70-min alkali-alone pretreatment. It may be that microwave/alkali pretreatment could remove more lignin and hemicellulose from rice straw with shorter pretreatment time compared with the alkali-alone one. Finally, the enzymic hydrolysis of pretreated rice straw (substrate concentration 50 g l^{-1}, enzyme loading 20 mg g^{-1} substrate) was also investigated and the results indicate that rice straw pretreated by microwave/alkali had a higher hydrolysis rate and glucose content in the hydrolysate in comparison with the one by alkali alone.

Keywords: Rice straw; Microwave/alkali pretreatment; Alkali pretreatment; Enzymic hydrolysis

1. Introduction

China is an agricultural country and rice is one of the most important agricultural corps in China. Associated with rice production is a corresponding annual production of nearly 200 million tons of rice straw (RS). RS has traditionally been used as animal feed for bovine and swine breeding, feedstock for paper industry, or organic fertilizer by burying it into the soil or burning it on the open field [1,2]. However, modern views of animal breeding practices and a growing interest in environmental problems tend to limit these uses, therefore negatively affecting its demand. In order to develop its new uses and increase its added value, the conversion of RS into fermentable sugars has been extensively studied to for utilization as feedstock material for ethanol and other chemicals [1,3]. The difficulties for RS to be effectively converted into fermentable sugars are from two facts: the strong crystalline structure of cellulose in RS and the presence of the complex structure of lignin and hemicellulose with cellulose, which together limit the accessibility of RS to hydrolytic enzymes. Therefore, various pretreatments of RS have been developed to disrupt the crystalline structure of cellulose and increase its exposure to hydrolytic enzymes and thus increase its enzymic hydrolysis. Although there have been many pretreatment methods, few can be used on an industrial scale based on economical and environmental consideration [3]. It is necessary to develop a cheap, efficient and environmental friendly pretreatment technique for the industrialization of the enzymic hydrolysis of RS.

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Microwave irradiation has been widely used in many areas because of its high heating efficiency and easy operation. Some studies have shown microwave irradiation could change the ultrastructure of cellulose [4], degrade lignin and hemicellulose in RS and increase the enzymic susceptibility of RS [5–7]. However, the reported microwave pretreatment of RS was carried out at an elevated temperature (>160 °C). This will limit its application to some extent because some useful components in RS might be decomposed at high temperature. Fortunately, microwave irradiation could be easily combined with chemical reactions and, in some case, accelerate the chemical reaction rate [8]. Alkali pretreatment is a typical chemical pretreatment method of RS and a combination of microwave treatment and alkali treatment might be an alternative for pretreatment of RS at lower temperature. To our knowledge, this combination has not been reported before. The objective of this work is to develop a novel pretreatment approach for RS and evaluate its suitability. In this work, the combination pretreatment of rice straw using microwave and alkali and its enzymic hydrolysis were investigated and compared with an alkali-alone pretreated process.

2. Materials and methods

All experiments were carried out three times. The data reported are expressed as the mean values ± standard deviation. The composition of RS or its hydrolyzed residues was expressed on a wet basis throughout this work.

2.1. Materials and chemicals

Raw RS was obtained from local farmers in Yichang, Hubei Province, China. Before any pre-treatment, it was cut to nominally 1–2 cm length and washed thoroughly with tap water until the washings were clean and colourless and then air dried for further treatment. The main composition of this RS was as follows: moisture 12.8 ± 0.2%, cellulose 38.6 ± 0.4%, lignin 13.6 ± 0.6% and hemicellulose 19.7 ± 0.5%.

The cellulase enzyme used in this study was a commercial *Trichoderma reesei* cellulase (formerly called *Trichoderma viride* cellulase) from Shanghai Boao Biotech. Corp., China. Its CMC-ase activity was 15 ± 0.6 IU/mg, measured as the initial rate of reducing sugars formation during hydrolysis of 0.5% CMC at pH 5.0 and 50 °C [9]. Its filter-paper activity was 0.53 ± 0.03 FPU/mg, determined following the standard procedure recommended by the Commission on Biotechnology, IUPAC [10]. The cellobiase activity was 0.19 ± 0.02 CBU/mg, measured as the initial rate of hydrolysis of 2 mM cellobiose to glucose at pH 5.0 and 50 °C [1].

All other chemicals employed in this study were of reagent grade and purchased from Wuhan Chemicals & Reagent Corp., China.

2.2. Alkali pretreatment

Twenty-gram samples of RS after cutting and washing were suspended in 160 ml of 1% NaOH aqueous solution and kept boiling in a 500 ml beaker for times ranging from 15 min to 2 h. The residues were collected and washed extensively with tap water until neutral pH, dried at 65 °C and weighed [11]. Then they were cut to 10–20 mesh for their composition analysis and subsequent enzymic hydrolysis.

2.3. Microwave/alkali pretreatment

A WD700 (MG-5062T) type domestic microwave oven produced by LG Electronics Tianjin Appliances Co., Ltd. was used in this study for microwave/alkali pretreatment. The microwave frequency was 2450 MHz and the power could be set at 700, 500 and 300 W, respectively. The microwave/alkali pretreatment was carried out as follows: 20 g of RS after cutting and washing was suspended in 160 ml of 1% NaOH aqueous solution in a 500 ml beaker and the beaker positioned at the centre of a rotating circular glass plate in the microwave oven for microwave treatment at a given power. The treatment times ranged from 15 min to 2 h. The residues were collected, and then treated as above alkali pretreatment for washing and drying. Their weight loss and composition were determined before their enzymic hydrolysis was carried out.

2.4. Enzymic hydrolysis

A typical hydrolysis mixture consisted of 1 g treated RS, 20 mg of enzyme powder and 20 ml 0.1 M citric acid/citric sodium buffer (pH 4.8), which was supplemented with antibiotics tetracycline (40 $\mu g\ ml^{-1}$) and cycloheximide (30 $\mu g\ ml^{-1}$) to prevent microbial contamination. The mixture was incubated at 45 °C in a rotary shaker at 160 rpm. Samples (1 ml) were taken from the reaction mixture at different times. Each sample taken from the hydrolysis solution was heated to 100 °C immediately for 3 min to denature the enzymes, cooled to room temperature, and then centrifuged for 20 min at 8000 rpm (TGL-16C from Shanghai Antin Science Instrument Corp., China). The supernatant was used for reducing sugar analysis. When the concentrations of reducing sugars reached a plateau, the residues were collected, washed, dried, weighed, and their composition determined. The glucose and xylose content in the hydrolysate was also determined.

2.5. Analysis

The moisture was measured as the weight loss of 1 g RS or its hydrolyzed residues dried at 105 °C for 24 h [12]. The cellulose content in RS or its hydrolyzed residues was determined by means of an HNO_3–ethanol method [11,12]. The lignin content in RS or its hydrolyzed residues was