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Comparison of Three Microwave/Chemical Pretreatment Processes for Enzymatic Hydrolysis of Rice Straw

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The microwave/chemical Pretreatment is a more effective rice-straw Pretreatment technique than the conventional heating chemical Pretreatment by accelerating reactions during the Pretreatment process. In this paper, three microwave/chemical Pretreatment processes of rice straw, *viz* microwave/alkali, microwave/acid/alkali and microwave/acid/alkali/H_2O_2 were investigated for its enzymatic hydrolysis and the xylose recovery from the Pretreatment liquor. Firstly, the effect of three microwave/chemical Pretreatment processes of rice straw on its weight loss and chemical composition was examined. The results show that the rice straw pre-treated by microwave/acid/alkali/H_2O_2 had the highest weight loss and cellulose content. Then, the xylose recovery from Pretreatment liquor was studied. The xylose could not be recovered during the microwave/alkali Pretreatment process, but it could be recovered as crystalline xylose during the microwave/acid/alkali and microwave/acid/alkali/H_2O_2 Pretreatment processes. Finally, the enzymatic hydrolysis of pre-treated rice straw (substrate concentration $50\,g\,l^{-1}$, pH 4·8 and enzyme loading $20\,mg\,g^{-1}$ substrate) was also investigated and the results indicate that the rice straw pre-treated by microwave/acid/alkali/H_2O_2 had the highest hydrolysis rate and glucose content in the hydrolysate.
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1. Introduction

Rice straw, an agricultural waste, has drawn much attention from researchers and farmers because its traditional uses tend to be limited (Vlasenko *et al.*, 1997; Zhu, 2005). To extend its uses and increase its added value, extensive research has been carried out. Among these studies, the most attractive is to convert it to fermentable sugars, which can subsequently be fermented to ethanol and other chemicals by suitable microorganism processes (Vlasenko *et al.*, 1997; Kitchaiya *et al.*; 2003; Zhu *et al.*, 2005a, 2005b, 2005c). There are three major components in rice straw; hemicellulose, lignin and cellulose. The complex structure of lignin and hemicellulose with cellulose limits the effective conversion of rice straw to fermentable sugars. Many Pretreatment techniques have been developed to improve its conversion (Sun & Cheng, 2002; Zhu, 2005). Among them, the microwave/chemical Pretreatment process was shown to be an efficient Pretreatment technique to enhance the enzymatic hydrolysis of rice straw (Zhu *et al.*, 2005a). Compared with the conventional heating chemical Pretreatment process, the microwave/chemical Pretreatment of rice straw improved its enzymatic hydrolysis by accelerating the reactions during the Pretreatment process (Zhu *et al.*, 2005c). However, in previous studies, the obtained hydrolysate from the microwave/chemical pre-treated rice straw enzymatic hydrolysis had relatively high pentose content although, it is much lower than that from the conventional heating chemical pre-treated rice straw enzymatic hydrolysis. This hydrolysate was still not suitable for the subsequent fermentation process because most of the microorganism used in fermentation processes are unable to use pentose as carbon sources (Dekker, 1986). Moreover, the xylose, which is the main

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feedstock used to synthesise xylitol, could not be recovered from the microwave/chemical Pretreatment liquor. Therefore, it is necessary to improve the previous microwave/chemical Pretreatment technique. Curreli *et al.* (2002) reported a two-step chemical Pretreatment technique with conventional heating for wheat straw by which the xylose was recovered as crystalline xylose from the Pretreatment liquor, and the enzymatic hydrolysis of pre-treated wheat straw obtained the hydrolysate with low pentose content. Combination of microwave irradiation and the two-step chemical Pretreatment technique of Curreli *et al.* (2002) used for rice straw might have the advantages of Zhu *et al.* (2005a) and Curreli *et al.* (2002). In this study, three microwave/chemical Pretreatment processes of rice straw, *viz* microwave/alkali, microwave/acid/alkali and microwave/acid/alkali/H_2O_2, were investigated for its enzymatic hydrolysis and the xylose recovery from the Pretreatment liquor during the Pretreatment processes.

2. Materials and methods

All experiments were carried out three times, and the data reported are expressed as the mean values $\pm$ SD. The composition of rice straw or its hydrolysed residues was expressed on the wet basis throughout this work.

2.1. *Materials and chemicals*

Raw rice straw was obtained from local farmers in Yichang, Hubei province, China. Before Pretreatment, it was cut to 1–2 cm length and washed thoroughly with tap water until the washings were clean and colourless, and then dried in air for further treatment. The main chemical composition of this rice straw was as follows: moisture $12{\cdot}8 \pm 0{\cdot}2\%$; cellulose $38{\cdot}6 \pm 0{\cdot}4\%$; lignin $13{\cdot}6 \pm 0{\cdot}6\%$ and hemicellulose $19{\cdot}7 \pm 0{\cdot}5\%$.

The cellulase enzyme used in this study was a commercial *Trichoderma reesei* cellulase (formerly called *Trichoderma viride* cellulase) from Shanghai Boao Biotech. Corp., China. The carbomethylcellulose (CMC) saccharification enzyme activity in international units (IU) was $15 \pm 0{\cdot}6$ IU/mg, measured as the initial rate of reducing-sugars formation during hydrolysis of 0·5% CMC at pH 5·0 and 50 °C (Mandels & Weber, 1969). Its filter-paper activity in filter paper units (FPU) was $0{\cdot}53 \pm 0{\cdot}03$ 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 $0{\cdot}19 \pm 0{\cdot}02$ CBU/mg, measured as the initial rate of hydrolysis of 2 mM cellobiase to glucose at pH 5·0 and 50 °C (Vlasenko *et al.*, 1997).

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

2.2. *Microwave/alkali Pretreatment*

A WD700 (MG-5062T)-type domestic microwave oven (LG Electronics Tianjin Appliances Co., Ltd, China) was used in this study for microwave Pretreatments, at a microwave frequency of 2450 MHz and with a pulsed radiation power of 300 W. The microwave/alkali Pretreatment was carried out under its optimal conditions as follows: 20 g of rice straw after cutting and washing was suspended in 160 m*l* of 1% NaOH aqueous solution in a 500 m*l* beaker and the beaker was positioned at the centre of a rotating circular glass plate in the microwave oven for microwave treatment for 60 min (Zhu *et al.*, 2005a). The residues were collected and washed extensively with tap water until neutral pH, dried at 65 °C and weighed. Then, they were cut to 10–20 mesh for chemical composition analysis and subsequent enzymatic hydrolysis.

2.3. *Microwave/acid/alkali Pretreatment*

The microwave/acid/alkali Pretreatment conditions were optimised in a previous study on the condition that the total microwave treatment time was the same as the above microwave/alkali Pretreatment (Zhu, 2005). It consisted of two stages: microwave/acid followed by microwave/alkali. The microwave/acid Pretreatment procedures were the same as the above microwave/alkali Pretreatment, except that the rice straw was suspended in 2% H_2SO_4 solution and treated for 30 min. After its weight loss and chemical composition were determined, the residue was treated by microwave/alkali for 30 min as described above. After weight loss and chemical composition were determined, the residue was used for subsequent microwave/acid/alkali/H_2O_2 Pretreatment or enzymatic hydrolysis.

2.4. *Microwave/acid/alkali/H_2O_2 Pretreatment*

The rice straw treated by microwave/acid/alkali was suspended in 160 m*l* of 1% NaOH containing 0·3% H_2O_2 and kept at room temperature in dark for 12 h (Zhu, 2005). The residues were collected, washed and dried as above microwave/alkali Pretreatment. The weight loss and chemical composition were determined before their enzymatic hydrolysis.

2.5. *Xylose extraction and purification*

The xylose extraction and purification were carried out following the procedures described by Curreli *et al.* (2002), except that the liquor obtained from the microwave/acid treatment was recycled five times.

2.6. *Enzymatic hydrolysis*

The hydrolysis mixture consisted of 5 g treated rice straw, 100 mg of enzyme powder and 100 m*l* citric acid 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 $160\,min^{-1}$. Samples were taken from the reaction mixture at different times for reducing-sugar analysis. When the concentrations of reducing sugars reached a plateau, the residues were collected, washed, dried, and weighed, and their chemical composition was determined. The glucose and xylose content in the hydrolysate was also determined.

2.7. *Analysis*

The chemical composition (moisture, ash, cellulose, lignin and hemicellulose) of rice straw and its hydrolysed residues was determined according to the method described by Liu (2004). Additionally, reducing sugar (Miller, 1959), glucose (McCleary *et al.*, 1988), and xylose (Ashwell, 1966) were determined in the hydrolysate.

3. Results and discussion

3.1. *Effect of Pretreatment of rice straw on its weight loss and chemical composition*

The weight loss and chemical composition change of rice straw are important indices for the effectiveness of its Pretreatment. Tables 1 and 2 list the weight loss and chemical composition of rice straw after each treatment stage for three microwave/chemical Pretreatment processes, respectively. The rice straw treated by microwave/acid/alkali/H_2O_2 had the highest total weight loss, cellulose content and lowest moisture, ash, lignin and hemicellulose content. The lowest lignin and hemicellulose content indicated that the microwave/acid/alkali/H_2O_2 Pretreatment efficiently removed these components in rice straw. The highest cellulose content came from the other components that were efficiently removed and its low loss in the microwave/acid/alkali/H_2O_2 Pretreatment. The lowest moisture probably arises from the enlarged pore size of rice straw by the

Table 1
Weight loss of rice straw after each treatment stage for three microwave/chemical pre-treatment processes

Pre-treatment method	*Weight loss, %*		
	Stage 1	*Stage 2*	*Stage 3*
Microwave/alkali	44·6 ± 0·5	—	—
Microwave/acid/alkali	29·7 ± 0·3	21·6 ± 0·4	—
Microwave/acid/alkali/H_2O_2	29·7 ± 0·3	21·6 ± 0·4	3·2 ± 0·2

Table 2
Chemical composition of rice straw after each treatment stage for three microwave/chemical pre-treatment processes

Pre-treatment method	*Treatment stage*	*Composition, %*				
		Moisture	*Ash*	*Hemicellulose*	*Lignin*	*Cellulose*
Microwave/alkali	1	4·9 ± 0·3	7·0 ± 0·4	10·3 ± 0·8	5·0 ± 0·4	69·3 ± 1·3
Microwave/acid/alkali	1	6·9 ± 0·2	8·2 ± 0·4	9·4 ± 0·5	17·8 ± 0·7	54·2 ± 0·6
	2	4·9 ± 0·4	6·5 ± 0·2	3·2 ± 0·3	5·3 ± 0·3	76·3 ± 0·8
Microwave/acid/alkali/H_2O_2	1	6·9 ± 0·2	8·2 ± 0·4	9·4 ± 0·5	17·8 ± 0·7	54·2 ± 0·6
	2	4·9 ± 0·4	6·5 ± 0·2	3·2 ± 0·3	5·3 ± 0·3	76·3 ± 0·8
	3	4·3 ± 0·1	4·6 ± 0·3	3·2 ± 0·2	3·8 ± 0·2	80·6 ± 0·4

microwave/acid/alkali/H_2O_2 Pretreatment, which leads to diminishing its combined water (Zhu *et al.*, 2005a). The enlarged pore size benefits the accessibility of rice straw to hydrolytic enzymes, and thus, increases its enzymatic hydrolysis. All these results show that the microwave/acid/alkali/H_2O_2 Pretreatment is an efficient rice-straw Pretreatment technique. In the microwave/acid/alkali/H_2O_2 Pretreatment, as shown in *Fig. 1*, the stage 1 successfully removed most of the hemicellulose and stages 2 and 3 mainly removed lignin, but cellulose had only a little loss in the whole Pretreatment process.

3.2. *Recovery of xylose from Pretreatment liquor*

Xylose is the main feedstock used to synthesise xylitol, a non-cariogenic and hypocaloric sweetener, which has a high demand in the market. Recovery of xylose from Pretreatment liquor is a useful method to increase the added value of rice straw. Curreli *et al.* (2002) reported an approach to recover xylose from the wheat-straw Pretreatment liquor. Following the procedure of Curreli *et al.* (2002), the xylose was successfully recovered from the microwave/acid Pretreatment liquor in the microwave/acid/alkali and microwave/acid/alkali/H_2O_2 Pretreatment (the Pretreatment liquor was recycled five times because its further recycle led to sharply decreasing its solubilising efficiency to rice straw). The overall xylose yield was 4·2±0·3% on the basis of the original rice straw. However, the xylose could not be recovered from other Pretreatment liquors such as the microwave/alkali Pretreatment, stage 2 in the microwave/acid/alkali Pretreatment, stages 2 and 3 in the microwave/acid/alkali/H_2O_2 Pretreatment, probably because of the high lignin-derived impurities in these Pretreatment liquors.

3.3. *Enzymatic hydrolysis of pre-treated rice straw*

Figure 2 shows the time course of enzymatic hydrolysis of rice straw for three microwave/chemical

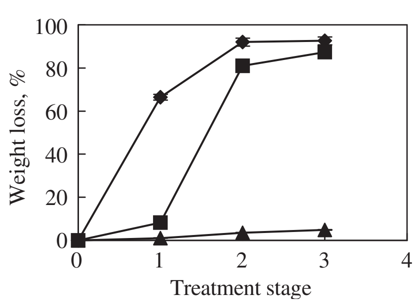

Fig. 1. Weight loss (%) for three components of rice straw after each treatment stage for microwave/acid/alkali/H_2O_2 Pretreatment: -◆-, hemicellulose; -■-, lignin; -▲-, cellulose

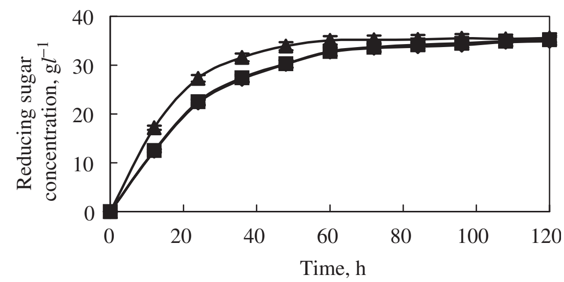

Fig. 2. Enzymatic hydrolysis of rice straw for three microwave/chemical Pretreatment processes: -◆-, microwave/alkali; -■-, microwave/acid/alkali; -▲-, microwave/acid/alkali/H_2O_2

Pretreatment processes (the curve a and b are overlapped in *Fig. 2* because the experiment data for microwave/alkali and microwave/acid/alkali Pretreatment are very close). The rice straw treated by microwave/acid/alkali/H_2O_2 had the highest hydrolysis rate. This result is consistent with the above discussion that the microwave/acid/alkali/H_2O_2 Pretreatment is an efficient rice-straw Pretreatment technique. The highest concentration of reducing sugar reached 34·9±0·5 and 35·2±0·4 g l^{-1}, after 72-h hydrolysis for rice straw treated by microwave/alkali and microwave/acid/alkali, respectively. The highest reducing sugar concentration for microwave/acid/alkali/H_2O_2-treated rice-straw was 35·6±0·4 g l^{-1} after 60-h hydrolysis. Tables 3 and 4 list the chemical composition of the hydrolysate and residues after the enzymatic hydrolysis for three microwave/chemical Pretreatment processes. The hydrolysate from the rice straw treated by microwave/acid/alkali/H_2O_2 had the highest glucose content and lowest xylose content, which is most suitable for subsequent fermentation process. The residues after hydrolysis still had relatively high cellulose content (Table 4). In order to investigate whether these residues can be reused for enzymatic hydrolysis, these residues were used in place of the above substrates and the enzymatic hydrolysis was carried out again under the same conditions. After 120-h hydrolysis, the reducing-sugar concentrations were less than 5 g l^{-1} for all above residues. This result shows these residues have very low reactivity. Their low reactivity, probably, arises from the high content of their inert components (lignin, ash and the like).

4. Conclusions

The microwave/chemical Pretreatment is a more effective rice-straw Pretreatment technique than the conventional heating chemical Pretreatment by accelerating reactions during the Pretreatment process. The microwave/acid/alkali/H_2O_2 Pretreatment is the most efficient rice-straw Pretreatment technique among these

Table 3
Chemical composition of the hydrolysate for three microwave/chemical pre-treatment processes

Pre-treatment method	*Reducing sugar, g*$^{-1}$	*Glucose, g l*$^{-1}$	*Xylose, g l*$^{-1}$
Microwave/alkali	34·9±0·5	24·8±0·4	2·6±0·2
Microwave/acid/alkali	35·2±0·4	27·4±0·3	1·5±0·2
Microwave/acid/alkali/H_2O_2	35·6±0·4	29·6±0·4	0·8±0·1

Table 4
Chemical composition of the residues after enzymatic hydrolysis for three microwave/chemical pre-treatment processes

Pre-treatment method	*Residue amount, g g*$^{-1}$ *pre-treated rice straw*	*Composition, %*			
		Ash	*Cellulose*	*Lignin*	*Hemicellulose*
Microwave/alkali	0·36±0·01	19·2±0·4	35·5±0·5	13·7±0·6	11·2±0·5
Microwave/acid/alkali	0·36±0·01	18·1±0·5	45·1±0·8	14·8±0·5	2·3±0·3
Microwave/acid/alkali/H_2O_2	0·34±0·01	13·6±0·3	53·6±0·4	4·6±0·3	5·5±0·3

three microwave/chemical Pretreatment processes. The rice straw treated by microwave/acid/alkali/H_2O_2 had the highest hydrolysis rate and its hydrolysate had the highest glucose content and lowest xylose content, which is most suitable for subsequent fermentation process. Moreover, the xylose could be recovered as crystalline xylose from the Pretreatment liquor when the microwave/acid/alkali/H_2O_2 Pretreatment was adopted.

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