Enzymatic Hydrolysis of Rice Straw Pretreated with Ammonia

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Abstract: In this study, Orthogonal methodology were employed to plan experiments and optimize the ammonia pretreatment of rice straw. Experimental results show that concentration of ammonia (CA), treatment time (TT) and powder size of rice straw (PS) were main factors governing the enzymatic saccharification of rice straw. The result showed that the influential extent of the affecting factors to the enzymatic hydrolysis was CA > PS >TT. The *F* value of CA was 8.42 and beyond $F_{0.01}(3,9)$ further in the orthogonal experiment. The reducing sugars produced by enzymes was increased from 0.8 g/L (untreated rice straw) up to 12.2 g/L (pretreated).The chemical composition analysis of straw confirmed that ammonia pretreatment could disrupt the silicified waxy surface, break down the lignin-hemicellulose complex and partially remove silicon and lignin.

Key words: rice straw, ammonia, pretreatment, enzymatic hydrolysis, orthogonal methodolog

1 Introduction

Rice is a major crop in the mainland of China at an annual yield of 0.18 billion tons. Associated with rice production is a corresponding annual production of 0.18 billion tons of rice straw. The straw has traditionally been removed from the field by the practice of open-field burning. This practice clears the field for new plantings and cleans the soil of disease-causing agents. Recently, the impact of open-field burning of rice straw on air quality has led to legislation which is strictly controlling this practice. The carbohydrate portion of the straw, 60% by weight, is being considered as a feedstock for fermentation, in a process that requires pretreatment of the lignocellulosic material resulting in a more efficient reaction despite the recalcitrant nature of the plant cell wall [1]. A number of different methods, including uncatalyzed steam explosion, liquid hot water, dilute acid, lime and ammonia[2-6], have been developed for the pretreatment of lignocellulosic biomass. The general idea of these methods if to remove or alter the hemicellulose or lignin, decrease the crystallinity of cellulose and increase the surface area. Additionally, most of these pretreatment methods require high-temperature or high-pressure reactions and the application of dose of chemicals which may be toxic to the enzymes or the microorganisms. The removal of these toxicants is always costly and complicated. Ammonia is a special agent in the chemical pretreatment of rice straw, because the C/N of rice straw is too high to ferment. When ammonia is employed in the pretreatment, ammonia can be an effective agent to decrease the C/N of medium containing rice straw for fermentation. Meanwhile, ammonia can disrupt the cell-wall matrix including the connection between carbohydrates and lignin, as well as depolymerising and solubilising hemicellulose polymers, which can promote the biotransformation in fermentation and be propitious to the growth of microorganisms.

Orthogonal methodology is a collection of statistical techniquies for designing experiments, building models, enaluating the effects of factors, which extracs the maximal information with the minimal number of runs[7]. In order to systemically study the effect of ammonia on digestibility of rice straw, the statistical methodology was applied to optimize the pretreatment conditons and investigate the effects of concentation of ammonia (CA), time of treatment (TT) and powder size of rice straw (PS) on the reducing sugars (RS) which was produced from cellulose saccharification as well as hemicellulose saccharification of rice straw. Changes of main chemical componds for pretreated straw were also determined to evaluate the influence of ammonia on recalcitrant structures.

2 Methods

2.1 Pretreatment

Rice straw was derived from a farm in Jiaxing in October 2008, and milled to particle. Ammonia pretreatment were carried out in a shaker. The following conditions were used for the pretreatment: ratio of rice straw with ammonia-10%; CA-variable; temperature-25°C; TT-varialbe; PA-variable; shake frequency-170 rpm. Solid was recovered by filtration and then washed repeatedly with distilled water until the wash water turned to pH 7.0. Subsequently this straw was dried at 80 °C to be constant weight in oven. The pretreated

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rice straw was used as the substrate for enzymatic hydrolysis.

2.2 Analytical Methods

The DNS method was used to monitor soluble RS formation during the enzymatic hydrolysis of rice straw. RS concentration in enzymatic hydrolyzated was measured with the using of DNS as a chromogen[8]. Cellulose, hemicellulose and lignin content were determined according to the procedures of Goering and Van Soest[9]

2.3 Orthogonal Experiments

For given ratio of rice straw with ammonia, hydrolysis temperature and enzyme loading, the main factors that affect enzymatic hydrolysis were the pretreatment conditions of CA,TT and PA. In order to optimze the pretreatment conditions, the $L16(4^5)$ orthogonal table[7] was applied in the study. The factors and their levels were designed in Table 1.

2.4 Enzymes

Enzymes used in the study were prepared from Coprinus 0901 by liquid fermentation in our laboratory. The activity of carboxymethyl-cellulase, filter paper eznyme, β-glucosidase and hemicellulose were 7.34×104IU/mL, 1098.23 FPU/mL, 123.45IU/mL and 578 IU/mL, respectively. Filter-paper activity of cellulase was measured according to the standard procedure reommended by the Commission on Biotechnology, IUPAC[10]. β-glucosidase activity was assayed for 30 min at 50 oC using an SBA-40C Biosensor to determine the glucose concentration released. Carboxymethyl-cellulase activity was determined according to Mandels et al.[11]. Xylanase activeity was analyzed according to the method of Mohana et al. [12].

2.5 Enzymatic Hydrolysis of Rice straw

Hydrolysis of rice straw was carried out in the 250-mL Erlenmeyer flasks at pH 5.0 and 50 ^oC for 2 h. The hydrolysis mixture was consisted of 1 g of powder of rice straw, 5 mL of liquid enzymatic mixture and 45 mL of 0.05 M sodium acetate buffer and was incubated in a shaker at 100 rpm. After incubation, samples were collected and centrifugaed for sugar analysis. The enzymatic hydrolysis experiments were performed in five replicates.

3 Results and Discussion

3.1 Orthogonal Experiments for Pretreatment

To obtain the optimal pretreatment conditions, orthogonal experiments were carried out and the production of RS and statistical analyis were shown in Table 1. The K, R_j and eΔ values in Table 1 and Table 2 were calculated following the procedures described by Du with some modification. As shown in Table 1, the Rj value for CA had the highest value, while that for TT had the lowest value, which was close to the average of e1 and e2. It indicated that the influential extent of the affecting factors to the enzymatic hydrolysis was CA > PS >TT. The F value of CA was 8.42 and beyond $F_{0.01}(3,9)$, which also meant that CA was the most important factor that affected the production of RS in the enzymatic hydrolysis. Further, the untreated rice straw (powder size 80 mesh) was employed in the enzymatic hydrolysis experiment, in which RS was 0.8 g/L.

Table 1 Orthogonal experiments for optimal pretreatment

Treatment No.	PS (mesh)	e_1	CA (%)	TT (h)	e_2	RS (g/L)
1	40	1	2	6	1	3.6
2	40	2	4	12	2	5.4
3	40	3	6	18	3	5.8
4	40	4	8	24	4	7.8
5	60	1	4	18	4	6.3
6	60	2	2	24	3	6.5
7	60	3	8	6	2	7.5
8	60	4	6	12	1	6.3
9	80	1	6	24	2	7.1
10	80	2	8	18	1	12.2
11	80	3	2	12	4	4.8
12	80	4	4	6	3	5.1
13	100	1	8	12	3	10.2
14	100	2	6	6	4	8.4
15	100	3	4	24	1	7.6
16	100	4	2	18	2	6.4
K_1	22.6	27.2	21.3	24.6	29.7	
K_2	26.6	32.5	24.4	26.7	26.4	
K_3	29.2	25.7	27.6	30.7	27.6	
K_4	32.2	25.6	37.7	29	27.3	
R_j	9.6	6.9	16.4	6.1	3.3	
S_j	11.52	5.95	33.62	4.65	1.36	

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Table 2 The variance analysis concerning to the orthogonal test

Resource	S_j	Freedom	$\bar{S_j}$	F	Notability
PS	11.52	3	3.84	2.89	
CA	33.62	3	11.2	8.42	**
TT	4.65	3	1.55		
e_1+e_2	7.31	6	1.22		
e^{Δ}	11.96	9	1.33		

*$F_{0.05}(3,9)=3.863$, $F_{0.01}(3,9)=6.992$.

3.2 Chemical Characteristics of Pretreated Rice Straw

For the recalcitrant nature of rice straw with polymerised complex structure, the biodegradtion of rice straw is difficult. Pretreatment of chmical reagents is efficient on the depolymising of cellulose, hemicellulose and lignin. As seen in Table 3, the main effect of the ammonia pretreatment on the composition of the biomass was the partial but substantial removal of lignin and silicified wax (equivalent of ash in Table 3). Cellulose was increased from 35.56% to 45.22, hemicellulose was reduced from 30.12% to 25.36, ash from 4.5% to 2.8%, as well as lignin from 25.22% to 20.69%, respectively, in the treatmet No. 10 compared with the untreated sample.

3.3 Physical Properties and Cellulose Micro-Structure of Pretreated Rice Straw

The chemical composition was not the sole factor influencing the enzymatic hydrolysis. Physical propertied and ligocellulose microstructure were among the potential factors influencing enzymatic hydrolysis.

FTIR spectroscopy was used as an analytical tool to qualitatively determine the chemical changes in the surface of pretreated straw to complement and understand the microscopic investigations. The FTIR spectra of untreated and pretreated rice straw samples are shown in Fig.1. One of the effects of the pretreatment is the removal of wax from the straw: Fig.1 shows that the CH2-stretching bands at approximately 2920 cm^{-1} were reduced for the pretreated straw sample, signifying a reduction in the amount of the aliphatic fraction of waxes[13]. The carbonyl bond at 1640 cm^{-1}, which has been ascribed to hemicellulose [14,15] is reduced for the pretreated straw. This is expected as the pretreatment is known to remove a large portion of the hemicellulosed as shown results of chemical components above. Lignin bands at approximately 1380 cm-[1] (aromatic ring stretch)[16] were reduced in the pretreated samle compared with untreated rice straw. The explanation for this could be a relative decrease in the amount of lignin due to the effect of ammonia.

Table 3 Chemical components of rice straw

	Cellulose	Hemicellulose	Lignin	Ash
Untreated	35.56%	30.12%	25.22%	4.5%
Treat 1	36.78%	29.565	24.86%	4.0%
Treat 10	45.22%	25.36%	20.69%	2.8%
Treat 14	41.55%	27.24%	22.54%	3.5%

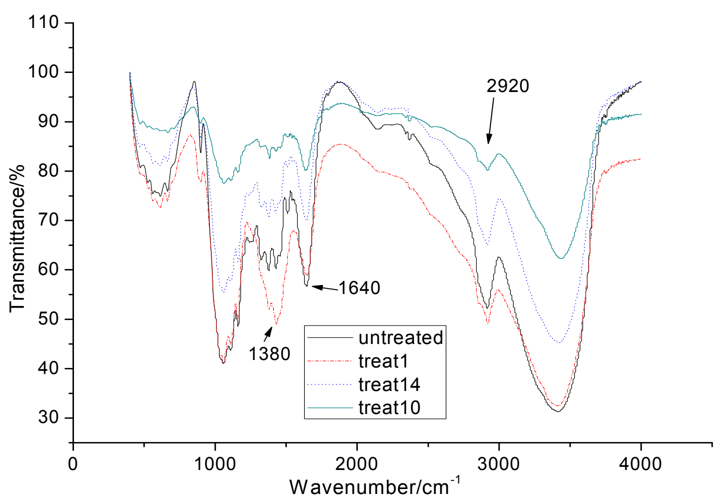

Figure 1 FTIR spectra of samples of untreated, treatment No.1, No.10 and No.14.

4 Conclusions and Discussion

The conditions for ammonia pretreatment of rice straw were examined by using orthogonal methodology. CA was the most important factor in the pretreatment of rice straw for enzymatic hydrolysis. Compared with the untreated straw, cellulose was increased 9.66%, hemicellulose, lignin and ash were decreased 4.67%, 4.35% and 1.7% under the pretreatment condions: PS at 80 mesh, CA at 8%, TT at 18h, respectively. The RS produced was increased from 0.8 g/L to 12.2 g/L. The result of FTIR analysis also confirmed that wax and lignin were partly removed by ammonia pretreatment. We will further study the effects of ammonia pretreatment on the bio-digestablity when the rice straw was applied as the feedstock for fermentaton. The cellulose and hemicellulose will be exposured and benefited to microorganism with the effect of ammonia. On the other hand, ammonia was a rapid available nitrogen resource for microorganism. We considered that ammonia was the most potential chemical reagent in the pretreatment of straw and other agricultural waste.

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