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Effect of the combined physical and chemical treatments with microbial fermentation on corn straw degradation

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HIGHLIGHTS

- Optimal reaction conditions of sodium hydroxide for lignin degradation were obtained.
- Physicochemical treatments were better than individual use for straw degradation.
- Combined physicochemical and microbial treatments could degrade straw significantly.

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ABSTRACT

In order to improve corn straw degradation, steam explosion, sodium hydroxide soaking and *Aspergillus oryzae* fermentation were used. The optimal sodium hydroxide pretreatment condition for lignin degradation was obtained. The degradation rates of hemicellulose, cellulose and lignin were 54.68%, 17.76% and 33.14% for the exploded straw ($P < 0.05$); 67.92%, 2.44% ($P > 0.05$) and 76.54% for the alkali-treated straw ($P < 0.05$); 75.98%, 39.93% and 77.88% for the exploded and alkali-treated straw ($P < 0.05$), respectively. The following microbial fermentation could degrade hemicellulose and cellulose further ($P < 0.05$). Cellulase, amylase and protease activities produced during microbial fermentation in the pretreated corn straw were lower than that in the untreated one ($P < 0.05$); however, glucose content was increased by microbial fermentation ($P < 0.05$). It can be concluded that the combined treatments of steam explosion, sodium hydroxide and microbial fermentation will be a good method for straw degradation.

1. Introduction

Lignocellulose is the largest renewable resources in the world, it is about 10 billion tons produced yearly, while about 70% is crop straw (Anchez and Cardona, 2008). Because the crystal structure of straw is composed of hemicellulose, lignin and cellulose, it is very difficult to be broken up and degraded. Therefore, a lot of straws are destroyed, buried or burned, resulting in resource waste and environmental pollution. How to use this resource effectively becomes more and more important.

At present, the main straw-degrading methods include physical, chemical, physicochemical and biological treatments (Brodeur et al., 2011). The steam explosion belongs to physicochemical treatment, which can break up and degrade the crystal structure of straw by explosion effect (Chang et al., 2012; Alvira et al., 2010). The chemical treatments include acid and alkali treatments or antioxidant treatments such as sodium hydroxide, hydrogen peroxide, sulfur dioxide, and so on. Sodium hydroxide cannot change the chemical structure of cellulose; however, the ester bonds formed among cellulose, hemicellulose and lignin are sensitive to alkali, which can cause fiber swell and make pectin, lignin, hemicellulose and other low-molecular weight components be dissolved (Koullas et al., 1992). Biological treatments include microbial fermentation and enzymatic hydrolysis, which are safe, environment friendly and economic, compared to other treatments (Liang et al., 2010; Dinis et al., 2009). However, it is limited by low-rate hydrolysis reaction and long-time microbial fermentation, so a great improvement is needed for its commercial application in the future (Brodeur et al., 2011; Sun and Cheng, 2002).

Generally, the individual treatment has some inevitable limitations and disadvantages for straw degradation due to its complex crystal structure. For example, steam explosion pretreatment could break up straw crystal structure but with low lignocellulosic degradation and some deleterious derivates or by-products (Chang et al., 2012; Brijwani and Vadlani, 2011). Sodium hydroxide could degrade lignin largely; however, it had no effect on cellulose degradation (Chesson, 1981). So the combined treatment methods are suggested (Brodeur et al., 2011). It was reported that the combined

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physicochemical and enzymatic hydrolysis treatments could significantly improve straw degradation (Kang et al., 2013; Huijgen et al., 2012). Nevertheless, the combined effects of steam explosion, sodium hydroxide treatment and microbial fermentation on straw degradation have not been reported. The objective of this research was to find an effective method to convert the straw to high-digestibility palatable feedstuffs for solving feed shortage in animal husbandry.

2. Methods

2.1. Corn straw preparation

The corn straw was obtained from the suburbs of Zhengzhou, China. The straw was ground in a mill, sieved to obtain pieces of 1 mm (accounting for 90%), and stored at room temperature for the following use.

2.2. Steam explosion treatment

The steam explosion of corn straw was carried out by steam explosion equipment (QBS-80), produced in Hebi Zhengdao Machine Factory, Hebi, China. About 50 kg corn straw was put into a steam chamber once. The steam was adjusted to 2.5 MPa pressure and kept for 200 s, and then suddenly released at the end of treatment to give explosion effect (Chang et al., 2012). The exploded samples were collected and dried at 65 °C before analysis.

2.3. Sodium hydroxide treatment

The orthogonal experimental design of three factors and three levels was listed in Table 1. Sodium hydroxide was assigned as factor A at 3 levels of 1.0%, 1.5% and 2.0%. Treatment temperature was assigned as factor B at 3 levels of 60, 80 and 100 °C. Treatment time was assigned as factor C at 3 levels of 1.0, 1.5 and 2.0 h. The ratio of corn straw and sodium hydroxide was 1:10 (w/v). There were 9 experimental groups and 1 control group in the design, 3 replicates for each group. At the end of each treatment, the pH values of samples were adjusted to 6.5–7.0 with H_2SO_4. The samples were dried at 65 °C before analysis.

2.4. Steam explosion–sodium hydroxide treatments

The corn straw was pretreated by steam explosion first, and then soaked in sodium hydroxide under the optimal conditions obtained from the above experiment. The other managements were the same as above.

Table 1
Factors and levels of orthogonal experiment for sodium hydroxide treatment.

Groups	Sodium hydroxide concentrations (%)	Soaking temperature (°C)	Soaking time (h)
	A	B	C
1	1.0	60	1.0
2	1.0	80	1.5
3	1.0	100	2.0
4	1.5	60	2.0
5	1.5	80	1.0
6	1.5	100	1.5
7	2.0	60	1.5
8	2.0	80	2.0
9	2.0	100	1.0
Control	0.0	25	0.0

2.5. Microbial fermentation of the different pretreated corn straws

The solid-state microbial fermentation media were composed of 90% pretreated or untreated corn straw, 4% corn meal, 3% soybean meal and 3% wheat bran. The fermentation was carried out in the 500 mL Erlenmeyer flasks with cotton plug. Twenty grams of samples were mixed with 30 mL mineral solution. The compositions of the mineral solution were as follows (g/L): $(NH_4)_2SO_4$ 1.4, KH_2PO_4 2.0, $MgSO_4$ 0.3, $CaCl_2$ 0.3, NaCl 0.5, $FeSO_4$ 0.005, $MnSO_4$ 0.0016, $ZnCl_2$ 0.0017, $CoCl_2$ 0.002. The solid-state media were autoclaved at 121 °C for 20 min. The designs of microbial fermentation experiment were as follows:

Group 1: the untreated corn straw with *Aspergillus oryzae* fermentation.
Group 2: the steam exploded corn straw with *A. oryzae* fermentation.
Group 3: the sodium hydroxide treated corn straw with *A. oryzae* fermentation.
Group 4: the pretreated corn straw with steam explosion and sodium hydroxide combination plus *A. oryzae* fermentation.

The pH value in group 2 was adjusted to 7.0 with $Ca(OH)_2$, and pH in group 3 and 4 was adjusted to 7.0 with H_2SO_4. The liquid seed culture was used at 4% (v/w). The experiments were performed in triplicates for each group at 30 °C for 6 d, and then dried for analyses.

2.6. Determinations of enzyme activity and corn straw compositions

The neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose, and lignin fractions of samples were determined according to the method of Van Soest et al. (1991). The enzyme activity and soluble carbohydrates were determined according to the following protocol: put 2 g fresh samples in 40 mL deionizer water, stirred for 2 h at room temperature, filtered through filter paper, and then the filtered liquid was kept for determining enzyme activity. The filter paper cellulase activity (FPA) and endoglucanase activity were measured with Whatman No. 1 filter paper and 1% (w/v) carboxymethyl cellulose (CMC) as substrates, respectively (Ghose, 1987). Amylase activity was determined by using soluble starch (Sigma Chemical, USA) as a substrate (Yasser et al., 2009). The units of FPA, CMC and amylase activities were defined as the amount of enzyme releasing 1 μmol glucose equivalent per min. Protease activity was determined by the method of Sandhya et al. (2005), 1 U of protease was defined as the amount of enzyme that liberated 1 μmol tyrosine per min.

2.7. Statistical analysis

Experimental data were expressed as the means and standard errors (SE) for calculating biological and chemical compositions of corn straw with different physicochemical and microbial treatments. The data were determined for each of the three replicates per treatment, which was averaged to give a single value of each sample for subsequent statistical analysis. The data were analyzed using the ANOVA procedures of Statistical Analysis Systems Institute (SAS 8.0). Duncan's multiple range test was used to compare treatment means. Differences were considered statistical significance at $P < 0.05$.

3. Results and discussion

3.1. Optimal condition of lignin degradation by sodium hydroxide treatment

Table 2 indicated that the best sodium hydroxide pretreatment condition for lignin degradation was to soak the sample in 1.5%