Bioresource Technology 194 (2015) 165–171

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effects of thermo-chemical pretreatment plus microbial fermentation and enzymatic hydrolysis on saccharification and lignocellulose degradation of corn straw

Ping Wang [a], Juan Chang [a], Qingqiang Yin [a,*], Erzhu Wang [a], Qun Zhu [b], Andong Song [c], Fushan Lu [d]

[a] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China
[b] Henan Delin Biological Product Co. Ltd., Xinxiang 453000, China
[c] College of Life Science, Henan Agricultural University, Zhengzhou 450002, China
[d] Henan Engineering and Technology Research Center of Feed Microbes, Zhoukou 466000, China

HIGHLIGHTS

- Response surface methodology for constructing model of alkali pretreatment corn straw.
- Combined chemical pretreatments with microbial fermentation and enzymatic hydrolysis.
- Combined treatment is better than the individual one for straw saccharification.

ARTICLE INFO

Article history:
Received 31 May 2015
Received in revised form 4 July 2015
Accepted 4 July 2015
Available online 11 July 2015

Keywords:
Corn straw
Thermo-chemical pretreatment
Microbial fermentation
Enzymatic hydrolysis
Saccharification

ABSTRACT

In order to increase corn straw degradation, the straw was kept in the combined solution of 15% (w/w) lime supernatant and 2% (w/w) sodium hydroxide with liquid-to-solid ratio of 13:1 (mL/g) at 83.92 °C for 6 h; and then added with 3% (v/v) H_2O_2 for reaction at 50 °C for 2 h; finally cellulase (32.3 FPU/g dry matter) and xylanase (550 U/g dry matter) was added to keep at 50 °C for 48 h. The maximal reducing sugars yield (348.77 mg/g) was increased by 126.42% ($P < 0.05$), and the degradation rates of cellulose, hemicellulose and lignin in pretreated corn straw with enzymatic hydrolysis were increased by 40.08%, 45.71% and 52.01%, compared with the native corn straw with enzymatic hydrolysis ($P < 0.05$). The following study indicated that the combined microbial fermentation and enzymatic hydrolysis could further increase straw degradation and reducing sugar yield (442.85 mg/g, $P < 0.05$).

1. Introduction

Straw lignocelluloses as structural carbohydrate are an abundant and renewable resource in the world (Thomas, 2008). However, a lot of them are burned or buried in the field as a waste due to the shortage of effective treatment methods (Jiang et al., 2012). Generally straw is mainly composed of cellulose, hemicellulose and lignin. Enzymatic hydrolysis and microbial fermentation are the promising methods to transfer cellulose and hemicellulose to glucose monomers, alcohol, furfural, animal feedstuffs, energy and other chemical raw materials (Lambert et al., 1990; Chang et al., 2012; Xu et al., 2012). However, the complex three-dimensional polyaromatic matrix of lignin prevents enzymes from accessing some regions of cellulose polymers (Hendriks and Zeeman, 2009). It is reported that pretreatment is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes for converting the carbohydrate polymers into fermentable sugars (Mosier et al., 2005). Pretreatment has been viewed as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion, and it has great potential for improvement of efficiency and lowering of cost through research and development (Mosier et al., 2005). Therefore, how to reduce the cost of straw pretreatment, degrade lignocellulose and increase reducing sugar yield becomes more and more important.

At present, there is a contradiction between human food and animal feed because animals always eat more grains which should belong to human being. How to solve this problem is important especially when population increases so quickly in the world. Turning cellulose and hemicellulose to reducing sugar instead of grain in animal diets is feasible. It is reported that straw can be

* Corresponding author. Tel.: +86 371 63558180; fax: +86 371 63558998.
E-mail address: qqy1964@126.com (Q. Yin).

http://dx.doi.org/10.1016/j.biortech.2015.07.012
0960-8524/© 2015 Elsevier Ltd. All rights reserved.

turned to animal feedstuffs by physical pretreatment and microbial fermentation (Chang et al., 2012), which will help to solve feed shortage for animal production.

For fracturing lignin and improving straw availability, many pretreatment strategies such as acid, alkali, steam explosion, and organic solvents have been developed to facilitate the enzymatic hydrolysis of corn stover (Mosier et al., 2005; Kim and Holtzapple, 2005; Chang et al., 2012; Perez-Cantu et al., 2013). Generally high chemical concentration will degrade straw better than the low concentration (Han et al., 2012), but high chemical concentration will cause environment pollution, increase cost, and damage animal health. It was reported that acid and alkali pretreatments of straw are unsuitable for feed production because chemical ion contents (Na^+, Ca^{2+} and Cl^-) are beyond animal feeding standards (Chen et al., 2009; Iroba et al., 2013). The organic solvents such as dimethylsulfoxide, N,N-dimethylformamide and 1,3-dimethy l-2-imidazolidinone have been used as co-solvents for dissolution of cellulose, but these materials are harmful to animal health (Mai et al., 2014).

In order to solve the above problems, low concentrations of lime (CaO) and sodium hydroxide (NaOH) were chosen according to animal feeding standards (NRC, 1994) in this study. It is reported that the requirements of Ca^{2+} and Na^+ for broilers are 0.8–1.0% and 0.12–0.20%, respectively (NRC, 1994). The residual Ca and Na ions in the pretreated corn straw can be used by animals with the optimal adding ratio of the treated straw in animal diet. Optimal lime concentration and pretreatment conditions were assessed by using response surface methodology (RSM) design. For increasing the effectiveness of straw degradation, the combined and independent pretreatments of lime, sodium hydroxide, hydrogen peroxide (H_2O_2) followed by microbial fermentation and enzymatic hydrolysis were used in this study to increase saccharification greatly for further applications.

2. Methods

2.1. Raw materials

The air-dried corn straw was ground in hammer crusher, sieved to a size of 1 mm, and stored at room temperature. The cellulase and xylanase powder was purchased from Shandong Zesheng Biological Technology Co. Ltd., China. The activities of cellulase and xylanase were 323 FPU/g (142 mg protein/g) and 5500 U/g (6 mg protein/g) estimated according to the NREL Laboratory Analytical Procedure (IUPAC, 1987). The enzyme protein content was determined using Bradford method (Bradford, 1976).

2.2. Experimental design for obtaining the optimal chemical acting condition

Design-Expert 8.0.5 software (Statease, Inc., Minneapolis, MS, USA) was used for the experimental design. Model fitting, statistical data analysis and Box–Behnken Design were applied. Based on the previous results of straw pretreatments, the optimal parameters of reaction temperature, reaction time, lime concentrations and liquid-to-solid ratios were selected and listed in Table 1 for RSM analysis. The chemical pretreatment process of straw was as following: (1) About 15 g mashed corn straw was put in a 500 mL Erlenmeyer flask. (2) The supernatants of different lime concentrations were prepared in the following process, i.e. lime amount (5%, 10% and 15%) was calculated based on the dry weight of corn straw (w/w), and then dissolved in the corresponding distilled water, stirred for 5 min and stood for 10 min for getting the supernatants. (3) About 2% NaOH was calculated based on the dry weight of corn straw (w/w) and added in the above lime supernatants, which was chosen according to animal feeding standards (NRC, 1994). (4) Corn straw was soaked in the above alkali solution at liquid-to-solid ratios of 9:1, 11:1 and 13:1 in water bath with 70, 80 and 90 °C for 6, 12 and 18 h, respectively. (5) The experiment was conducted with RSM design (4 factors and 3 levels). (6) All the samples were treated with 3% H_2O_2 (v/v) at 50 °C for 2 h. (7) The biomass was dried at 65 °C, and then enzymatic hydrolysis and RSM analysis were conducted.

Table 1
The levels of variables for the design.

Factors	Coded levels		
	−1	0	1
X_1: temperature (°C)	70	80	90
X_2: time (h)	6	12	18
X_3: lime concentrations (lime/straw) (%)	5	10	15
X_4: liquid-to-solid ratios	9:1	11:1	13:1

In order to correlate the relationship between variables and response, a quadratic polynomial equation was used for fitting. The general form of the predictive polynomial quadratic equation was in the following:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4$$

Y is the predicted production of reducing sugar in the chemically pretreated straw with enzymatic hydrolysis (mg/g dry matter); X_1, X_2, X_3, X_4 are the independent variables corresponding to reaction temperature, reaction time, lime concentration, liquid-to-solid ratio, respectively; β_0 is the intercept; β_1, β_2, β_3, β_4 are linear coefficients; β_{11}, β_{22}, β_{33}, β_{44} are square coefficients; β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34} are cross product coefficients.

2.3. Straw pretreatments

The following experiments were conducted based on the above ideal result (liquid-to-solid ratio of 13:1 at 84 °C for 6 h). The experimental design was as following:

Group 1: untreated corn straw.
Group 2: corn straw treated with the supernatant of 15% lime (w/w).
Group 3: corn straw treated with 2% sodium hydroxide (w/w).
Group 4: corn straw treated with the supernatant of 15% lime (w/w) + 2% sodium hydroxide (w/w).
Group 5: corn straw treated with the supernatant of 15% lime (w/w) + 3% hydrogen peroxide (v/v).
Group 6: corn straw treated with 2% sodium hydroxide (w/w) + 3% hydrogen peroxide (v/v).
Group 7: corn straw treated with the supernatant of 15% lime (w/w) + 2% sodium hydroxide (w/w) + 3% hydrogen peroxide (v/v).
Group 8: corn straw treated with 3% hydrogen peroxide (v/v).

2.4. Cellulase and xylanase hydrolysis of the pretreated corn straw

Enzymatic hydrolysis was carried out at straw consistency of 5% (w/v) in distilled water in shaking incubator at 180 rounds per min (RPM) and 50 °C for 48 h, and then the reaction was terminated by heating at 100 °C for 30 min. The biomass after enzymatic hydrolysis was dried at 50 °C to 90% dry matter, and then ground for analysis. The pH value of enzymatic hydrolysis system was adjusted to 4.8 with 0.05 M HCL or supernatant of $Ca(OH)_2$. Enzyme activity