



## Full Length Article

# Bioethanol production from cotton stalk: A comparative study of various pretreatments



Meixia Wang<sup>a,1</sup>, Dayun Zhou<sup>b,1</sup>, Yanqin Wang<sup>b</sup>, Shoujun Wei<sup>b</sup>, Weihua Yang<sup>b</sup>, Meng Kuang<sup>b</sup>, Lei Ma<sup>b</sup>, Dan Fang<sup>b</sup>, Shuangjiao Xu<sup>b</sup>, Shuang-kui Du<sup>a,\*</sup>

<sup>a</sup> College of Food Science and Engineering, Northwest A&F University, Yangling 712100, Shaanxi, China

<sup>b</sup> Institute of Cotton Research of Chinese Academy of Agricultural Sciences, State Key Laboratory of Cotton Biology, Anyang 455000, Henan, China

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## ABSTRACT

Cotton stalk (CS) is a potential biomass for bioethanol production, but the direct conversion without pretreatment always results in an extremely low yield because of the recalcitrant nature of lignocellulose. In this study, the effects of various methods, i.e. dilute sulfuric acid pretreatment (DSAP), ultrasound-assisted alkali pretreatment (UAAP), and high pressure-assisted alkali pretreatment (HPAP), on chemical composition, physical structure, and subsequent enzymatic hydrolysis and ethanol fermentation for bioethanol production from CS have been explored. It was found that the intact structures of pretreated CS were obviously disrupted. The hemicellulose and lignin of biomass were removed and the crystallinity of cellulose increased after pretreatments. HPAP led to the highest reducing sugar and ethanol yields (271.70 mg g<sup>-1</sup> and 45.53%, respectively) compared with UAAP and DSAP. HPAP proved to be a potential and effective pretreatment method for ethanol production from CS.

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## 1. Introduction

Growing concerns over the global energy shortage and rapid depletion of fossil fuels associated with environmental damages, such as global warming, acid rain, and urban smog, have led to the extensive exploration of alternative and renewable energy sources [1,2]. Bioethanol produced from lignocellulosic biomass is one of the most promising biofuels as it is abundant, renewable, and relatively inexpensive [3,4].

Cotton stalk (CS), a by-product of cotton production, is a renewable lignocellulosic biomass. It is rich in cellulose (32–46%) and hemicellulose (20–28%), which make it a potential raw material for the conversion of cellulose to ethanol [5]. Globally, more than 12 million hectares of cotton is planted across 80 countries [6]. As the world's largest producer of cotton, China annually produces 40 million tons of CS [5]. However, a large portion of CS is used as firewood for household energy needs or burned on the ground, causing serious environmental pollution and biomass waste [7,8].

Some attempts have been made to investigate the potential of employing the cellulose of CS for fuel ethanol. CS is a complex and compact network structure consisting primarily of cellulose,

hemicellulose, and lignin bonded to one another, so native CS is recalcitrant to enzymatic accessibility. Therefore, pretreatment is essential to change the recalcitrant structure [9] and chemical composition of biomass to facilitate the production of fermentable sugars and cellulosic ethanol. Pretreatment is necessary to reduce production and processing costs.

Through many researchers' efforts, current existing pretreatment techniques of lignocellulosic biomass, including dilute acid, alkali, ionic liquid, and biological pretreatment or various combinations, have been extensively investigated in laboratories and under development [10–14]. Although numerous pretreatment methods exist, each one has its own advantages and disadvantages. Various pretreatments are better suited for specific feedstocks [15]. Hence, much effort must be made to develop a cheap, efficient, and environmentally friendly pretreatment technique for CS.

Recently, acid- and alkali-based pretreatment technologies were extensively used for lignocellulosic biomass, although both methods demonstrate distinct action mechanisms for cell wall destruction. Generally, acid-based pretreatment hydrolyzes hemicellulose components and exposes cellulose for enzymatic digestion [16]. Dilute acid, such as dilute sulfuric, nitric, and hydrochloric acids, is commonly used to cost-effectively and environmentally friendly pretreat lignocellulosic biomass [13,16]. Various lignocellulosic feedstocks were subjected to dilute acid pretreatment to enhance the production of fermentable sugars

\* Corresponding author.

E-mail address: [dushuangkui@hotmail.com](mailto:dushuangkui@hotmail.com) (S.-k. Du).

<sup>1</sup> The authors contributed equally to this work.

via enzymatic hydrolysis, such as cotton gin trash [6] and mustard stalk [10]. Kapoor et al. [10] investigated dilute acid, steam exploded, and alkali pretreated mustard stalk and found that dilute acid pretreatment was the best methodology in terms of the maximum sugar yield at low enzyme loading. Dilute acid is favorable for industrial applications [15]. Alkali-based pretreatment causes the breakdown of ester bonds cross-linking lignin and xylan, removal of lignin, cellulose swelling, and partial decrystallization of cellulose [10,16]. Among alkali-based pretreatments, sodium hydroxide has been studied by many researchers. Kaur et al. [8] reported that the enzymatic hydrolysis of 4% alkali-treated CS (121 °C, 60 min) after 48 h resulted in 65% of the theoretical glucose yield from cellulose. Silverstein et al. [16] compared four chemical pretreatment methods (sulfuric acid, sodium hydroxide, hydrogen peroxide, and ozone pretreatments) for improving saccharification of CS, and sodium hydroxide pretreatment resulted in the highest level of cellulose conversion (60.8%, for 2% NaOH, 90 min, 121 °C/15 psi). However, a single pretreatment method alone is not feasible in consideration of cost and efficiency. Therefore, complex pretreatment methods need to be extensively investigated. Excellent efficiency of hydrolysis can be obtained by integrating acid or alkali with suitable thermomechanical techniques, such as ultrasound and high pressure.

Ultrasound, a sound wave, can produce energy in the form of cavitation and agitation in liquid [13], which has the potential to destroy the surface structure of lignocellulosic biomass. Ultrasound has been applied to assist in the pretreatment of various lignocellulosic feedstocks with different reaction solutions [13,17]. Although researches on high-pressure pretreatment of biomass are limited, some researchers have shown that saccharification is enhanced efficiently with the help of high pressure. Du et al. [5] reported that HPAP led to the maximum reducing sugar of 0.293 g/g from CS. The above mentioned studies showed that ultrasound-assisted alkali and high pressure-assisted alkali are promising pretreatment methods for CS. As different lignocellulosic feedstocks have different physicochemical characteristics, suitable pretreatment techniques based on the properties of each raw material must be adopted [15].

CS is one of the most abundant agricultural wastes in China [18] and has the potential to act as a low-cost feedstock for bioethanol production. To date, studies on the pretreatment of CS are very limited and lack depth. Dilute sulfuric acid pretreatment (DSAP), ultrasound-assisted alkali pretreatment (UAAP), and high pressure-assisted alkali pretreatment (HPAP) were studied respectively for ethanol production from lignocellulosic materials [5,6,17]. However, further evaluate on the effectiveness of various pretreatments on specific biomass was required. The purposes of this work were to compare the effects of these pretreatments on: (1) the chemical composition and physical structures of pretreated CS; (2) reducing sugar yields after enzymatic hydrolysis; (3) ethanol yields after yeast fermentation. The pretreatments were comparatively studied to improve ethanol production from CS. All such efforts on adding value to CS are becoming increasingly necessary in tackling environmental pollution and global energy shortage.

## 2. Materials and methods

### 2.1. Materials and chemicals

CS (*Gossypium hirsutum*) was obtained from the Institute of Cotton Research of CAAS. The CS were air dried to reduce the moisture content to 8–9%, shredded to 1–2 cm, and milled to pass through a 40 mesh screen (~0.7 mm) using a sawtooth mill. All samples were stored in air-tight containers at room temperature for composition analysis and for further use. All the chemical reagents used were of analytical grade.

### 2.2. Pretreatment

Three pretreatments (DSAP, UAAP, and HPAP) were employed according to the following parameters and untreated CS was used as the control in this study. All of the experiments were carried out in duplicate.

#### 2.2.1. DSAP

Approximately 2.00 g of dried and ground cotton stalk powders were mixed with 50 mL of 3.5% H<sub>2</sub>SO<sub>4</sub> to obtain a solid loading of 4% (w/v, grams dry weight per 100 ml). The mixtures were placed in 250 mL Erlenmeyer flasks, kept at 135 °C for 2.0 h in a laboratory autoclave, and cooled. The residual solid biomass was collected by vacuum filtration using a Buchner funnel lined with filter paper and washed repeatedly with distilled water to pH of 7. The neutralized residues were dried at 60 °C to a constant weight. After cooling, they were weighed to determine weight loss before and after pretreatment. Finally, the residues were sealed in polybags and used for composition analysis and enzymatic hydrolysis.

#### 2.2.2. UAAP

Approximately 2.00 g of dried and ground cotton stalk powders were mixed with 40 mL of 3.5% NaOH to obtain 5% solid loading. The mixtures were placed in a sealed conical flask and subjected to ultrasound pretreatment in an ultrasound cleaning bath (KQ-700DE, Ultrasound Instrument Co., Ltd., Kunshan, China), which acted as a thermo-stated ultrasound generator for 90 min with ultrasound at 420 W, and the temperature was controlled at 25 °C. After pretreatment, the pretreated solid biomass was filtered, washed, dried, and collected.

#### 2.2.3. HPAP

Approximately 2.00 g of dried and ground cotton stalk powders were mixed with 40 mL of 3.0% NaOH to obtain a solid loading of 5% (w/v). The mixtures were placed in a sealed conical flask and treated at 121 °C by high pressure using a commercial autoclave (ES-315, Tomy Kogyo Co., Ltd., Japan) within an operating pressure of 130 kPa for 40 min. After pretreatment, the pretreated solid biomass was filtered, washed, dried, and collected.

### 2.3. Enzymatic saccharification of CS

Enzymatic saccharification of untreated or pretreated CS was carried out using commercial cellulase (activity of 60 ± 3.1 FPU/g, FPU-Filter Paper Unit) from Shanghai Boao Biotech. Corp., China. The amount of enzyme used was 30 FPU/g dried substrate. The hydrolysis was performed in a 150 mL flask containing 25 mL 50 mM sodium acetate buffer (pH 5.0, at room temperature). 0.50 g (dry weight) of pretreated residues was added to the acetate buffer with a resultant substrate concentration of 2% (w/v). The mixture containing 10 mM sodium azide to prevent microbial contamination was incubated at 48 °C for 24 h with 120 rpm. After hydrolysis, the samples were filtered and centrifuged at 3000g for 10 min to remove unhydrolyzed residues. The reducing sugar (measured as glucose) content of the supernatant was determined using the 3,5-dinitrosalicylic acid method [19]. Results were expressed as mg reducing sugar per g dry biomass using the following equation:

$$\text{reducing sugar yield (mg g}^{-1} \text{ dry biomass)} = (\rho \times V)/m$$

where  $\rho$  is the concentration (mg/mL) of reducing sugars in the sample hydrolyzed,  $V$  is the total volume (mL) hydrolyzed,  $m$  is the initial dry weight (g) of native or pretreated CS.

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