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Improved enzymatic saccharification of steam exploded cotton stalk using alkaline extraction and fermentation of cellulosic sugars into ethanol

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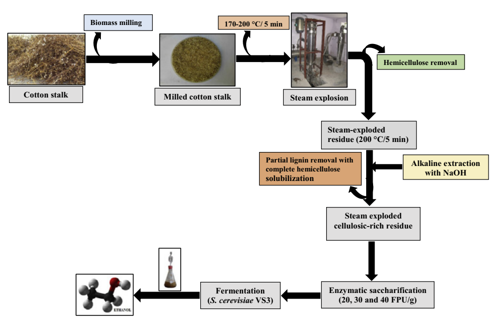
HIGHLIGHTS

- Steam exploded cotton stalk can be promising substrate for ethanol production.
- Steam explosion at 200 °C and 5 min led to 71.90% hemicellulose solubilization.
- SECOH showed 85.07% lignin reduction with complete hemicellulose solubilization.
- Steam explosion coupled with NaOH extraction showed promising sugar yield.
- Fermentation with *S. cerevisiae* strain produced 0.44 g/g ethanol yield.

GRAPHICAL ABSTRACT



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ABSTRACT

Cotton stalk, a widely available and cheap agricultural residue lacking economic alternatives, was subjected to steam explosion in the range 170–200 °C for 5 min. Steam explosion at 200 °C and 5 min led to significant hemicellulose solubilization (71.90 ± 0.10%). Alkaline extraction of steam exploded cotton stalk (SECOH) using 3% NaOH at room temperature for 6 h led to 85.07 ± 1.43% lignin removal with complete hemicellulose solubilization. Besides, this combined pretreatment allowed a high recovery of the cellulosic fraction from the biomass. Enzymatic saccharification was studied between steam exploded cotton stalk (SECS) and SECOH using different cellulase loadings. SECOH gave a maximum of 785.30 ± 8.28 mg/g reducing sugars with saccharification efficiency of 82.13 ± 0.72%. Subsequently, fermentation of SECOH hydrolysate containing sugars (68.20 ± 1.16 g/L) with *Saccharomyces cerevisiae* produced 23.17 ± 0.84 g/L ethanol with 0.44 g/g yield.

1. Introduction

The growing demand for energy and decreasing petroleum-based transportation fuels have stimulated tremendous interest in finding alternative renewable energy sources. Lignocellulosic biomass produced from photosynthesis, has the potential to serve as a sustainable supply of fuels and chemicals (Huang et al., 2011). Ethanol produced from lignocellulosic biomass has gained considerable interest in order to supply energy security and has shown to involve up to 85% net reduction in greenhouse gas emissions (García-Aparicio et al., 2011). Presently, a very limited amount of lignocellulosic biomass is successfully employed for ethanol production while most of it is discarded as waste, causing widespread environmental issues. One promising technology to exploit this

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abundant and renewable agricultural residue is to convert to ethanol through an enzyme-based process (Chen et al., 2007).

Cotton plant is an important agricultural crop, after harvesting the cotton, stalks and leaves left in the fields have no suitable use and are usually burnt by farmers causing environmental pollution. In India, cotton crop was cultivated over an area of 9.4 million hectares in the year 2009–2010, with 11.4 million metric ton available as a feedstock, which can be converted to biobased products such as ethanol (Binod et al., 2012). Unlike other agricultural residues, cotton stalk is recalcitrant to pretreatment due to its high lignin content in the plant cell wall (Akpinar et al., 2011). Conversion of lignocellulose to bioethanol is a complex process, which imposes an appropriate pretreatment step to fractionate different cell wall components (hemicelluloses and lignin) of the biomass, leaving the cellulosic surface exposed to the enzymic attack (Camesasca et al., 2015). Besides being viewed as a critical step in the bioconversion to ethanol, biomass pretreatment identifies one of the major economic costs in the process (Alvira et al., 2010).

To improve the yield of reducing sugars and minimize the formation of inhibitors from lignocellulosic biomass, a specific or a combination of the pretreatment methods are necessary (Chaudhary et al., 2012). Steam explosion, also known as autohydrolysis, is the most extensively used physico-chemical pretreatment process for the fractionation of any lignocellulosic biomass components. The treatment results in substantial breakdown of the lignocellulosic structure with hemicellulose removal and lignin transformation (Singh et al., 2015). Among the different chemical pretreatments, alkali pretreatment with NaOH is the most widely used method to enhance the enzymatic digestibility of various lignocelluloses. The alkaline treatment disrupts the ester bonds between lignin and the carbohydrate polymers, thus increasing the porosity of biomass. The pretreatment leads to solubilization of lignin with an increased access for cellulase enzymes to inner structure of biomass (Silverstein et al., 2007; Singh et al., 2015; Xing et al., 2013).

Enzymatic saccharification is an eco-friendly method that consists of a group of cellulolytic enzymes, which are capable of degrading carbohydrate fraction of pretreated biomass with high specific catalyst activity. During enzymatic saccharification of biomass, access to cellulose is hampered by other cell wall polymers such as hemicelluloses and lignin. The degradation of noncellulosic components through pretreatment has the major effect on digestibility of cellulose fraction of biomass (Öhgren et al., 2007; da Costa et al., 2015). Different factors affecting the cellulose accessibility and saccharification include, the type of feedstock, biomass structure, chemical composition, substrate loading, enzyme loading and inhibitors. Among these factors, substrate to enzyme loading was identified as a critical factor in determining the efficiency of enzymatic saccharification of biomass (da Costa et al., 2015). To achieve high ethanol yields and productivity, the choice of fermenting microbial strain lies upon its ethanol tolerance and ability to withstand against various fermenting inhibitors. *Saccharomyces cerevisiae*, is the most widely used hexose fermenting yeast in the industrial processes owing to their high conversion efficiency and easy adaptability under various fermentation conditions (Camesasca et al., 2015).

Although, there are few studies on cotton stalk (Huang et al., 2015; Wei et al., 2016), that reported the study of combined effect of steam explosion and dilute NaOH treatment followed by enzymatic saccharification and fermentation to ethanol, it remains to test the efficiency of this pretreatment on enhancing the sugar and ethanol yields. The main objective of this work was to study the bioconversion of cotton stalk using combined pretreatment strategy to maximize the sugar yield for ethanol production. An effective pretreatment process that combines steam explosion and alkaline extraction with NaOH (named SECOH pretreatment) has been studied to improve the cellulosic fraction while separating the noncellulosic components from the biomass. The two pretreatment methods namely, steam explosion and SECOH were evaluated and compared on the basis of the sugar yields from subsequent enzymatic saccharification with different cellulase loadings. Fig. 1 shows the experimental strategy used for the bioconversion of cotton stalk into ethanol.

2. Material and methods

2.1. Feedstock preparation

Cotton stalk samples were obtained from the farming fields of Karimnagar district in the state of Telangana, India. Stalks were air dried at room temperature to an equilibrium moisture content less than 10% and milled using a laboratory grinder to a particle size lesser than 3 mm, and stored in sealed plastic bags until used.

2.2. Pretreatment

2.2.1. Steam explosion of cotton stalk

The steam explosion of cotton stalks was carried out in a capacity of 10 L reactor vessel designed to reach a maximum operating pressure of 4.13 Mpa (600 psi). The reactor was loaded with 100 g of dried biomass per batch and heated to selected temperatures (170, 180, 190 and 200 °C) and the desired temperature was maintained for 5 min. The steam exploded material was recovered in a cyclone separator and after cooling to about 40 °C filtered for solid fraction. The collected steam exploded biomass was water-washed, air-dried and analyzed for the composition of different cell wall polymers such as cellulose, hemicellulose and lignin.

The severities of the various steam explosion pretreatments were calculated by using the severity factor (SF), which aggregates the pretreatment temperature and residence time in a single factor (Agudelo et al., 2016). The following expression was used to define severity factor of different steam explosion pretreatments:

$$SF = \log_{10}\left(t \times \exp\left(\frac{T - 100}{14.75}\right)\right)$$

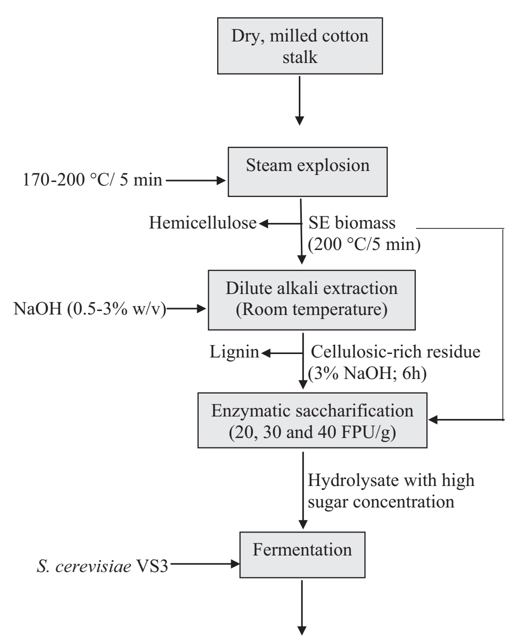

Fig. 1. Overview of ethanol production from steam exploded cotton stalk.