

# Bioethanol production from corn stover using *Saccharomyces cerevisiae*

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**Abstract**— Corn stover hemicellulose acid hydrolysate has been utilized as a substrate for ethanol production using *Saccharomyces cerevisiae*. This study on the one hand reviews ethanol production from lignocellulosic materials and on the other hand, through a process of Separate Hydrolysis and Fermentation, produces ethanol from a corn stover. The maximum value of ethanol yield was 143.15mg/L at conditions of 1.08%, 3.32hrs, 14.32hrs, 6.43g/l, 39.34 oC and 7.64 for H<sub>2</sub>SO<sub>4</sub> Concentration, Hydrolysis Time, Fermentation Time, Enzyme Concentration, Fermentation Temperature and pH respectively.

**Index Terms**—Corn stover, Ethanol, Fermentation, Sugar, *Saccharomyces cerevisiae*

## 1 INTRODUCTION

Bioethanol production from food sources such as corn, cassava etc. is estimated to increase to 113.6 billion liters (approximately 30.29 billion gallons) by 2022 [1]. Production of bioethanol from corn starch received much criticism in the feed, food and fuel debate [2]. Armah and Colleagues [3] reported that the rise in food prices in some countries is attributed to the production of biofuels such as bioethanol and biodiesel. Thus, attention was shifted from the use of food-based materials for bioethanol production to non-food sources such as lignocelluloses biomass.

Various studies on biofuels production consider bioethanol from lignocellulosic biomass as a viable alternative for reducing oil dependences while protecting food crops. Corn stover and switch grass are very abundant therefore, possess the potential to be utilized as lignocellulosic feed stock. However, the cost associated with production of bioethanol from the lignocellulosic biomass is very high compared with bioethanol production from food materials. Hence, conversion of lignocellulosic materials to ethanol in a cost-effective way remains a central technological challenge to fully unlock its commercial potential. The two common methods for production of ethanol from cellulose are: separate hydrolysis and fermentation (SHF), and simultaneous saccharification and fermentation (SSF). Otulugbu [4] reported that the yield of ethanol is higher with the SSF method but the difference in the ethanol yield does not account for the difference in cost of production making the SHF method more attractive. The separate hydrolysis and fermentation (SHF) process uses either concentrated or dilute acid for breaking the cellulose into sugar.

In the production of bioethanol from lignocellulosic materials, Taherzadeh and Karimi [5] reported the possibility of byproducts such as furfural (pentose sugars byproduct), hydroxyl methyl furfural (hexose sugar byproduct), phenolic acid (lig-

nin byproduct) and acetate (diacetylation product of hemicellulose). However, each of these byproducts at concentrations  $\geq 5$  mM have been shown to have significant inhibitory effect on the fermentation process [6], [7], [8]. Hamelinck and colleagues [9] reported that, the use of concentrated acid in comparison with dilute acid offers several advantages such as: (i) very high yield of sugar (90%), (ii) can handle diverse feedstock, (iii) be relatively rapid (10 to 12 hours), and (iv) can cause less degradation. Unfortunately, the use of concentrated acid will require more expensive corrosion resistant equipment. Perlack and colleagues [10], Hess and colleagues [11], and Templeton and colleagues [12] all carried out extensive studies on corn stover. From their findings, corn stover (residue after harvesting corn - leaves and stalk) is a substantial source of inexpensive and abundant lignocellulosic biomass. It is also one of the most abundant agricultural residues in Europe and China.

*Saccharomyces cerevisiae* is a well-established fermenting strain in existing commercial-scale ethanol industries [13]. This informs its choice in this study. Therefore, in this study ethanol is produced from the fermentation of hemicellulose acid hydrolysate of alkaline pretreated corn stover using *Saccharomyces cerevisiae*.

## 2 MATERIALS AND METHODS

### 2.1 Sample Preparation

The corn stover was collected from a farm in Udu L.G.A of Delta State and taken to a standard laboratory for analysis. The corn stover was cleaned, chopped and oven-dried at 60 °C for 48 hours at moisture content of 10 % dry basis. The oven dried corn stover was then milled with a Retsch mill from between 10 - 25mm to 0.1 - 0.5mm particle. The milled corn

stover was sieved to produce a uniform particle size between 0.180 – 0.250mm and kept in a sealed plastic jar at room temperature until required for treatment.

## 2.2 Compositional Analysis

500g of the sieved corn stover was taken to a standard laboratory to analyze for its composition by TAPPI (Technical Association of Pulp and Paper Industry) method. TAPPI method was used for the determination of the chemical compositions in weight percent (wt. %); moisture content (mc. %) on wet basis, ash, cellulose, hemicelluloses and lignin in the lignocellulosic material.

## 2.3 Alkaline Pretreatment of Corn Stover

The milled corn stover was pretreated with 2% w/w dilute sodium hydroxide solution, after which the mixture was autoclaved at 121°C for 25 min. Then the autoclaved mixture was filtered to separate the solid residue and the filtrate fraction. The solid residue was thoroughly washed with distilled water to remove the residual alkali. This was done repeatedly until the pH of the residue was neutral. Furthermore, the solid residue was then oven dried at 65°C, and analyzed for its compositions.

## 2.4 Hydrolysis

The alkaline pretreated corn stover was hydrolyzed with dilute sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) at different concentrations of H<sub>2</sub>SO<sub>4</sub> (i.e. 1, 2 and 4 % H<sub>2</sub>SO<sub>4</sub> in distilled water (v/v) respectively). In order to break down the cellulose and hemicelluloses into simple sugar, 20mg of the pretreated corn stover sample was maintained at solid to liquid ratio of 1:10, in 250 mL round bottom flask, and refluxed. Samples (60mls each), were retained after 2, 4, and 6 hours of hydrolysis for subsequent fermentation experiments. After hydrolysis the liquid fraction of the hydrolysate samples was cooled, filtered, collected, and adjusted to pH 5 by adding concentrated sulphuric acid and 2N Sodium hydroxide, and the solutions prepared for fermentation.

## 2.5 Microorganism

The yeast, *Saccharomyces cerevisiae*, which was purchased from local market in Warri, Nigeria, was used in all experiments throughout this work. The yeast, *Saccharomyces cerevisiae* was used for fermentation reaction; which is a process whereby microorganism such as yeast feeds on sugar and converts it to ethanol and carbon dioxide.

## 2.6 Fermentation

After hydrolysis, the flasks containing the hydrolyzed samples was covered with cotton wool, wrapped in aluminium foil, autoclaved for 15 minutes at 121 °C and allowed to cool at room temperature. Fermentation was carried out in 250 mL Erlenmeyer flask with 3 g/L of yeast (*S. cerevisiae*) at an incubation temperature of 30 °C [14]. Ethanol concentration was analyzed by gas chromatography at different fermentation times (12, 24, and 48 hours). Samples (15 – 20ml each) were withdrawn every 12 hours and the fermentation was carried out up to 48 hours. Plate 3 shows fermented samples

ready for bioethanol analysis by Gas Chromatography.

## 2.7 Analytical Methods

All the fermented solutions were each centrifuged at 10,000 rpm for 5 minutes to separate the supernatants. After centrifugation, the supernatants were filtered and then analyzed for ethanol concentration by gas chromatography.

### 2.7.1 Gas Chromatographic Determination of Bioethanol

The ethanol concentration was determined by gas chromatography. Gas chromatograph (GC 600) equipped with flame ionization detector (FID) was employed for the separation and quantification of ethanol. A fused silica capillary column (30 - 32mm) coated with 95% methylpolysiloxane (stationary phase) was fitted to the instrument to provide for column injection. The injector and detector temperature were maintained at 210 and 250°C, respectively. The oven starting temperature was set at 50 °C, one minute hold time with heating rate of 30 °C per minute to 155°C. Nitrogen was used as carrier gas at a flow rate of 1 kg/cm<sup>2</sup> /min and for Hydrogen at 1.5 kg/cm<sup>2</sup> /min, was adjusted [15]. The concentration of ethanol in the samples was determined using isopropanol as internal standard.

### 2.7.2 Standard Solution of Ethanol

Internal standard concentration spiking solution was prepared, using reagent grade isopropanol (99%). The internal standard spiking solution was added in the same proportion to every standard and sample. The internal standard concentration was 0.9 g/L universally throughout the experiment. Standard solutions of ethanol were prepared containing 2, 3, 5 and 7.5% (v/v) of ethanol (96%) in distilled water and all containing the internal standard of 0.9 g/l isopropanol. 1 microlitre of each of the standard solutions were analyzed and the chromatogram recorded as the response ratio. A calibration curve of ethanol standard solution was made from a plot of response ratio versus to amount ratio. The area under the peak was determined for 1 microlitre of each sample by comparing with a standard curve and the concentration of ethanol (mg/l) was calculated [16].

## 3 RESULTS AND DISCUSSION

### 3.1 Corn Stover Compositions

Corn stover is considered to be a potential biomass source of cellulose for conversion to useful biofuel feedstock [17]. Table 1 gives the composition of the corn stover determined using standard laboratory procedure in line with the TAPPI method, at the Nigerian Institute for Oil Palm Research central laboratory.

Table 1: Composition of the corn stover

Constituent	Present study	Lee and colleagues [18]
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	(wt. %)	(wt. %)
Cellulose	36.23	38
Lignin	18.69	19
Crude Protein	4.95	5
Ash	4.42	6
Moisture	6.6	-
Unknown soluble	1.83	-

These results agreed fairly well with the data reported by other investigators [19], [20]-[25]. The result indicated that corn stover could be a good source of cellulose for bioconversion to ethanol. Corn stover also contains appreciable amounts of crude protein, which could provide nitrogen source in any bioconversion process.

### 3.2 Corn Stover Pretreatment

Corn stover pretreatment helps to increase accessibility to plant cell wall polysaccharides for carbohydrate-active enzymes to produce sugars for bioethanol fuels. Thus, 98g of the milled corn stover was mixed with 800ml of distilled water containing 2g of anhydrous NaOH crystals. This resulted in a solid to liquid weight ratio of 1:8. The mixture was autoclaved, filtered, the residue washed and oven dried.

### 3.3 Corn Stover Hemicellulose Acid Hydrolysate Preparation

Dilute sulfuric acid hydrolysis (1, 2 and 4% v/v) under reflux was used to release a good amount of simple sugar from the alkaline pretreated corn stover. After hydrolysis the liquid fraction of the hydrolysate samples was cooled, filtered, collected, and adjusted to pH 5 by adding concentrated sulphuric acid and 2N Sodium hydroxide, and the solutions prepared for fermentation.

### 3.4 Fermentation

The detoxification of hemicellulose acid hydrolysate took place before fermentation. The flasks containing the detoxified hydrolysate samples was covered with cotton wool, wrapped in aluminium foil, autoclaved for 15 minutes at 121 °C and allowed to cool at room temperature. Fermentation was carried out in 250 mL Erlenmeyer flask with 3 g/L of yeast (*S. cerevisiae*) at an incubation temperature of 30 °C [14]. The concentration of the produced ethanol was analyzed by gas chromatography at different fermentation times. The optimum yield of ethanol was 143.15mg/L at conditions of 1.08%, 3.32hrs, 14.32hrs, 6.43g/l, 39.34 oC and 7.64 for H<sub>2</sub>SO<sub>4</sub> Concentration, Hydrolysis Time, Fermentation Time, Enzyme

Concentration, Fermentation Temperature and pH respectively.

## 4 CONCLUSION

This study on the one hand reviews bioethanol production from lignocellulosic materials and on the other hand, through a process of Separate Hydrolysis and Fermentation (SHF), produces ethanol from a lignocellulosic material (corn stover). The maximum value of ethanol yield was 143.15mg/L at conditions of 1.08%, 3.32hrs, 14.32hrs, 6.43g/l, 39.34 °C and 7.64 for H<sub>2</sub>SO<sub>4</sub> Concentration, Hydrolysis Time, Fermentation Time, Enzyme Concentration, Fermentation Temperature and pH respectively.

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