# Production of Bioethanol from Corn Stalks Through Fermentation Process

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**Abstract**

This study focuses on the fermentation-based production of bioethanol from corn stalks. As a widespread agricultural waste product, corn stalks are used to produce bioethanol, a sustainable replacement for fossil fuels. The aim of this study was to investigate the effect of varying enzymatic treatment temperatures and varying feedstock loads on ethanol production. The concentration of ethanol produced was analyzed as a response to different enzymatic temperatures. The results showed that at an enzymatic temperature of 50°C, the sample exhibited the highest concentration of ethanol, reaching 48.90%. This finding suggests that 50°C is the optimal temperature for enzyme treatment. Further analysis revealed that enzymatic temperatures of 30°C, 40°C, and 60°C resulted in decreasing ethanol concentrations, indicating reduced efficiency of ethanol production compared to the optimal temperature of 50°C. These results highlight the importance of selecting the appropriate enzymatic temperature for achieving maximum ethanol yield.

**Introduction**

First generation bioethanol (FGB) is the conventional term for bioethanol produced from edible sources. FGB has a disadvantage since it depends on the use of edible feedstocks like maize and sugarcane. Recent developments in ethanol production technology have switched to the use of leftover lignocellulosic materials in an effort to overcome this constraint and reduce production costs.

The aim of this research is to produce ethanol from maize stalks by fermenting them using yeast and commercial enzymes. Enzymatic hydrolysis is used to break down lignocellulosic materials in the process, and enzymatic saccharification has been shown to be a more efficient means to produce sugar than chemical methods like acid hydrolysis. The metabolic activity of Saccharomyces cerevisiae, a kind of yeast often utilized in fermentation operations, then transforms the released carbohydrates into ethanol. This method makes it possible to transform maize stalks, a lignocellulosic waste product, into ethanol, providing a sustainable and affordable option for the manufacturing of bioethanol.

Pentosic polysaccharides, which are readily hydrolyzable, are abundant in corn stalks. Additionally, a compositional examination of maize stalks showed that they include 35.06% pentosans, 19.35% lignin, and 40.28% cellulose. Corn stalks are a possible source for ethanol production due to their composition. The cost of producing ethanol may be reduced by using maize stalks as a feedstock, which helps to make ethanol manufacturing techniques more affordable.

The cellulosic ethanol market is actively being addressed by industry initiatives aimed at enhancing affordability and competitiveness in comparison to ethanol derived from sugar and starch sources. These efforts focus on optimizing the production process to make cellulosic ethanol more economically viable. In line with this, the objective of this research is to examine the enzymatic hydrolysis of corn stalks, a potential feedstock, for the production of bioethanol. The enzymatic hydrolysis process involves breaking down the complex cellulose and hemicellulose components of corn stalks into simpler sugars. These sugars are then fermented into ethanol using specialized microorganisms or yeast. To assess the effectiveness of the enzymatic hydrolysis and fermentation, the composition of the resulting ethanol will be analyzed using High Performance Liquid Chromatography (HPLC).

This analytical technique provides detailed information about the ethanol composition, including the concentrations of various chemical compounds present. The findings of this research will contribute to a better understanding of the feasibility and potential of utilizing corn stalks for cost-effective bioethanol production, thereby advancing the development of sustainable and economically viable alternatives to conventional ethanol sources.

**Methodology**

**Corn stalk Preparation**

In this study, corn stalks were collected from Rajasthan, India, and were specifically chosen for being fully matured stalks that had been harvested for maize production. To prepare the corn stalks for further experimentation, the leaves were removed, and the stalks were subjected to a drying process at a temperature of 60-70°C until a consistent weight was achieved. Once dried, the corn stalks were manually fragmented into small pieces measuring 10-30 mm in length. Finally, the corn stalks were finely ground to produce smaller particles ranging in size from 0.2-2 mm. This grinding process aimed to create a suitable substrate for the subsequent enzyme treatment, ensuring optimal conditions for the enzymatic breakdown of the corn stalk material.

**Treatment with enzyme**

To prepare the corn stalk samples, ground corn stalks were mixed with acetate buffer (0.05M, pH 4.8) to create a slurry with a substrate loading of 5% (w/v). The slurry underwent autoclaving at 121°C for 15 minutes to ensure sterilization. After cooling to 50°C, 2% Tween 20 (v/v) was added as a surfactant. Enzymatic treatment was conducted using 0.3% (v/v) Viscozyme® L Cellulolytic Enzyme Mixture V2010 from Sigma Aldrich. The sample was incubated on an orbital shaker for 48 hours at temperatures of 30°C, 40°C, 50°C, and 60°C. Different amounts of corn stalk loading (10g, 20g, and 30g) were used for each temperature condition. This process aimed to break down cellulose into fermentable sugars for ethanol production.

**Utilization of Microorganisms in Batch Fermentation**

Mauripan, a commercial baker's yeast derived from Saccharomyces cerevisiae, was utilized as the source of microorganisms. The dry yeast was added to a nutrient-rich medium comprising glucose

(50g/L), peptone (5g/L), $MgSO_4.7H_2O$ (1g/L), $K_2HPO_4$, and yeast extract (5g/L). The medium was sterilized through autoclaving at 121°C for 15 minutes. Following sterilization, the yeast was inoculated in the medium and incubated on an orbital shaker at 30°C and 50rpm for 18 hours. Subsequently, the yeast-containing medium was aseptically introduced to the fermentation broth in a 1:10 volume ratio. The fermentation process occurred at 30.5°C and 150rpm using an orbital shaker for duration of 48 hours. This controlled environment facilitated the conversion of fermentable sugars into ethanol by the yeast.

**Sample Distillation Procedure**

First, the sample form the fermentation process was filtered through muslin cloth to separate the liquid from the solid source components. A rotating vacuum evaporator was then used to further separate the filtrate. At 78°C, vacuum conditions, and 100 rotations per minute were used for the evaporation process.

**Analysis Using High Performance Liquid Chromatography (HPLC)**

To prepare the samples for analysis, 1.5 ml of the samples treated with a rotary evaporator were carefully transferred into an HPLC vial. From this, a precise volume of 20µl was injected into the HPLC system to assess the presence of ethanol in the samples. The HPLC analysis was conducted using specific parameters configured as follows: a C18 RP Hyper Sil column was utilized, and once again, 20µl of the sample was injected into the HPLC system. The mobile phase employed consisted of 0.1M phosphoric acid with pH adjusted to 2.5. A flow rate of 1.5ml/min was maintained, and the detection wavelength was set at 254nm. These parameters ensured the accurate separation and quantification of ethanol in the samples through HPLC analysis.

**Result and Discussion**

**Analysis Using High Performance Liquid Chromatography (HPLC)**

Table 1 presents the retention time and peak area data obtained for ethanol using absolute ethanol at concentrations of 25%, 50%, 75%, and 100% in relation to the mobile phase. These different concentrations were employed to establish a calibration linear equation for determining ethanol concentrations in all the samples. Figure 1 displays the linear plot derived from the ethanol calibration, which yielded a correlation coefficient ($R^2$) value of 0.9516. The linear equation generated from the plot can be used to accurately calculate ethanol concentrations in the samples.

$$y=367.98x-2854.5 \quad (1)$$

The results presented in Table 2 indicate the average retention period, peak area, and peak height for samples conducted at a temperature of 50°C with variable weights of the sample. For the sample with a weight of 10 grams, the mean retention time was 3.76 minutes, and the corresponding mean peak area was 2,547. The mean maximal height was recorded as 480. At a weight of 20 grams, the mean retention time decreased marginally to 3.65 minutes, while the mean peak area increased considerably to 9,728. The mean maximal height also increased to 1,530.The sample with the

maximum weight of 30 grams exhibited the minimum mean retention time of 3.21 minutes. The mean peak area for this sample was the highest among all weights, measuring 15,131. The mean maximal height recorded for this sample was 1,672.5.

These results suggest that as the weight of the sample increased, there was a decrease in the mean retention time, indicating a quicker elution of the ethanol compound. Additionally, both the mean peak area and mean peak height increased with increasing sample weight, indicating a higher concentration of ethanol.

The data from Table 2 emphasizes the relationship between sample weight and the chromatographic parameters, which can be useful in assessing the ethanol content and quality of the samples. These findings contribute to a better comprehension of the ethanol production process and can aid in optimizing conditions for ethanol production and analysis. Subsequently, the ethanol concentrations were calculated by substituting the peak areas obtained from the HPLC analysis into equation 1. The ethanol concentrations were then plotted in a linear graph (Figure 3).

Table 3 presents the average retention time, peak area, and peak height for the samples treated at a temperature of 30°C. The retention time indicates the duration it took for the ethanol peaks to elute from the HPLC column. The average retention durations ranged from 3.46 to 3.43 minutes, demonstrating consistent elution behavior. The peak area and peak height represent the concentration and intensity of the ethanol peaks, respectively. With increasing sample weight from 10 to 30 grams, there was a noticeable increase in the mean peak area, indicating higher ethanol concentrations in the samples. This suggests that a larger quantity of corn stalks resulted in a greater supply of fermentable carbohydrates, leading to a higher ethanol yield. These findings emphasize the importance of sample weight in ethanol production from corn stalks. Optimizing the corn stalk loading can enhance ethanol production efficiency. Further research should investigate additional factors such as enzyme concentration and process optimization to advance bioethanol production from corn stalks. The insights from Table 3 provide valuable guidance for increasing the sustainability and efficiency of ethanol production processes.

**Table 1: HPLC Analysis Results for Absolute Ethanol**

| Concentration of Ethanol (%) | Time of Retention (min) | Peak Area Measurement | Area (%) |
|---|---|---|---|
| 100 | 2.86 | 30,417 | 87.38 |
| 75 | 2.78 | 29,515 | 90.18 |
| 50 | 2.93 | 17,890 | 4.52 |
| 25 | 3.01 | 6998 | 1.55 |

**Table 2: Average Retention Time and Peak Area for Samples Conducted at 50°C**

| Sample Weight Variation (g) | Average Retention Time (min) | Average Peak Area | Average peak height |
|---|---|---|---|
| 10 | 3.76 | 2547 | 480 |
| 20 | 3.65 | 9728 | 1530 |
| 30 | 3.21 | 15,131 | 1672.5 |

**Table 3: Average Retention Time and Peak Area for Samples Conducted at 30°C**

| Sample Weight Variation (g) | Average Retention Time (min) | Average Peak Area | Average peak height |
|---|---|---|---|
| 10 | 3.46 | 3210 | 471.5 |
| 20 | 3.42 | 6740 | 805 |
| 30 | 3.43 | 8613 | 1075 |

**Table 4: Average Retention Time and Peak Area for Samples Conducted at 40°C**

| Sample Weight Variation (g) | Average Retention Time (min) | Average Peak Area | Average peak height |
|---|---|---|---|
| 10 | 2.12 | 5452 | 1209 |
| 20 | 2.01 | 1782 | 325 |
| 30 | N/A | N/A | N/A |

**Figure 1: Linear Plot of Ethanol Calibration**

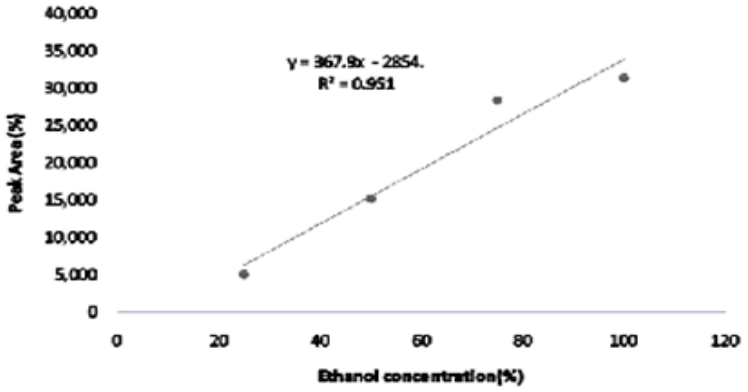

**Figure 2: Plot of Peak Area for Sample Treatment at 50°C**

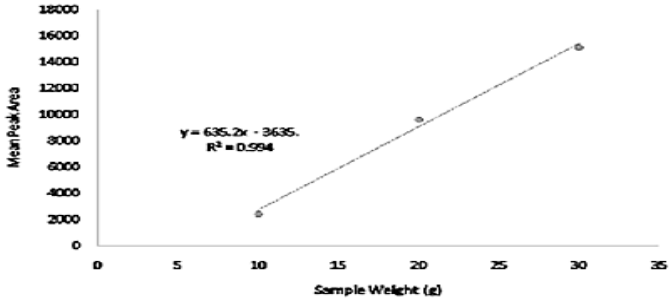


**Figure 3: Plot of Ethanol Concentration for Sample Treatment at 50°C**

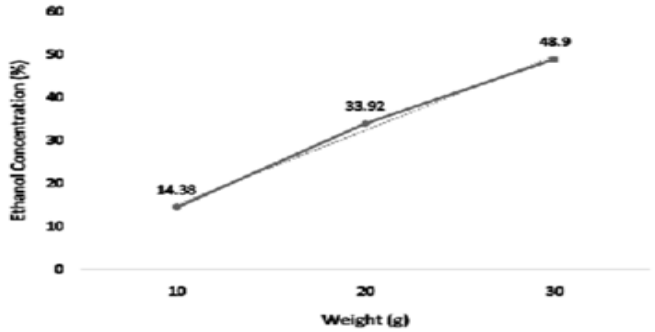


**Figure 4: Plot of Peak Area for Sample Treatment at 30°C**

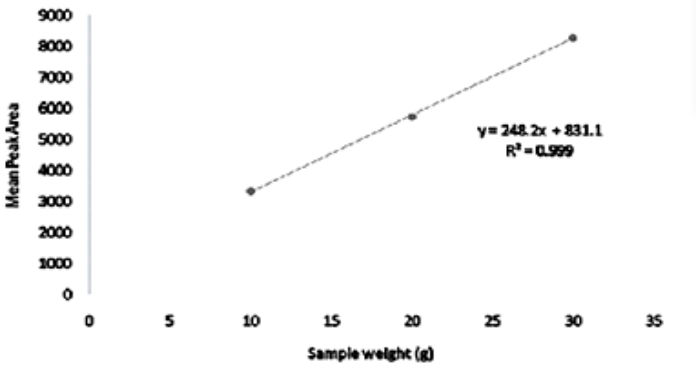


**Figure 5: Plot of Ethanol Concentration for Sample Treatment at 30°C**

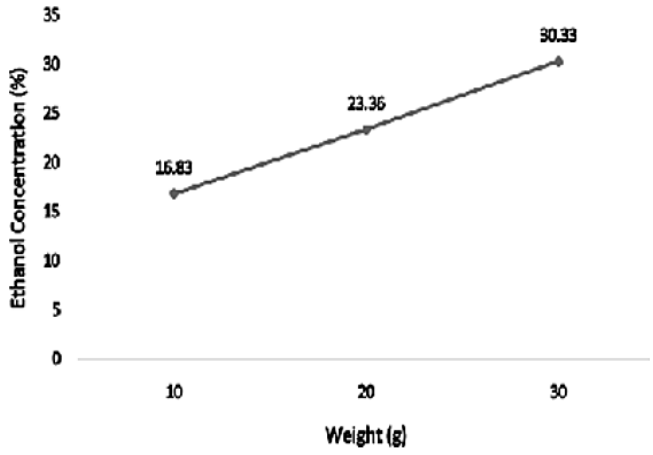


**Figure 6: Plot depicting the ethanol concentration in samples treated at a temperature of 40°C.**

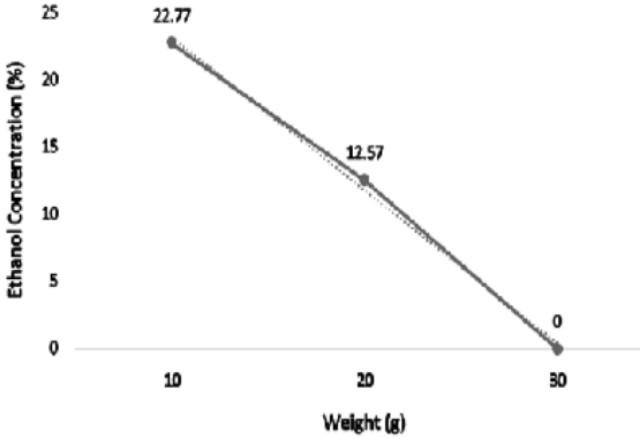


Figure 4 demonstrates the interaction between the peak areas of the 10, 20, and 30-gram corn stalk samples, forming a straight line with a correlation coefficient ($R^2$) value of 0.9996, indicating a strong relationship among all data points. The ethanol concentration in the samples was then determined using the peak area values. Figure 5 represents a linear graph depicting the ethanol content of the samples at various weights. The ethanol content increased as the weight of the corn stalks increased, ranging from 16.83% to 30.33% after enzyme treatment at 30°C.

The comparison between Table 2 (samples at 50°C) and Table 3 (samples at 30°C) demonstrates the influence of temperature on ethanol production from corn stalks. At 50°C, increasing sample weight leads to a decrease in mean retention time, indicating quicker elution of ethanol compounds. The mean peak area and peak height also increase with greater sample weight, indicating higher ethanol concentrations. In contrast, at 30°C, the mean retention time remains comparatively consistent, while the mean peak area and peak height progressively increase with sample weight. This suggests that lower temperatures are less affected by sample weight but still result in larger ethanol concentrations with greater substrate quantities. These findings emphasize the importance of optimizing both temperature and sample weight for efficient ethanol production from corn stalks.

The use of a temperature of 40°C, the mean retention time and mean peak area are provided, along with the mean peak height. Notably, the 10g sample demonstrates the highest mean peak area and peak height, indicating a relatively higher concentration of ethanol. In contrast, the 20g sample exhibits lower values for both mean peak area and peak height, suggesting a slightly lower ethanol concentration compared to the 10g sample. Unfortunately, no data is available for the 30g sample, implying that no peak was detected during the analysis. This absence of data for the 30g sample prevents us from making any conclusive observations regarding its ethanol concentration. Overall, the results indicate a weight-dependent effect on the ethanol concentration, with higher weights potentially leading to higher concentrations. This deviation in ethanol concentration can be attributed to the oxidation of ethanol to acetic acid, which is likely caused by contamination from microorganisms, possibly Acetobacter species. The conversion of ethanol into acetic acid is a common occurrence in various biological processes.

The application of enzyme treatment at 60°C resulted in the absence of ethanol production across all corn stalk loadings. This consequence can be attributed to the fact that the temperature exceeded the optimal range for the enzyme responsible for converting the lignocellulosic materials. Consequently, no peaks were observed during the HPLC analysis, indicating that the fermentation process was unable to convert any fermentable sugars present in the samples.

**CONCLUSION**

In conclusion, the analysis of the data presented in Tables 1, 2, and 3 provides valuable insights into the ethanol production process. Table 1 establishes a calibration linear equation for accurately determining ethanol concentrations in the samples based on retention time and peak area measurements. The results from Table 2 demonstrate that increasing the weight of the sample leads to decreased retention time and increased peak area and peak height, indicating higher ethanol concentrations. Similarly, Table 3 demonstrates a consistent trend of increasing ethanol concentrations with increased sample weights. These findings underscore the significance of sample weight in ethanol production and emphasize the need for optimizing sample loading conditions to enhance ethanol yields. The observed relationships between sample weight and chromatographic parameters provide valuable guidance for increasing the efficiency and sustainability of ethanol production processes. Further research should focus on investigating additional factors, such as

enzyme concentration and process optimization, to further enhance ethanol production from corn stalks. By advancing our understanding of ethanol production and optimizing key variables, we can contribute to the development of more efficient and environmentally favorable bioethanol production methods.

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