Investigating the Impact of Enzymatic Treatment Temperature and Feedstock Load on Bioethanol Production from Corn Stalks through Fermentation

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Abstract

This study focuses on the utilization of corn stalks, a widely available agricultural waste material, for the production of bioethanol through fermentation. Bioethanol serves as a sustainable alternative to fossil fuels. The objective of this study was to investigate the influence of different enzymatic treatment temperatures and feedstock loads on ethanol production. Specifically, the concentration of ethanol was analyzed in response to varying enzymatic temperatures. The findings revealed that the highest ethanol concentration, reaching 48.90%, was achieved at an enzymatic temperature of 50°C. This result indicates that 50°C is the optimal temperature for enzyme treatment. Conversely, ethanol concentrations decreased when enzymatic temperatures deviated from the optimal range, such as at 30°C, 40°C, and 60°C. These results underscore the significance of selecting the appropriate enzymatic temperature to maximize ethanol yield.

Introduction

First generation bioethanol (FGB) refers to bioethanol produced from edible sources, such as maize and sugarcane. However, FGB has a drawback as it relies on edible feedstocks. To overcome this limitation and reduce production costs, recent advancements in ethanol production technology have shifted towards using leftover lignocellulosic materials. The aim of this research is to produce ethanol from maize stalks by fermenting them with yeast and commercial enzymes. Enzymatic hydrolysis is employed to break down the lignocellulosic components of the maize stalks, and enzymatic saccharification has proven to be a more efficient method for sugar production compared to chemical approaches like acid hydrolysis. The carbohydrates released from the maize stalks are then converted into ethanol through the metabolic activity of Saccharomyces cerevisiae, a commonly used yeast in fermentation processes. This approach allows for the conversion of maize stalks, a waste product rich in pentosic polysaccharides, into ethanol, offering a sustainable and cost-effective option for bioethanol production. Maize stalks are a viable feedstock for ethanol production due to their composition, which includes a significant amount of pentosans, lignin, and cellulose. Utilizing maize

stalks as a feedstock can potentially reduce the cost of ethanol production and make the manufacturing process more affordable. Efforts are being made in the cellulosic ethanol market to improve affordability and competitiveness compared to ethanol derived from sugar and starch sources. These initiatives focus on optimizing the production process to enhance the economic viability of cellulosic ethanol. In alignment with these objectives, this research aims to investigate the enzymatic hydrolysis of corn stalks, a potential feedstock, for bioethanol production. The enzymatic hydrolysis process involves breaking down the complex cellulose and hemicellulose components of corn stalks into simpler sugars, which are then fermented into ethanol using specialized microorganisms or yeast. The composition of the resulting ethanol will be analyzed using High Performance Liquid Chromatography (HPLC) to evaluate the effectiveness of enzymatic hydrolysis and fermentation. HPLC provides detailed information about the ethanol composition and 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, promoting the development of sustainable and economically viable alternatives to traditional ethanol sources.

Methodology

Corn stalk Preparation

The corn stalks used in this study were sourced from Rajasthan, India. Fully matured stalks that had been harvested for maize production were specifically selected. To prepare the corn stalks for further experimentation, the leaves were removed, and the stalks underwent a drying process at a temperature range of 60-70°C until a consistent weight was achieved. Subsequently, the dried 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 with sizes ranging from 0.2-2 mm. The grinding process aimed to create an appropriate substrate for the subsequent enzyme treatment, ensuring favorable conditions for the enzymatic breakdown of the corn stalk material.

Treatment with enzyme

To prepare the corn stalk samples, ground corn stalks were combined with acetate buffer (0.05M, pH 4.8) to create a slurry with a substrate loading of 5% (w/v). The slurry was then subjected to autoclaving at 121°C for 15 minutes to ensure sterilization. After cooling to 50°C, a surfactant, 2% Tween 20 (v/v), was added. Enzymatic treatment was performed 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 to facilitate 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

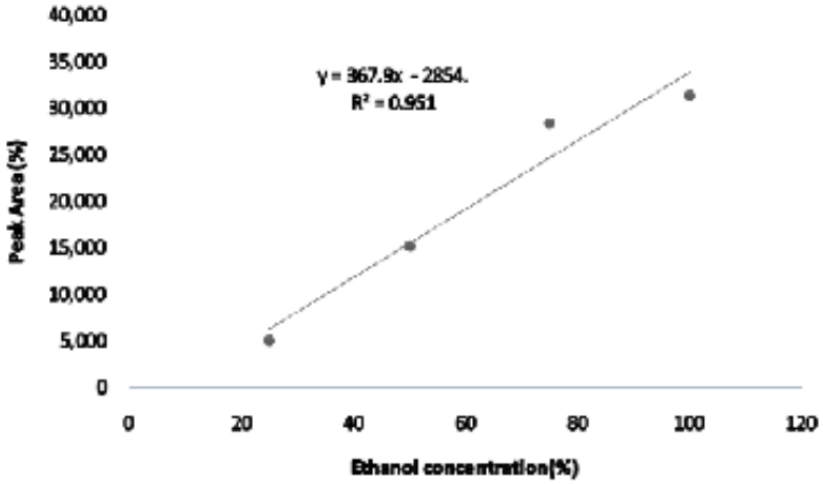

Figure 2: Plot of Peak Area for Sample Treatment at 50°C

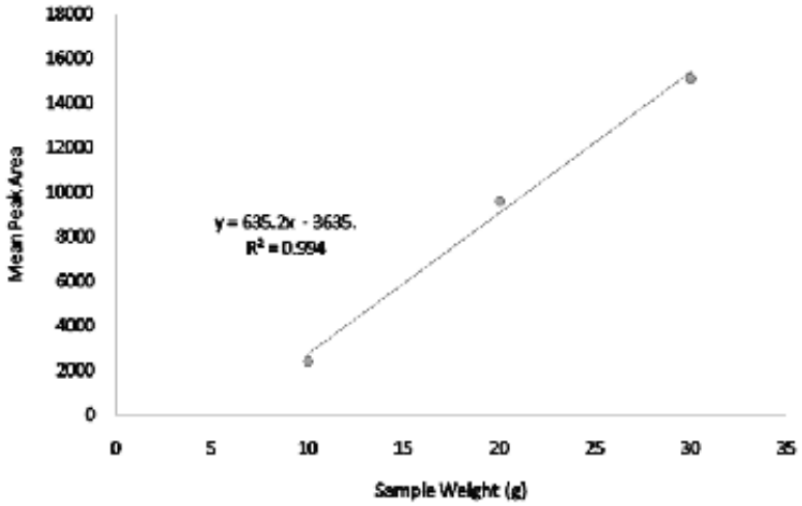

Figure 3: Plot of Ethanol Concentration for Sample Treatment at 50°C

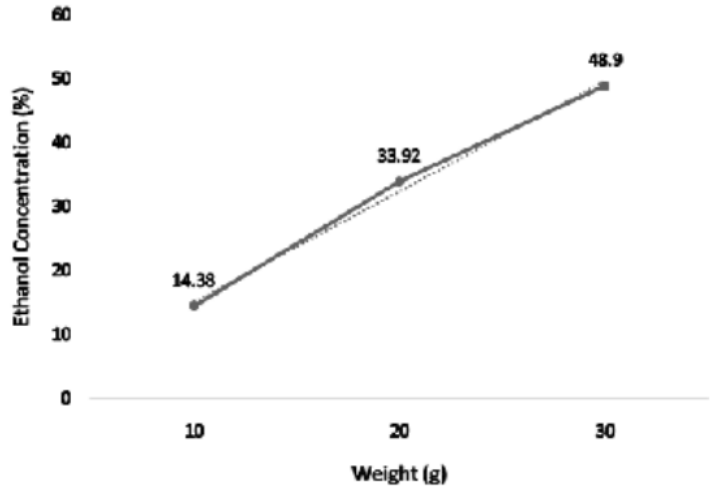

Figure 4: Plot of Peak Area for Sample Treatment at 30°C

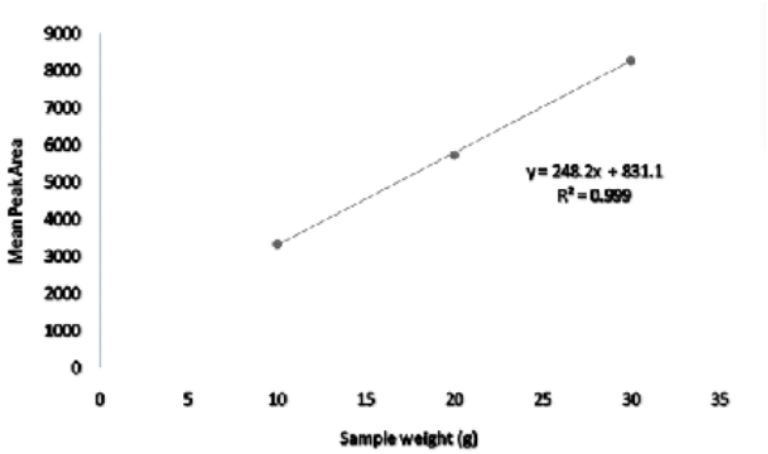

Figure 5: Plot of Ethanol Concentration for Sample Treatment at 30°C

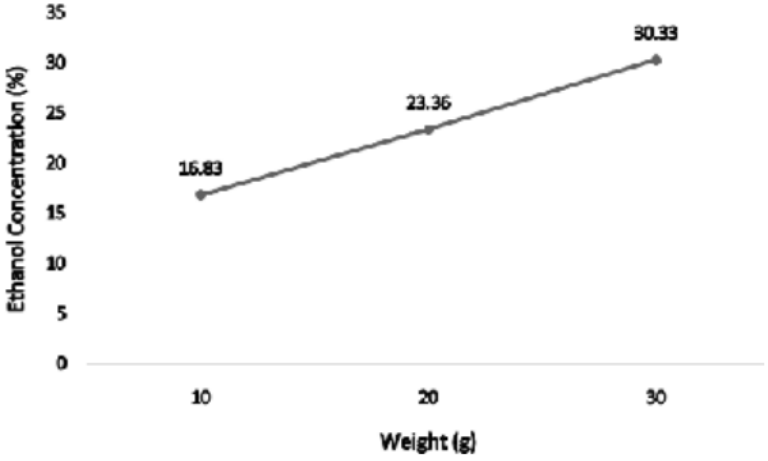

Figure 6: Plot depicting the ethanol concentration in samples treated at a temperature of 40°C.

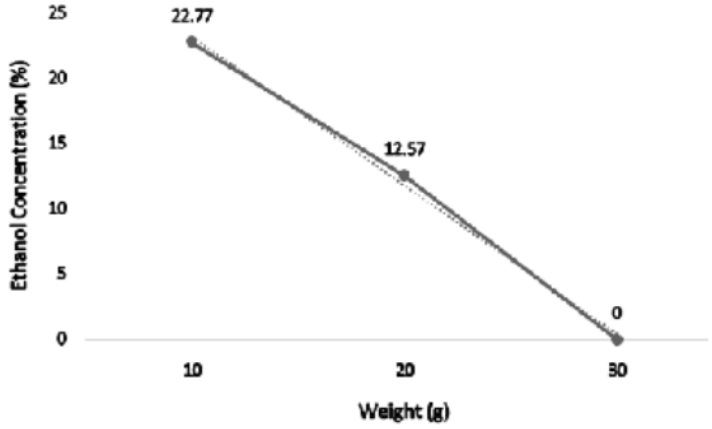

Figure 4 demonstrates the interaction between the peak areas of the 10, 20, and 30-gram corn stalk samples, forming a straight line with a correlation coefficient (R^2) value of 0.9996, indicating a strong relationship among all data points. The ethanol concentration in the samples was then determined 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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REFRENCES

1. Goh, C. S.;Kok, T. T.; Lee, K. T.; Bhatia, S.; Bioresource Technology 2010.
2. Howard, R. L.;Abotsi, E.; Rensburge, E. L.; Howard, S.; African Journal of Biotechnology, 2003, 2(12)
3. Hassan, O.; Ling, T. P.; Maskat, M. Y.;Illias, R. M.; Badri, K.; Jahim, J.;Mahadi,N. M.; Biomass and Bioenergy, 2013, 56, 137-146.
4. Valechev, I.;Nenkova, S.; Tsekova, P.; Lasheva,V.; Bioresource, 2009,4(1), 285-291.
5. Maniatis, K.; Chiaramonti,D.;Thornley, P.; Biomass & Bioenergy, 2012,46, 1-4.
6. Karagoz, P.; Rocha, I. V.; Ozkan, M.; Angelidaki, I.; Bioresource Technology, 2012,104, 349- 357
7. Cao, W.; Sun, C.; Liu, R.; Yin, R.; Wu, X.; Bioresource Technology, 2012, 111, 215- 221.
8. McFeeters, R. F.; Thompson, R. L.; Fleming, H. P.;Journal of the Association of Official Analytical Chemists,1984,67, 4.

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