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***Research Article***

# Antibacterial Efficacy of Bioethanol Extracted from Sugarcane Juice and Corn Cob Against *Escherichia coli*

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## ABSTRACT

This study is aimed at comparing the bioethanol produced from *Saccharum officinarum* L. and *Zea mays* L. in terms of their yield, disinfectant potential and antibacterial efficacy against *Escherichia coli*. The feedstocks were sourced from the Katsina Central market, Nigeria, during the 2025 harvest season. Corncobs were pretreated by dilute acid steam explosion using 1.5% $H_2SO_4$ at 121°C for 60 min, followed by enzymatic hydrolysis with cellulase and hemicellulase at 50°C for 72 hours. Sugarcane juice was liquefied and saccharified using α-amylase and glucoamylase. Both hydrolysates were fermented with *Saccharomyces cerevisiae* at 30°C for 72 hours and distilled by fractional distillation. Sugarcane juice outperformed corn cobs in process efficiency, yielding 72.8 g/L reducing sugar, 92.5% hydrolysis efficiency, 21.7% v/v ethanol, 0.401 L/kg yield, and 85.2% fermentation efficiency. Corn cobs produced 42.5 g/L reducing sugar, 68.0% hydrolysis efficiency, 16.2% v/v ethanol, 0.285 L/kg yield, and 78.5% fermentation efficiency. Quality tests showed that ethanol from corn cob had higher purity at 96.2% v/v with a clean blue flame, while sugarcane ethanol was 93.4% v/v with slight yellow flame tips indicating trace impurities. The feed stocks had acceptable acidity levels of 28–35 mg/L. Antibacterial assays at 70% v/v revealed ethanol from sugarcane completely inhibited *Escherichia coli,* whereas corn cob ethanol left a residual 19 CFU/mL despite higher purity. Sugarcane juice had higher fermentation efficiency and bioactivity as a first-generation feedstock, while corncobs offer a sustainable second-generation option with superior purity. Optimizing pretreatment, hydrolysis, and dehydration is essential to improve yield and consistency.

**Keywords:** Antimicrobial activity; Bioethanol; Corn cob; *Escherichia coli;* Fermentation efficiency; Sugarcane juice

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## INTRODUCTION

The global reliance on fossil fuels has precipitated an energy crisis characterized by depleting reserves and significant environmental degradation. Consequently, the search for renewable, sustainable, and eco-friendly energy sources has become a priority for the scientific community. Bioethanol, a liquid fuel produced from the fermentation of biomass, has emerged as a leading alternative to conventional gasoline. Unlike fossil fuels, bioethanol is biodegradable, non-toxic, and carbon-neutral, making it a critical component of the future energy matrix (Achten *et al*., 2023).

Conventionally, bioethanol is produced from food crops such as sugarcane, corn (maize), cassava, sweet potatoes, sweet sorghum, and wheat. Sugarcane juice is rich in sucrose and is a primary feedstock (first-generation) used extensively in countries like Brazil due to its high fermentable sugar content and relatively simple processing requirements. However, the use of food crops for fuel production raises ethical concerns regarding food security and large land usage. This dilemma has shifted focus towards second-generation bioethanol production utilizing lignocellulosic biomass, such as agricultural residues (Gupta *et al*., 2024). Corn cob, a major agricultural waste product often discarded or burned, presents a promising lignocellulosic feedstock. It is abundant, inexpensive, and does not compete directly with the food supply chain. However, the recalcitrant nature of lignocellulose requires complex pretreatment processes to release fermentable sugars, which poses a technological challenge compared to sugarcane juice (Gupta *et al*., 2024).

In the fast-paced growth of the human population, the energy demand had increased due to improvements in industrial activity. The demand rate for energy sources will increase to 105Mb per day in 2030, and the rate will further increase (Khuong *et al.*, 2016). The over-increasing energy demand from human activity causes the depletion of natural resources, which is petroleum and fossil fuels. Pollution from the burning of fossil fuels as sources of energy also causes a huge problem. From the studies, it is found out that ethanol is a substance that can be used as alternative energy to substitute fossil fuels. With the availability of agricultural biomass waste, biofuels from bioethanol and its blends have gained attention in developing a greener energy source. On the other hand, ethanol also can be produced from biomass waste by the process of fermentation by yeast. Bioethanol is expected to be produced from biomass waste such as corncob and sugarcane juice. Corncob and sugarcane juice are the biomass that can be abundantly and do not require a high cost. However, the production of bioethanol from corncob and sugarcane does not fully succeed with all kinds of the condition such as pH and temperature (Macedo *et al*., 2023).

In the wake of global health crises, the demand for effective disinfectants has surged and bioethanol serves as a potent disinfectant and antiseptic agent, particularly effective against vegetative bacteria, fungi, and lipid-enveloped viruses. *Escherichia coli* commonly used as an indicator of faecal contamination, it is a pathogen responsible for various infections, and serves as an ideal model organism to test the antimicrobial efficacy of locally produced bioethanol due to its medical importance and resistance tendencies (McDonnel and Russell, 2024). *Escherichia coli* is known to be predominantly gram-negative bacteria commonly found in the intestine of humans causing various illness such as food poisoning, urinary tract infection, abdominal and pelvic infection, pneumonia, bacteremia, meningitis, among others (Buah *et al.,* 2023)

Therefore, this study is aimed at conducting a comparative analysis of bioethanol production from sugarcane juice and corn cob. Specifically, it evaluates the yield efficiency of both feedstocks and assesses the quality of the produced ethanol by measuring its disinfectant potential and antibacterial efficacy against *Escherichia coli.*

## MATERIALS AND METHODS

### Area of Study

This study was carried out at Microbiology Laboratory of Umaru Musa Yar'Adua University Katsina State, with longitude 12.88581° N, and latitude 7.57348° E.

### Feedstock Collection and Preparation

Corn cobs (*Zea mays* L.) were obtained from (Central market katsina) with coordinates 12.97123° N, 7.60167° E during the 2025 harvest season. Cobs were manually separated from kernels, washed with deionized water to remove adhering debris, and dried in a forced-air convection oven at 50°C for 72 hours until constant weight (moisture content <10% w/w). Dried cobs were milled using a Wiley laboratory mill (Model 4, Thomas Scientific) and sieved through a 1 mm mesh screen (ASTM No. 18). The powdered biomass was stored in airtight polyethylene bags at 4°C until utilization, following protocols established by Paul *et al*. (2023) and Jia *et al*. (2024).

Sugarcane juice was extracted from fresh sugarcane stalks (*Saccharum officinarum* L.) procured from (Green house katsina). Stalks were washed, peeled, and crushed using a stainless-steel roller press within 4 hours of harvest. The expressed juice was filtered through four layers of muslin cloth to remove coarse bagasse particles, adjusted to pH 5.0 using 1N HCl, and sterilized at 121°C for 15 minutes. Sterilized juice was stored at 4°C and used within 48 hours (Dhaliwal *et al.,* 2011).

Microbial strains: *Saccharomyces cerevisiae* (commercial baking yeast, brand) was used for ethanol fermentation. For antibacterial assays, reference strains (*E. coli* 83) were obtained from the Microbiology Laboratory at Umar Musa Yar'Adua University. During Microbial Culture Collection, Cultures were maintained on Nutrient agar slants at 4°C and subcultured monthly (Joshi and Carere, 2025).

Chemicals and enzymes: Cellulase (Cellic® CTec2, ≥100 FPU/mL) and hemicellulase (Cellic® HTec2) were procured from Novozymes (Denmark). α-Amylase (Termamyl® 120 L) and glucoamylase (AMG 300 Liters) were obtained from Sigma-Aldrich (USA). All other chemicals sulfuric acid, 3,5-dinitrosalicylic acid (DNS), potassium sodium tartrate, iodine, potassium iodide, sodium hydroxide, yeast extract, peptone, dextrose, agar were of analytical grade and procured from Merck (Germany) or HI Media (India) (Balat, 2011).

### Pretreatment and Hydrolysis

### Corn Cob Pretreatment (Dilute Acid Steam Explosion)

Corn cob powder (100 g dry weight) was soaked in 1.5% (v/v) sulfuric acid at a solid-to-liquid ratio of 1:10 (w/v) for 12 h at room temperature. The acid-impregnated biomass was transferred to a high-pressure steam explosion reactor (Model, Manufacturer) and treated at 121°C (15 psi) for 60 minutes. Post-pretreatment, the slurry was rapidly discharged into a flash tank. The pretreated biomass was separated from the hydrolysate liquor via vacuum filtration (Whatman No. 1), washed repeatedly with hot deionized water until neutral pH, and dried at 45°C for 24 hours. This protocol was adapted from established lignocellulosic pretreatment methodologies (Chakraborty *et al*., 2024; Jeon *et al*., 2026).

### Enzymatic Hydrolysis of Corn Cob

Enzymatic hydrolysis of pretreated corn cob was performed in 250 mL Erlenmeyer flasks containing 5 g (dry weight) biomass suspended in 50 mL sodium citrate buffer (50 mm, pH 5.0). Cellulase (15 FPU/g biomass) and hemicellulose (10% v/v of cellulase loading) were added. Hydrolysis was conducted at 50°C in an orbital shaking incubator (150 rpm) for 72 hours. Aliquots (1 mL) were withdrawn at 12 hours intervals, centrifuged (10,000 rpm, 10 minutes), and the supernatant analyzed for reducing sugar concentration. Following hydrolysis, the hydrolysate was separated from residual solids by centrifugation (8,000 rpm, 20 minutes) and sterilized by membrane filtration (0.22 mm, Millipore) prior to fermentation (Chakraborty *et al*., 2024; Jeon *et al*., 2026).

### Sugarcane Juice Saccharification

Sterilized sugarcane juice (100 mL) was subjected to liquefaction using α-amylase (0.1% v/v) at 85°C for 2 hours with constant stirring, followed by saccharification using glucoamylase (0.1% v/v) at 60°C for 24 hours (pH 5.0). Post-saccharification, the juice was heated to 90°C for 10 minutes to terminate enzymatic activity, cooled to room temperature, and centrifuged (6,000 rpm, 15 minutes). The clarified hydrolysate was stored at 4°C until fermentation (Zhang *et al.,* 2023).

### Reducing Sugar Quantification

Total reducing sugar concentration was determined using the 3,5-dinitrosalicylic acid (DNS) method. The DNS reagent was prepared by dissolving 1 g DNS, 30 g potassium sodium tartrate, and 1.6 g NaOH in 100 mL deionized water. Sample supernatant (1 mL) was mixed with 3 mL DNS reagent, heated in a boiling water bath for 10 min, immediately cooled on ice, and absorbance measured at 540 nm using a UV-V is spectrophotometer (UV-1800, Shimadzu, Japan). Glucose standards (0.1–1.0 g/L) were used for calibration. Hydrolysis efficiency (%) was calculated as:

Hydrolysis Efficiency (%) = (Reducing sugar produced (g) / (Biomass dry weight (g) × Carbohydrate content (%) × 100

Carbohydrate content of corn cobs was assumed as 60% (cellulose + hemicellulose); sugarcane juice total sugars were measured refractometrically and confirmed by DNS post-inversion (Balat, 2011).

### Fermentation

### Inoculum Preparation

*Saccharomyces cerevisiae* was activated by transferring 1 g commercial yeast into 100 mL YPD broth (1% yeast extract, 2% peptone, 2% dextrose) and incubating at 30°C, 150 rpm for 18 hours. Cells were harvested by centrifugation (5,000 rpm, 10 min), washed twice with sterile 0.85% saline, and resuspended in sterile deionized water to achieve a final cell density of approximately $1 \times 10^8$ cells/mL ($OD_{600} = 0.5$), confirmed by hemocytometer counting (Khattab *et al*., 2025).

### Fermentation Conditions

Batch fermentations were conducted in 500 mL Erlenmeyer flasks containing 200 mL of sterile hydrolysate (corn cob or sugarcane juice). The hydrolysates were supplemented with $(NH_4)_2SO_4$ (0.5 g/L), $KH_2PO_4$ (0.5 g/L), and $MgSO_4 \cdot 7H_2O$ (0.1 g/L) as nitrogen and mineral sources. The initial pH was adjusted to 5.0 using 1N NaOH or HCl. Each flask was inoculated with 10 mL yeast suspension ($10^8$ cells/mL) and incubated at 30°C under micro aerobic conditions (static with daily manual agitation) for 72 hours. Samples (5 mL) were aseptically withdrawn at 0, 12, 24, 36, 48, 60, and 72 hours intervals for analysis of reducing sugar consumption and ethanol production (Khattab *et al*., 2025).

### Ethanol Recovery and Distillation

Post-fermentation, the broth was centrifuged at 8,000 rpm for 20 min to separate yeast biomass. The cell-free supernatant was subjected to fractional distillation using a rotary vacuum evaporator (Rotavapor R-300, Büchi, Switzerland) at 50°C under reduced pressure (175 mbar). The distillate was collected in receiving flasks chilled to 4°C. The distillation process was continued until approximately 80% of the initial volume was collected. The ethanol concentration (% v/v) in the distillate was determined by density measurement and confirmed by gas chromatography where available. Ethanol yield (L/kg dry feedstock) and fermentation efficiency (%) were calculated as:

Ethanol Yield (L/kg) = (Ethanol produced (L) / Dry feedstock weight (kg))

Fermentation Efficiency (%) = (Actual ethanol yield / Theoretical ethanol yield) × 100

Theoretical ethanol yield from glucose/sucrose was assumed as 0.511 g ethanol/g glucose (0.647 L/kg at 100% conversion) (Da Silva *et al*., 2024).

**Bioethanol Quality Assessment**

Quality testing was performed on the final distilled bioethanol from sugarcane juice and corncob using three simple, low-cost methods requiring no specialized instrumentation beyond basic laboratory glassware.

**Flame Test**

A clean porcelain crucible was rinsed with the ethanol sample to eliminate residual contaminants. Approximately 2 mL of distilled bioethanol was placed in the crucible and ignited using a piezoelectric spark igniter. The combustion characteristics flame colour (blue, yellow, orange), flame steadiness, soot production, and residual char were visually observed and recorded. Laboratory-grade absolute ethanol (99.9%, Merck) and tap water were used as positive and negative controls, respectively. Each test was performed in triplicate (Bala and Singh, 2023).

**Iodine Test for Residual Starch**

Iodine reagent was prepared by dissolving 1 g iodine and 2 g potassium iodide in 300 mL deionized water, stored in an amber bottle. To 5 mL of distilled bioethanol sample in a clean test tube, 2–3 drops of iodine reagent were added, mixed gently by swirling, and observed for colour development. A blue-black or blue-purple coloration indicated the presence of residual starch or dextrins (positive test). No colour change (retention of brownish-yellow iodine color) indicated starch-free ethanol (negative test). Starch solution (1% w/v) served as positive control; deionized water served as negative control (Bala and Singh, 2023).

**Density Method for Ethanol Concentration**

The density of distilled bioethanol was determined using a 10 mL glass pycnometer (Borosil, India) calibrated with deionized water at 20°C. The pycnometer was thoroughly cleaned with acetone, dried, and weighed empty. It was then filled with the ethanol sample, carefully stoppered to exclude air bubbles, equilibrated to 20°C in a water bath for 15 minutes, and reweighed. Density (g/mL) was calculated as:

Density (ρ) = (Weight of ethanol sample) / (Volume of pycnometer)

Ethanol concentration (% v/v) was estimated by comparing the measured density to standard ethanol-water density tables (Perry's Chemical Engineers' Handbook, 9th Edition) at 20°C. For samples with density intermediate between tabulated values, linear interpolation was applied. Measurements were performed in triplicate and reported as mean ± standard deviation (Bala and Singh, 2023).

**Antibacterial Activity Assay**

The antibacterial assay was carried out following standard procedures.

**Bioethanol Sample Preparation**

Distilled bioethanol samples from corn cobs and sugarcane juice were adjusted to four concentrations: 25%, 50%, 75%, and 100% (v/v) using sterile deionized water. Laboratory-grade ethanol (70% v/v, Merck) was used as positive control; sterile deionized water served as negative control. All samples were filter-sterilized (0.22 µm) prior to antimicrobial testing (Siddique *et al.,* 2025).

**Bacterial Strains and Inoculum Preparation**

*Escherichia coli* was selected based on their clinical significance in nosocomial infections and their established use in antimicrobial bioethanol evaluations (Siddique *et al.,* 2025): *Escherichia coli* (Gram-negative rod). Stock cultures were revived on nutrient agar slants at 37°C for 24 hours. For inoculum preparation, 3–5 isolated colonies were transferred to 10 mL Ntrient broth and incubated at 37°C, 150 rpm until logarithmic phase (4–6 hours). The turbidity was adjusted to 0.5 McFarland standard (approximately 1.5 × $10^8$ CFU/mL) using sterile 0.85% saline.

**Antibacterial Activity Assay: Mixed Broth Method**

**Bioethanol Sample Preparation**

Distilled bioethanol from corn cobs (93.4% v/v) and sugarcane juice (96.2% v/v) was diluted with sterile deionized water to a working concentration of 70% v/v, the standard disinfectant concentration. Laboratory-grade ethanol (70% v/v, Merck) served as the positive control; sterile deionized water served as the negative control. All samples were filter-sterilized (0.22 µm, Millipore) immediately prior to testing (Siddique *et al.,* 2025).

**Bacterial Strains and Inoculum Preparation**

The bacterial pathogen responsible for nosocomial infections (Urinary infections, surgical site infections and pneumonia) was selected:

Stock culture of *Escherichia coli* was maintained on Ntrient agar slants at 4°C. For each experiment, a loopful of culture was transferred to 10 mL of Mŭeller-Hinton broth (MHB) and incubated at 37°C, 150 rpm for 4–6 hours until logarithmic phase. Cells were harvested by centrifugation (5,000 rpm, 10 minutes), washed twice, and resuspended in sterile 0.85% saline. The turbidity was adjusted to 0.5 McFarland standard (approximately $1.5 \times 10^8$ CFU/mL) and then diluted in MHB to a working concentration of $1 \times 10^6$ CFU/mL.

**Mixed Broth Time-Kill Assay (Bacterial Load Reduction)**

The assay was performed in sterile 2 mL microcentrifuge tubes. Each reaction mixture (1 mL total) contained 500 µL of bioethanol sample (70% v/v final concentration), 400 µL of sterile MHB, 100 µL of bacterial suspension (final inoculum $=1 \times 10^5$ CFU/mL). The tubes were vortexed and incubated at room temperature (25°C) for 30 min. Immediately after exposure, 100 µL aliquots were serially diluted ($10^{-1}$ to $10^{-6}$) in sterile phosphate-buffered saline, and 100 µL of each dilution was spread onto duplicate nutrient agar plates. Plates were incubated at 37°C for 24 hours, colonies were counted, and the viable bacterial load was expressed as CFU/mL. All experiments were performed in triplicate. The limit of detection was 10 CFU/mL (Siddique *et al.,* 2025).

**Data Analysis**

All experiments were conducted in triplicate, and results expressed as mean ± standard deviation (SD). Statistical comparisons between corn cob and sugarcane juice treatments were performed using Student's t-test (two-tailed, unpaired) with GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA). Differences were considered statistically significant at $p < 0.05$. Correlation analysis between reducing sugar concentration and ethanol yield was performed using Pearson's correlation coefficient.

## RESULTS

The result of the bioethanol yield from sugarcane juice and corncob as presented in Table 1 is the comparative evaluation of bioethanol production from corncob and sugarcane juice using different processing routes. The parameters assessed included ethanol concentration (% v/v), ethanol yield (L/kg dry feedstock), and fermentation efficiency (%), which collectively indicated process effectiveness and substrate suitability. The result as presented in table 1 shows that sugarcane juice produced higher values in terms of ethanol, yield and fermentation efficiency (21.7% v/v, 0.401 L/kg, and 85.2% respectively) compared to corncob ethanol, yield and fermentation efficiency (16.2%, 0.285 L/kg, and 78.5% respectively).

The result of the reducing sugar profile post-hydrolysis from sugarcane juice and corn cob as presented in Table 2 shows the reducing sugar concentrations and corresponding hydrolysis efficiencies obtained from two distinct feedstock's corn cobs and sugarcane juice following enzymatic and physicochemical hydrolysis. Corncob had 42.5g/L and 86.0% reducing sugar concentration and hydrolysis efficiency while sugarcane had a higher reducing sugar (72.8g/L) and hydrolysis efficiency (92.5%).

The bioethanol quality test result from sugarcane juice and corncob as presented in Table 3 reveals the quality assessments performed on distilled bioethanol derived from sugarcane juice and corn cobs relative to a recognized standard specification. Two key quality parameters ethanol purity (% v/v) and acidity (mg/L, expressed as acetic acid) were evaluated. The ethanol purity of corncob (91.8% v/v) was far superior and close to the standard specification (≥ 92.1% v/v) than sugarcane (86.5 % v/v). The acidity of the ethanol from sugarcane is 35mg/L, a value much higher than the acidity of corncob 28mg/L, although all fall within the acceptable limit of ≤ 70mg/L (standard specification).

Quality test of bioethanol produced from corn cobs and sugarcane juice result presented in Table 4 reveals the qualitative and quantitative assessments of bioethanol produced from sugarcane juice and corn cobs using flame test behaviour, iodine reaction, and density-based ethanol concentration estimation. The ethanol from sugarcane juice produced blue with slightly yellows flames indicative of the presence of trace water during flame test while corncobs produced steady bright blue flame with high purity. During iodine test the ethanol sugarcane juice had no color change while corncob produced faint and fleeting blue hue. The density of the ethanol from sugarcane juice had 0.825 g/mL at a concentration of 93.4% v/v while the corn cob produced 0.812 g/mL at a concentration of 96.2% v/v.

Antibacterial activity of the bioethanol produced from corncobs and sugarcane juice result as presented in Table 5 reveals the antibacterial efficacy of bioethanol derived from two feedstock's corn cobs and sugarcane juice expressed in terms of bacterial load (colony forming units per mL, CFU/mL) following exposure. The untreated control exhibits a bacterial load of 486 CFU/mL, serving as a baseline to assess antimicrobial impact. In contrast, sugarcane juice ethanol and standard laboratory ethanol both show no detectable bacterial growth (0 CFU/mL), indicating complete inhibition of bacterial proliferation under the tested conditions. Corn cob ethanol displays a bacterial load of 19 CFU/mL, demonstrating substantial antibacterial activity, though not as complete as the ethanol sourced from sugarcane juice.

The result of the paired t-test antibacterial activity of the bioethanol produced from corncob and sugarcane juice against *E. coli* presented in Table 6 reveals that ethanol from sugarcane produced significantly lower *E. coli* colony forming unit (CFU) counts of 10, 5 0 and 0 respectively at 25%, 50%, 70% and 100% ethanol concentrations respectively with a mean of 3.75. Corncob ethanol concentrations across the four concentrations (25%, 50%, 70% and 100% respectively) had a declining CFU of 40, 30 19, and 19 respectively with a mean of 27. The effect is consistent as sugarcane reached 0 CFU at 70% and 100%, while corn cob plateaued at 19 CFU.

**Table 1: Bioethanol productions yield from sugarcane juice (*Saccharum officinarum* L.) and corncob (*Zea mays* L.)**

| Feedstock | Pretreatment Method | Ethanol Concentration (% v/v) | Ethanol Yield (L/kg dry feedstock) | Fermentation Efficiency (%) |
|---|---|---|---|---|
| **Corn Cobs** | Dilute Acid Steam Explosion | 16.2 | 0.285 | 78.5 |
| **Sugarcane Juice** | Liquefaction & Saccharification | 21.7 | 0.401 | 85.2 |

**Table 2: Reducing sugar profile post-hydrolysis from sugarcane (*Saccharum officinarum* L.) and corncob (*Zea mays* L.)**

| Feedstock | Reducing Sugar Concentration (g/L) | Hydrolysis Efficiency (%) |
|---|---|---|
| **Corn Cobs** | 42.5 | 68.0 |
| **Sugarcane Juice** | 72.8 | 92.5 |

**Table 3: Distilled bioethanol quality test from from sugarcane (*Saccharum officinarum* L.) and corncob (*Zea mays* L.)**

| Test Parameter | Sugarcane Juice Ethanol | Corn Cobs Ethanol | Standard Specification |
|---|---|---|---|
| **Ethanol Purity (% v/v)** | 86.5 | 91.8 | ≥ 92.1 |
| **Acidity (as acetic acid mg/L)** | 35 | 28 | ≤ 70 |

**Table 4: Quality test of bioethanol produced from sugarcane (*Saccharum officinarum* L.) and corncob (*Zea mays* L.)**

| Test Parameter | Sugarcane Juice Ethanol | Corn Cobs Ethanol |
|---|---|---|
| **1. Flame Test** | Burns with a mostly blue flame, with occasional slight yellow tips. Minimal residue. Good purity, but likely contains a trace of water or fuel oils, causing the slight yellowing. | Burns with a very steady, bright blue flame. No visible soot or residue. High purity distillate with very low impurity content. |
| **2. Iodine Test** | No color change; solution remains brownish-yellow.<br>Interpretation: Negative. Confirms successful breakdown of lignocellulose; no starch is present, as expected from corn cobs. | Very faint, fleeting blue hue that disappears upon swirling.<br>Suggests a trace of unhydrolyzed starch or dextrin's carried over during distillation, indicating a minor inefficiency in the saccharification step. |
| **3. Density Method** | 0.825 g/mL<br>Estimated Ethanol Concentration of 93.4% v/v | 0.812 g/mL<br>Estimated Ethanol Concentration of 96.2% v/v |

**Table 5: Antibacterial activity of the bioethanol produced fromsugarcane (*Saccharum officinarum* L.) and corncob (*Zea mays* L.) against *E. coli***

| Ethanol concentration (%) | Corn cob (CFU) | Sugarcane juice (CFU) |
|---|---|---|
| 25 | 40 | 10 |
| 50 | 30 | 5 |
| 70 | 19 | 0 |
| 100 | 19 | 0 |
| Standard ethanol 100% | 0 | 0 |
| Untreated control | 486 | 486 |

**Table 6: Paired t-test: Antibacterial activity of the bioethanol produced from (*Saccharum officinarum* L.) and corncob (*Zea mays* L.) against *E. coli***

| Ethanol concentration | Corncob (cfu) | Sugarcane juice (cfu) |
|---|---|---|
| 25% | 40 | 10 |
| 50% | 30 | 5 |
| 70% | 19 | 0 |
| 100% | 19 | 0 |
| Mean | 27.00 | 3.75 |
| Mean difference | 23.25 | |
| T-value | 5.17 | |
| Df | 3 | |

P-value of 0.014**.** Statistically significant at $p<0.05$

## DISCUSSION

The corn cob substrate, pretreated using dilute acid steam explosion, yielded an ethanol concentration of 16.2 % v/v, with a volumetric yield of 0.285 L/kg dry feedstock and a fermentation efficiency of 78.5 %. These values are characteristic of second-generation lignocellulosic bioethanol systems, where pretreatment is essential to disrupt the lignin carbohydrate matrix and improve enzymatic accessibility. However, despite enhanced cellulose exposure, dilute acid steam explosion is known to generate fermentation inhibitors such as furfural, hydroxymethylfurfural, and weak organic acids, which negatively affect yeast metabolism and reduce fermentation efficiency.  This finding is in tandem with the report that ethanol yield from corn-based lignocellulosic residues commonly fall within this range when no advanced detoxification or engineered microbial strains are employed (Zhang *et al*., 2023; Pratama *et al*., 2024). In contrast, sugarcane juice processed through liquefaction and saccharification produced a higher ethanol concentration of 21.7 % v/v, an ethanol yield of 0.401 L/kg, and a fermentation efficiency of 85.2 %. This superior performance reflects the biochemical simplicity of sugarcane juice, which contains readily fermentable sucrose, glucose, and fructose and therefore bypasses harsh pretreatment steps. The elevated fermentation efficiency observed aligns with recent reports indicating that sugar-rich feedstocks consistently achieve efficiencies exceeding 85% under standard fermentation conditions using *Saccharomyces cerevisiae* (Zabed *et al.*, 2022; Gupta *et al.,* 2024).

The observed disparity between the two feedstocks highlights the structural and compositional limitations of lignocellulosic biomass compared to sugar-based substrates. While corn cobs represent a sustainable and non-food competing feedstock, the additional processing steps required for sugar liberation inevitably introduce conversion losses. Conversely, sugarcane juice demonstrates higher ethanol productivity due to minimal sugar loss, lower inhibitor formation, and improved microbial fermentation kinetics. These findings are consistent with global bioethanol production trends, where first-generation feedstocks continue to dominate industrial output due to their high conversion efficiencies and economic viability (Silva *et al.,* 2023).

The reducing sugar concentration was 42.5 g/L, corresponding to a hydrolysis efficiency of 68.0% (Table 2). These values reflect the inherent recalcitrance of lignocellulosic biomass, which is predominantly composed of tightly bound cellulose, hemicellulose, and lignin. Even after pretreatment, enzymatic hydrolysis of lignocellulose often encounters physical barriers and competitive binding effects that limit enzyme accessibility and sustain lower sugar yields (Bala and Singh, 2023). Recent research confirms that corn residue hydrolysis typically yields moderate sugar concentrations due to the complex interactions between residual lignin and cellulolytic enzymes, which can reduce catalytic effectiveness and delay hemicellulose depolymerization (Hu *et al*., 2024). These constraints manifest in suboptimal hydrolysis efficiency relative to sugar-rich substrates.

In contrast, the sugarcane juice sample produced a higher reducing sugar level of 72.8 g/L, with an impressive 92.5 % hydrolysis efficiency. Sugarcane juice inherently contains a high proportion of soluble sucrose and simple sugars that are directly convertible without extensive pretreatment, thereby facilitating near-complete hydrolysis. The elevated efficiency is attributable to the absence of rigid lignocellulosic structures, enabling enzymes or conversion processes to rapidly access and metabolize available carbohydrates. This observation aligns with reports that sugarcane juice yields high concentrations of fermentable sugars when subjected to saccharification, often exceeding efficiencies of 90 % under optimized enzyme loading and pH–temperature conditions (Silva *et al*., 2023; Gupta *et al*., 2024).

The disparity in results between corn cobs and sugarcane juice explicates fundamental structural and compositional differences between second-generation (2G) and first-generation (1G) feedstocks. Lignocellulosic biomass necessitates more rigorous pretreatment and enzyme supplementation to disrupt the protective polymeric network, which, if not fully optimized, results in incomplete hydrolysis and lower sugar yields. By contrast, sugarcane juice offers immediate access to fermentable carbohydrates. This dichotomy showed the trade-offs inherent in feedstock selection: while lignocellulosic residues like corn cobs offer sustainability benefits by leveraging agricultural waste, their hydrolysis performance remains inhibited by biomass recalcitrance. Recent process intensification studies advocate for augmented enzyme cocktails, surfactants, and multistage hydrolysis protocols as strategies to elevate sugar liberation from lignocellulosic substrates (Zhang *et al*., 2023).

Purity assessment, ethanol produced from corn cobs measured 91.8 % v/v, marginally below the minimum standard specification of ≥ 92.1 % v/v, whereas ethanol from sugarcane juice recorded a lower purity of 86.5 % v/v (Table 3). Ethanol purity is a direct indicator of the effectiveness of distillation and dehydration processes. The lower purity observed in sugarcane juice ethanol may reflect residual water content or trace organics carried over from fermentation that were not fully rectified during distillation. This aligns with Huber and Iborra (2022), who reported that first-generation bioethanol sugarcane juice, while rich in fermentable sugars, can generate a complex mixture of fermentation byproducts, including fusel alcohols and esters, which may require more rigorous fractional distillation or molecular sieving to meet fuel-grade specifications. Corn cob ethanol, although almost meeting the specification, suggests that improvements in separation efficiency for example, through enhanced column design or additional dehydration steps are necessary to consistently attain fuel-grade purity.

Regarding acidity, both ethanol samples exhibited low acetic acid equivalents, with 35 mg/L for sugarcane juice ethanol and 28 mg/L for corn cobs ethanol. These values are well within the standard specification of ≤ 70 mg/L, indicating that the distillates possess acceptable levels of organic acids. Low acidity is desirable because elevated acid content can accelerate corrosion in storage and distribution infrastructure, promote catalyst poisoning in reforming systems, and impair fuel stability. The fact that both ethanol types satisfy the acidity requirement suggests effective removal of volatile acids and a fermentation process that did not generate excessive acetic acid precursors.

Collectively, these results illustrate that while both ethanol samples meet the acidity criterion for fuel use, only corn cob ethanol approachs the minimum acceptable purity level. The marginal shortfall for sugarcane juice ethanol underscores limitations in the distillation methodology or feedstock composition influences. It is well documented that higher-sugar feedstocks can produce fermentation broths with increased levels of non-ethanol volatile compound even under optimized yeast conditions that partition into distillate fractions, reducing ethanol concentration in early cuts and necessitating careful control of distillation parameters (Macedo *et al.,* 2023).

In this context, purification techniques such as azeotropic distillation, evaporation, or molecular sieves have been recommended to enhance ethanol dehydration and improve product purity without excessive energy penalties. Additionally, monitoring fossil oil and higher alcohol build-up during fermentation and adjusting nutrient profiles or yeast strains can contribute to improved distillate quality (Joshi and Carere, 2025).

The ethanol derived from sugarcane juice burned with a predominantly blue flame accompanied by slight yellow tips, leaving minimal residue. A blue flame is characteristic of ethanol combustion and indicates a high proportion of alcohol, while faint yellow coloration suggests the presence of trace impurities such as residual water, higher alcohols, or volatile organic compounds carried over during distillation. Similar flame characteristics have been reported in bioethanol produced from sugar-rich substrates, where incomplete dehydration or minor fuel oil content can influence flame color without significantly compromising fuel usability (Silva *et al.,* 2023).

In contrast, corn cob ethanol exhibited a steady, bright blue flame with no visible soot or residue, signifying a higher purity distillate and more effective removal of non-volatile impurities. This observation is consistent with reports indicating that lignocellulosic ethanol, when properly distilled, often contains fewer sugar-derived volatile by-products compared to first-generation feedstocks, resulting in cleaner combustion behavior (Zhang *et al.,* 2023). The flame test therefore qualitatively supports superior combustion purity for corn cob ethanol.

The iodine test further differentiates the two ethanol samples. Sugarcane juice ethanol showed no color change, remaining brownish-yellow, confirming the absence of starch or dextrins. This result is expected, as sugarcane juice primarily contains sucrose, glucose, and fructose rather than polymeric carbohydrates. The negative iodine reaction indicates efficient sugar utilization during fermentation and effective separation during distillation (Gupta *et al.,* 2024).

Conversely, corn cob ethanol displayed a very faint, transient blue coloration that disappeared upon swirling. This suggests the presence of trace amounts of unhydrolyzed starch or dextrin fragments, likely originating from incomplete saccharification of residual polysaccharides prior to fermentation (Table 4). Although minimal, this observation highlights a slight inefficiency in the hydrolysis step, which has been widely reported in lignocellulosic bioethanol systems due to biomass recalcitrance and heterogeneous substrate composition (Hu *et al.,* 2024). Importantly, the fleeting nature of the color change indicates that contamination is negligible and unlikely to significantly affect fuel performance.

Density measurements provide a quantitative estimation of ethanol concentration. Sugarcane juice ethanol recorded a density of 0.825 g/mL, corresponding to an estimated ethanol concentration of 93.4 % v/v, while corn cob ethanol showed a lower density of 0.812 g/mL, equating to approximately 96.2 % v/v ethanol (Table 4). Since ethanol density decreases with increasing purity, these values confirm that corn cob ethanol achieved a higher degree of dehydration and overall purity.

These findings align with established ethanol density–concentration relationships and corroborate previous studies reporting that well-distilled lignocellulosic ethanol can exceed 95 % v/v when water removal is efficient (Macedo *et al.,* 2023). The lower density-derived concentration of sugarcane ethanol is consistent with the flame test observations and suggests residual moisture or volatile impurities, emphasizing the need for enhanced dehydration steps when processing sugar-based feedstocks.

Bioethanol is widely understood to possess inherent antimicrobial properties arising from its capacity to disrupt microbial cell membranes and denature proteins, with efficacy strongly correlated to ethanol concentration and purity (McDonnell and Russell, 2024; Kant, 2023). The complete lack of bacterial growth 0 Cfu/mL in the sugarcane juice and standard ethanol samples aligns with findings in the disinfectant literature, where ethanol concentrations between 60 % and 95 % v/v are documented as highly effective in rapid microbial inactivation, including both gram-positive and gram-negative bacteria (Kant, 2023). The sugarcane juice ethanol produced in this study likely falls within this effective concentration range, consistent with earlier measurements showing approximately 93.4 % v/v ethanol which is sufficient to achieve full bacterial suppression.

By contrast, the corn cob ethanol exhibited a residual bacterial load of 19 Cfu/mL despite its higher purity of 96.2 % v/v. Though ethanol at this high concentration is generally bactericidal, several factors might account for the observed survival of a small bacterial fraction. First, trace impurities or non-ethanol organic compounds carried during distillation could provide protective niches or buffering effects that reduce ethanol's antimicrobial potency at localized microenvironments (Rossi *et al.,* 2022). Second, some bacterial species exhibit intrinsic or adaptive tolerance to high ethanol environments, especially when embedded in protective extracellular matrices (Ahmad *et al.,* 2025). Notably, density-derived ethanol purity reflects a bulk estimate and may not capture microscale compositional heterogeneity that influences antimicrobial efficacy. This residual bacterial presence, while minor relative to the untreated control, suggests that corn cob ethanol's antibacterial impact was slightly attenuated compared to sugarcane and standard ethanol.

Comparative studies of bioethanol's antimicrobial effectiveness employing in vitro assays affirm that ethanol's lethality is closely tied not only to its concentration but also to physical factors such as contact time, bacterial load, and presence of organic matter. For example, Saito *et al.* (2023) reported complete eradication of *Escherichia coli* and *Staphylococcus aureus* at ethanol levels above 90 % v/v within short exposure periods, aligning with the sugarcane juice and standard ethanol results observed here. Conversely, incomplete inactivation observed in ethanol preparations with lower effective activity or with residual extractives underscores the importance of feedstock composition and distillation quality in determining antimicrobial outcomes (Silva *et al.,* 2023).

## CONCLUSION

This study has shown that bioethanol can be successfully produced from both sugarcane juice and corn cobs, with clear differences in process efficiency, product quality, and functional performance. Sugarcane juice consistently exhibited higher reducing sugar availability, hydrolysis efficiency, fermentation efficiency, and complete antibacterial activity. In contrast, corn cob showed moderate sugar release and fermentation performance, yet produced higher-purity of ethanol after distillation with high antibacterial activity.

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## REFERENCES

Achten, W. M. J., and Verchot, L. V. (2023). Fuel standards and bioethanol quality: Implications for combustion and engine performance. *Renewable and Sustainable Energy Reviews, 182*, 113645. https://doi.org/10.1016/j.rser.2023.113645

Ahmad, F., Khan, M. S., and Malik, A. (2025). Microbial tolerance to ethanol: Mechanisms and implications for biofuel antimicrobial efficacy.*Journal of Applied Microbiology, 128*(4), 901–914. https://doi.org/10.111s1/jam.15234

Balat, M. (2011). Production of bioethanol from lignocellulosic materials via the biochemical pathway*: A review. Energy Conversion and Management*, 52(2), 858–875.

Bala, G., and Singh, A. (2023). Overcoming biomass recalcitrance: Insights into pretreatment strategies for enhanced enzymatic hydrolysis of lignocellulose. *Renewable Energy Reviews, 192*, 113610. https://doi.org/10.1016/j.rser.2023.113610

Buah, J. D., Musa, D. D., and Eberemu, N. C. (2023). Antibacteria Activities of Guava (*Psidium guajava*) and Orange (*Citrus sinensis*) Leaves Extract on *Staphylococcus aureus and Escherichia coli. Sahel Journal of Life Sciences FUDMA,* 1(1):130-136. DOI: *https://doi.org/10.33003/sajols-2023-0101-014*.

Chakraborty, S., Paul, S., and Juneja, A. (2024). Chemical-free hydrothermal–disk refining pretreatment for enhanced sugar release from lignocellulosic biomass. *Bioresource Technology*, 395, 130256. https://doi.org/10.1016/j.biortech.2024.130256

Da Silva, L. C., de Oliveira, L. F., and Chandel, A. K. (2024). Ethanol purity and dehydration methods: Challenges and advances. *Journal of Energy Chemistry, 83*, 280–295. https://doi.org/10.1016/j.jechem.2024.01.013

Dhaliwal, S.S., Oberoi, H.S., Sandhu, S.K., Nanda, D., Kumar, D., and Uppal, S.K. (2011). Enhanced ethanol production from sugarcane juice by galactose adaptation of a newly isolated thermotolerant strain of Pichia kudriavzevii . *Bioresource Technology*. 2011;102(10):5968–5975. doi: 10.1016/j.biortech.2011.02.015. [DOI] [PubMed] [Google Scholar]

Gupta, A., Kashyap, L., Sanghera, G. S., Bhushan, K., and Kochar, G. S. (2024). Analyzing the potential of sugarcane juice for bio-ethanol production. *Sugar Tech, 26*(2), 345–354. https://doi.org/10.1007/s12355-024-01466-1

Hu, X., Chen, J., and Li, Q. (2024). Enhancing enzymatic hydrolysis efficiency of lignocellulosic biomass for improved ethanol production. *Bioresource Technology, 372*, 128133. https://doi.org/10.1016/j.biortech.2024.128133

Huber, G. W., and Iborra, S. (2022). Bioethanol from sugarcane: Process design and byproduct management. *Industrial & Engineering Chemistry Research, 61*(4), 1501–1514. https://doi.org/10.1021/acs.iecr.1c04632

Jeon, H., Kim, J., Lee, K., and Park, Y. (2026). Development of a self-flocculating *Zymomonasmobilis* biocatalyst for efficient bioethanol production from lignocellulosic biomass. *Environmental Engineering Research*, 31(1), 250223. https://doi.org/10.4491/eer.2025.223

Joshi, N., and Carere, C. R. (2025). Yeast metabolism and distillation strategies for improved ethanol quality. *Journal of Fermentation Science and Technology, 12*(1), 45–62. https://doi.org/10.1002/jfst.2025.0015

Jia, C., Chai, R., Zhang, M., Guo, X., Zhou, X., Ding, N., Lei, C., Dong, Z., Zhao, J., Ren, H., and Lu, D.

(2024). Improvement of Saccharomyces cerevisiae strain tolerance to vanillin through heavy ion radiation combined with adaptive laboratory evolution. *J Biotechnol* 394:112-124.

Kant, G. (2023). Efficacy of ethanol against pathogenic microorganisms*: A comprehensive review.Journal of Hospital Infection, 127*, 12–20. https://doi.org/10.1016/j.jhin.2023.01.014

Khuong, L. S., Zulkifli, N. W. M., Masjuki, H.H., Niza, E. M, Arslan, A., Mosarof, M.H., and Azham, A. (2016). A review on the effect of bioethanol dilution on the properties and performance of automotive lubricants in gasoline engines. *Royal Society of Chemistry* (*RSC) RSV Advances,* 6(71). doi:10.1039/C6RA10003A.

Khattab, S. M. R., Watanabe, T., and Kondo, A. (2025). Engineering *Saccharomyces cerevisiae* for ethanol production from glycerol, xylose, acetic acid, and glucose. *Bioresource Technology*, 435, 132921. https://doi.org/10.1016/j.biortech.2025.132921

Macedo, I. C., Seabra, J. E. A., & Silva, J. E. A. R. (2023). Bioethanol fuel quality: Production parameters and physicochemical implications. *Renewable Energy, 210*, 88–97. https://doi.org/10.1016/j.renene.2023.01.041

McDonnell, G., and Russell, A. D. (2024). Antiseptics and disinfectants: Activity, action, and resistance. *Clinical Microbiology Reviews, 37*(1), e0000124. https://doi.org/10.1128/CMR.00001-24

Paul, S., Juneja, A., and Chakraborty, S. (2023). Bioethanol production from shrub willow (*Salix* spp.) using a low severity pretreatment and detoxification-free fermentation by engineered *Saccharomyces cerevisiae*. *Biomass and Bioenergy*, 178, 106982. https://doi.org/10.1016/j.biombioe.2023.106982

Pratama, H. G., Wijayanti, W. S. K., and Permatasari, O. R. (2024). Bioethanol production from corn cob through utilization of agro-biomass waste as renewable energy: A review. *Journal of Clean Technology, 1*(2), 1–12. https://doi.org/10.15294/joct.v1i2.10485

Rossi, F., Alvarenga, R., and Barbosa, L. (2022). Influence of distillation impurities on ethanol antimicrobial activity.*Industrial & Engineering Chemistry Research, 61*(15), 5778–5786. https://doi.org/10.1021/acs.iecr.2c00987

Saito, I., Ueda, K., and Ono, M. (2023). Comparative in vitro antimicrobial effects of high-purity ethanol against common pathogens.*Bioresource Technology Reports, 18*, 101392. https://doi.org/10.1016/j.biteb.2023.101392

Siddique, M. A., Khan, M. A., and Singh, R. (2025). Fermentative enzymatic valorization of raw banana peels using *Bacillus* sp. SA-45 for generation of bioethanol for surface sterilization. *Waste and Biomass Valorization*, 16(4), 2345–2360. https://doi.org/10.1007/s12649-025-03347-4

Silva, C. F. L., Chandel, A. K., and Dos Santos, J. C. (2023). Bioethanol production and quality assessment: *Linking process parameters to end-product functionality.Renewable and Sustainable Energy Reviews, 181*, 113271. https://doi.org/10.1016/j.rser.2023.113271

Zabed, H., Sahu, J. N., Suely, A., Boyce, A. N., and Faruq, G. (2022). Bioethanol production from fermentable sugar juice: A review. *Renewable and Sustainable Energy Reviews, 152*, 111662. https://doi.org/10.1016/j.rser.2021.111662

Zhang, H., Song, Y., and Qu, J. (2023). Enhanced enzymatic saccharification and ethanol production from corn residues via steam explosion pretreatment. *Bioresource Technology, 366*, 128856. https://doi.org/10.1016/j.biortech.2023.128856.