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ORIGINAL ARTICLE

# Bioconversion of Lignocellulosic Biomass into Xylitol and Ethanol Using *Kluyveromyces* and *Saccharomyces cerevisiae*

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## Abstract

The sustainable utilization of lignocellulosic agricultural residues represents a promising approach to producing renewable bio-based chemicals and reducing environmental pollution caused by open-field burning. This study aimed to evaluate sugarcane bagasse, wheat straw, and corn cob as low-cost substrates for the biotechnological production of Xylitol and Ethanol. The biomass was subjected to sequential Acid and Alkali Hydrysis, and Enzymatic Saccharification to maximize the release of fermentable sugars, followed by fermentation with *Kluyveromyces* (pentose-fermenting yeast) and *Saccharomyces cerevisiae* (hexose-fermenting yeast). Alkali-enzyme pretreatment of sugarcane bagasse liquor released the highest concentrations of xylose (36.27 g/L) and glucose (6.21 g/L). Fermentation with *Kluyveromyces* yielded a maximum xylitol concentration of 28.54 g/L with 78.68 % yield and volumetric productivity of 0.016 g/L/h, whereas *S. cerevisiae* fermentation of sugarcane bagasse residue produced 4.26 g/L ethanol with 69.40 % yield and 0.236 g/L/h productivity. Wheat straw alkali–enzyme hydrolysate demonstrated the highest Xylitol yield (121.29 %) at pH 6, indicating the influence of pH on process efficiency. These findings demonstrate that integrated chemical hydrolysis and enzymatic saccharification substantially enhances sugar recovery and subsequent microbial conversion into value-added products. The results highlight the potential of using agro-industrial residues as a sustainable feedstock for biorefineries. Future studies should focus on process optimization, detoxification of hydrolysates, and techno-economic assessments to enable industrial-scale, cost-effective production of Xylitol and Ethanol.

**Keywords** Yeast Fermentation, Sugarcane Bagasse, Wheat Straw, Corn Cobs, Enzymatic Saccharification, Acid and Alkali hydrolysis

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## 1. Introduction

Lignocellulosic biomass is the most abundant renewable carbon source on Earth, accounting for nearly 57-60% of global biogenic carbon and representing about 181.5 billion tons of biomass annually, of which only a small fraction is currently utilized (1, 2). Major agricultural residues like Wheat Straw, Sugarcane Bagasse, and Corn Cob are produced in large quantities in agricultural economies such as Pakistan, where a significant amount of this biomass is often discarded or openly burned. This practice contributes substantially to environmental pollution and greenhouse gas emissions (3). Efficient utilization of lignocellulosic agricultural residues is critical for advancing sustainable bioeconomy goals and mitigating climate change. Pakistan's biomass residues, including wheat straw, rice husks, corn stalks, and sugarcane bagasse, hold significant potential for renewable energy production, such as biofuels, syngas, and biohydrogen, which can replace fossil fuels and reduce carbon footprints (4). Innovative bioprocessing technologies and integrated biorefineries are being developed to convert these residues into biofuels, bioplastics, and other high-value bioproducts, supporting a circular bioeconomy while reducing waste (5).

Lignocellulosic biomass primarily consists of cellulose, hemicellulose, and lignin, which form a complex and highly

recalcitrant structure that is resistant to microbial degradation (6, 7). Typically, cellulose accounts for 40-50% of this biomass and provides structural strength as a crystalline polysaccharide composed of β-1,4-glucopyranose units. Hemicellulose, representing about 20-30%, is a heterogeneous matrix polymer composed of various sugar monomers including xylose and glucose, and intricately binds cellulose fibers. Lignin, making up 10-25%, is a complex aromatic polymer that encrusts cellulose and hemicellulose, conferring rigidity and making the biomass highly resistant to enzymatic attack (6, 7). Due to this intricate structure, pretreatment is essential to break down lignin and hemicellulose to enhance accessibility to cellulose, thereby releasing fermentable sugars such as xylose and glucose. These sugars can then be converted into valuable bioproducts including xylitol and ethanol. Xylitol is a natural, low-calorie sweetener with dental and metabolic health benefits, while ethanol serves as a renewable biofuel and a versatile platform chemical in industry. Conventional chemical synthesis of these compounds is energy-intensive and costly, underscoring the importance of developing efficient microbial fermentation pathways (2).

Several pretreatment methods have been explored. Acid pretreatment is effective at hemicellulose depolymerization but risks generating inhibitory compounds like furfural and hydroxymethylfurfural (HMF) detrimental to fermentation. Alkali pretreatment excels at removing lignin and increasing cellulose accessibility. Enzymatic hydrolysis offers high specificity and mild conditions, producing higher yields of fermentable sugars without generating toxic by-products. Despite progress, integrated approaches combining chemical pretreatment with enzymatic saccharification and comprehensive evaluation of both hydrolysate and residual fractions for microbial fermentation remain underutilized and warrant further research (6, 7).

This study aims to evaluate Sugarcane Bagasse, Wheat Straw, and Corn Cob as lignocellulosic feedstocks for the integrated production of Xylitol and Ethanol. Acid and Alkali hydrolysis, and Enzymatic treatment strategies are compared to maximize xylose and glucose recovery, followed by fermentation using *Kluyveromyces* spp. for pentose sugars and *Saccharomyces cerevisiae* for hexose sugars. The study further investigates the effects of pH and substrate type on fermentation efficiency, product yield, and volumetric productivity. By combining optimized pretreatment and microbial fermentation, this work seeks to enhance the bioconversion of agricultural residues into value-added bio-products, supporting sustainable biomass utilization.

## 2. Materials and Methods

### 2.1. Raw Materials and Sample Preparation

The agricultural residues including Sugarcane Bagasse, Wheat Straw, and Corn cob were collected from local farms in Faisalabad, Pakistan, and from industrial sources such as Rafhan Maize Products (Pvt.) Ltd. for corn cob and Chanar Sugar Mills (Pvt.) Ltd. for sugarcane bagasse. The samples were carefully washed with tap water to remove soil and dust, then oven-dried at 55 °C for 24 hours until reaching a constant weight. The dried biomass was milled to a particle size of 2–5 mm using a Wiley mill, then stored in airtight polyethylene bags under dry conditions to maintain stability until further use. This standard preparation method ensures sample uniformity and optimal processing for subsequent pretreatment and fermentation steps, as consistent particle size and moisture content are critical to pretreatment efficacy and enzymatic saccharification (8, 9).

### 2.2 Acid Pretreatment

Acid hydrolysis was performed to depolymerize hemicellulose and release monomeric sugars efficiently. For each biomass type (200 g dry weight), 1% (v/v) sulfuric acid ($H_2SO_4$), prepared from 72% concentrated sulfuric acid, was used at a solid-to-liquid ratio of 1:5 (w/v) in 2 L Erlenmeyer flasks. The mixture was autoclaved at 121 °C for 90 minutes to promote hydrolysis of polysaccharides. After cooling, the slurry was filtered through double-layered muslin cloth to separate the hydrolysate liquor from the solid residue. The hydrolysate pH was adjusted to 5.5–6.0 using calcium hydroxide ($Ca(OH)_2$), and the precipitated calcium sulfate was removed by vacuum filtration. To remove colored inhibitors, the clarified liquor was treated with 1% (w/v) activated charcoal and filtered through Whatman No. 1 filter paper before use in fermentation (10, 11).

### 2.3 Alkali Pretreatment

For Alkali treatment, 200 g of biomass was treated with 2–4% (w/v) sodium hydroxide (NaOH) at a solid-to-liquid ratio of 1:5 (w/v) in 2 L Erlenmeyer flasks. The mixture was autoclaved at 121 °C for 90 minutes. After cooling, the pH was adjusted to 6.0–6.5 using concentrated hydrochloric acid (HCl), and lignin precipitates were removed by filtration. Both the liquor and residual solids were thoroughly washed with distilled water until neutral pH was achieved and then stored at 4 °C for subsequent saccharification. This alkali pretreatment disrupts lignin-carbohydrate complexes, solubilizes lignin, swells biomass particles, and reduces cellulose crystallinity, thereby enhancing enzymatic digestibility (12-14).

### 2.4 Enzymatic Saccharification

Enzymatic hydrolysis was performed on acid- and alkali-pretreated liquor and residual solids to maximize sugar recovery. Commercial cellulase (≥10 FPU/mL) and recombinant xylanase from Pichia pastoris (≥20 U/mL) were procured from the Industrial Biotechnology Division, NIBGE, Faisalabad. The enzymatic saccharification was carried out in 250 mL Erlenmeyer flasks containing 10 g (dry weight equivalent) of pretreated biomass or liquor, 25 mL of 0.1 M citrate buffer (pH 5.0), and the enzyme solution. Flasks were incubated in a shaking incubator at 50 °C and 120 rpm for 24 hours. Samples were collected every 6 hours, and reducing sugar concentrations were quantified using the DNS (3,5-dinitrosalicylic acid) assay, which is based on the reduction of DNS reagent by reducing sugars to form a colored complex measurable at 540 nm. Enzymatic reactions were halted by heating at 95 °C for 5 minutes, followed by centrifugation at 10,000 × g for 10 minutes to separate unhydrolyzed solids (15).

### 2.5 Microorganisms and Inoculum Development

*Kluyveromyces* spp. (pentose-fermenting yeast) and *Saccharomyces cerevisiae* (hexose-fermenting yeast) were obtained from the culture collection of the Industrial Biotechnology Laboratory, NIBGE, Faisalabad. Strains were maintained on yeast extract peptone dextrose (YPD) agar slants at 4 °C and sub-cultured monthly. For inoculum preparation, single colonies were transferred to 500 mL Erlenmeyer flasks containing 200 mL YPD broth supplemented with 0.5 % $(NH_4)_2SO_4$, 0.05 % $MgSO_4 \cdot 7H_2O$, 0.01 % $CaCl_2 \cdot 2H_2O$, 0.1 % $KH_2PO_4$, and 3 % D-xylose. Cultures were incubated at 37 °C with agitation (120 rpm) for 12 h until reaching an $OD_{600}$ of 1.3–1.5 ($10^6$ cells/mL). The inoculum was centrifuged (5,000 × g, 5 min), washed twice with sterile saline, and resuspended in sterile distilled water for use in fermentation experiments (16, 17).

### 2.6 Fermentation Setup

Fermentation experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of hydrolysate liquor or resuspended residue. Flasks were inoculated with 10% (v/v) yeast inoculum, resulting in a final concentration of $10^5$ to $10^6$ cells/mL, and incubated at 30 °C with shaking at 120 rpm for 72 hours. The experiments were performed in duplicate for each condition and repeated twice to ensure reproducibility. Samples of 5 mL were collected at 0, 4, 8, 12, 24, 48, and 72 hours for analysis of sugars and fermentation products. Throughout the experiment, pH was monitored and maintained between 5.5 and 6.0 using 0.1 M phosphate buffer when necessary. This incubation regime aligns with typical yeast fermentation protocols for lignocellulosic hydrolysates, balancing optimal yeast growth and product formation while managing stresses from potential inhibitory compounds present in pretreated biomass hydrolysates (18-20) .

### 2.7 Analytical Methods

Sugar analysis was performed using two methods. Reducing sugars were quantified using the DNS (3,5-dinitrosalicylic acid) method, a widely used spectrophotometric assay that measures the presence of reducing sugars by their ability to reduce DNS reagent into a colored complex detectable at 540 nm. Although the DNS method is simple and sensitive, it may be influenced by the presence of sugar degradation products and buffer components, requiring careful calibration and controls for accurate measurements (21, 22).

Monomeric sugars including xylose, glucose, and cellobiose were quantified by high-performance liquid chromatography (HPLC) using a PerkinElmer system equipped with a Bio-Rad Aminex HPX-87H column and guard column. The mobile phase consisted of 0.001 N sulfuric acid at a flow rate of 0.6 mL/min, and the column temperature was maintained between 65–75 °C. Detection was carried out with a diode-array detector at 210 nm. Calibration curves for sugars and fermentation products such as xylitol, ethanol, glycerol, and acetic acid demonstrated excellent linearity ($R^2 \geq 0.99$), ensuring precise quantification.

Fermentation parameters calculated included:

**Xylitol/Ethanol yield (%) = (product formed / theoretical yield) × 100**

**Volumetric productivity (Qp) = product concentration / fermentation time (g/L/h)**

**Specific sugar consumption rate (Qs) = sugar consumed per unit cell mass per hour**

These methodologies enable accurate tracking of sugar consumption and product formation vital for evaluating fermentation efficiency (21, 22).

### 2.8 Statistical Analysis

All experiments were performed in duplicate, and mean values ± standard deviation (SD) were reported. Statistical analysis was performed using one-way ANOVA, and differences with $p < 0.05$ were considered statistically significant.

## 3. Results and Discussion

### 3.1 Carbohydrate Recovery from Pretreated Biomass

Acid and alkali pretreatments released substantial amounts of fermentable sugars from all three biomass types. Sugarcane bagasse showed the highest xylose concentrations in both treatments, reaching 34.10 g/L with alkali hydrolysis compared with 33.21 g/L under acid hydrolysis (Table 1). Glucose was the second most abundant sugar fraction, with the highest value (6.19 g/L) recorded for alkali-treated sugarcane bagasse liquor. Alkali pretreatment consistently released slightly more sugars than acid pretreatment, suggesting more effective delignification and cellulose accessibility.

**Table 1:** Carbohydrate composition (g/L) of acid- and alkali-hydrolyzed biomass liquors.

| Substrate | Treatment | Xylose (g/L) | Glucose (g/L) | Cellobiose (g/L) |
|---|---|---|---|---|
| **Sugarcane bagasse** | Acid | 33.213 | 5.71 | 0.748 |
| | Alkali | 34.105 | 6.19 | 0.550 |
| **Wheat straw** | Acid | 25.183 | 1.98 | 0.458 |
| | Alkali | 26.890 | 2.79 | 0.810 |
| **Corn cob** | Acid | 24.339 | 2.43 | 0.414 |
| | Alkali | 25.870 | 2.61 | 0.706 |

### 3.2 Effect of Enzymatic Saccharification

Enzymatic saccharification with cellulase and xylanase further enhanced sugar release from pretreated biomass. The highest xylose concentration (36.27 g/L) was observed in alkali-enzyme-treated sugarcane bagasse liquor, an improvement of ~6 % compared with alkali pretreatment alone. Glucose concentration in the residue also increased to 6.21 g/L after alkali-enzyme treatment (Figure 1 (A-D)).

As shown in Figure 1A-B (top left to right), both acid and alkali hydrolysis released considerable amounts of xylose and glucose from Sugarcane Bagasse, Wheat Straw, and Corn cob. Alkali treatment consistently resulted in slightly higher sugar concentrations compared

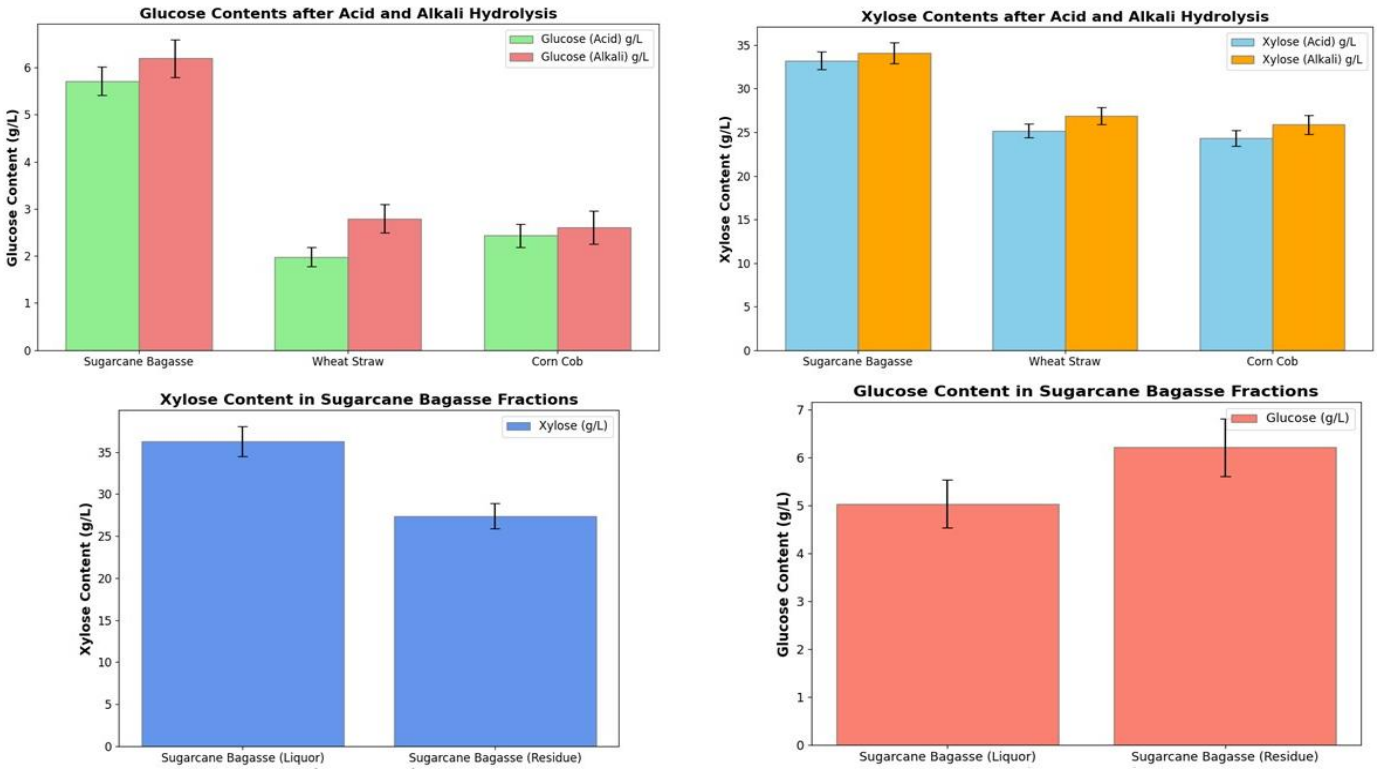


Figure 1. Carbohydrate contents in acid-, alkali-, and alkali + enzyme-treated biomass

with acid treatment, with sugarcane bagasse liquor showing the highest xylose (34.10 g/L) and glucose (6.19 g/L) contents among all tested biomasses. Wheat straw and corn cob yielded lower sugar concentrations, with xylose ranging from 24.34–26.89 g/L and glucose from 1.98–2.79 g/L.

The impact of Enzymatic Saccharification on sugar recovery from sugarcane bagasse is illustrated in Figure 1C-D (bottom left to right). Combined alkali–enzyme treatment further enhanced xylose release in the liquor fraction (36.27 g/L) and glucose release in the residue fraction (6.21 g/L) compared to chemical treatment alone. These results confirm that integrating chemical pretreatment with enzymatic hydrolysis improves cellulose and hemicellulose digestibility, thereby increasing the availability of fermentable sugars for subsequent microbial fermentation.

### 3.3 Xylitol Production

*Kluyveromyces* fermentation efficiently converted xylose into xylitol. Maximum Xylitol production (28.54 g/L) was achieved with alkali–enzyme-treated sugarcane bagasse liquor, yielding 78.68 % with volumetric productivity of 0.016 g/L/h. Wheat straw alkali–enzyme hydrolysate residue showed the highest xylitol yield of 121.29 % at pH 6 **(Table 2).** Small amounts of glycerol and acetic acid were detected as secondary metabolites.

### 3.4 Ethanol Production

Ethanol production was significantly higher in *S. cerevisiae* fermentations than in *Kluyveromyces*. The maximum ethanol titer (4.26 g/L) was obtained from sugarcane bagasse alkali-enzyme residue, with a yield of 69.40 % and productivity of 0.236 g/L/h. Wheat straw and corn cob hydrolysates produced lower ethanol levels (<1.3 g/L).

**Table 2:** Xylitol and ethanol production from alkali + enzyme hydrolysates after 72 h fermentation.

| Substrate & Fraction | pH | Yeast | Xylose (g/L) | Xylitol (g/L) | Xylitol Yield (%) | Ethanol (g/L) | Ethanol Yield (%) |
|---|---|---|---|---|---|---|---|
| **Sugarcane bagasse liquor** | 6 | *Kluyveromyces* | 36.27 | **28.54** | 78.68 | – | – |
| **Sugarcane bagasse residue** | 6 | *S. cerevisiae* | 27.39 | 20.10 | 73.38 | **4.26** | 69.40 |
| **Wheat straw residue** | 6 | *Kluyveromyces* | 20.27 | 24.59 | **121.29** | 0.81 | 36.74 |
| **Corn cob liquor** | 6 | *Kluyveromyces* | 26.11 | 3.38 | 12.95 | 0.18 | 53.82 |

### 3.5 Comparative Substrate Performance

Overall, sugarcane bagasse demonstrated the highest sugar release, Xylitol production, and ethanol yield, confirming its suitability as a preferred feedstock for biotechnological conversion. Wheat straw, while lower in total sugar content, exhibited the highest xylitol yield at optimized pH, indicating its potential for targeted xylitol production processes. Corn cob performed comparatively poorly, suggesting that detoxification or nutrient supplementation may be necessary to improve its fermentability.

### 3.6 HPLC Analysis

Representative HPLC chromatograms confirmed the presence of Xylose, Glucose, Xylitol, Ethanol, Glycerol, and Acetic acid in the fermentation broth (Figures 2A and 2B). Minimal peaks for furfural and HMF were observed, suggesting that pH adjustment and charcoal treatment effectively reduced fermentation inhibitors.

## 4. Discussion

The present study demonstrates that integrating chemical pretreatment with enzymatic saccharification significantly improves fermentable sugar recovery from lignocellulosic biomass, leading to enhanced production of xylitol and ethanol through microbial fermentation. Among the three biomass types tested, sugarcane bagasse consistently outperformed wheat straw and corn cob in terms of xylose and glucose release, as well as final product yields, confirming its potential as a preferred feedstock for biorefinery applications.

### 4.1 Carbohydrate Recovery and Pretreatment Efficiency

The slightly higher sugar yields observed after Alkali treatment compared with Acid hydrolysis suggest more efficient delignification and improved cellulose accessibility, which aligns with previous reports demonstrating that Alkali pretreatment selectively removes lignin and acetyl groups, thereby enhancing enzymatic digestibility of hemicellulose (23, 24). Alkali pretreatment effectively disrupts lignin-carbohydrate complexes and swells cellulose fibers, reducing crystallinity and increasing

surface area for enzyme action. This method also operates under milder conditions than acid pretreatment, minimizing sugar degradation to inhibitory compounds such as furfural and HMF, and is more economical due to simpler processing and alkali reuse (24, 25). The improvement in sugar release following enzymatic saccharification (up to a 6% increase in xylose concentration) highlights the importance of combining chemical and biological approaches for maximizing sugar recovery. Integrated systems leveraging chemical delignification followed by enzymatic depolymerization synergistically enhance fermentable sugar availability, as documented in numerous studies (25, 26). These findings reinforce the efficacy of alkali pretreatment coupled with enzymatic hydrolysis as a promising strategy for efficient bioconversion of lignocellulosic biomass.

### 4.2 Xylitol Fermentation

The ability of *Kluyveromyces* spp. to convert xylose to Xylitol with high yield (up to 121.29% in wheat straw hydrolysate) confirms its metabolic efficiency and tolerance to complex lignocellulosic substrates. This exceptional yield likely reflects efficient pentose metabolism and reduced inhibition by lignocellulosic hydrolysate components. The slightly higher Xylitol yield observed at pH 6 compared with pH 7 suggests optimal activity of xylose reductase, the key enzyme catalyzing xylose reduction to Xylitol, under mildly acidic conditions. This is consistent with prior studies demonstrating maximal xylose reductase activity and xylitol production in *Kluyveromyces* species within a similar pH range (27, 28).

Although Wheat Straw hydrolysate had relatively lower total sugar content, its higher relative proportion of pentose sugars contributed to the highest xylitol yield, emphasizing the importance of substrate composition in targeted xylitol production. This finding is particularly significant for developing processes tailored to maximize high-purity xylitol production, which is valued in pharmaceutical and food industries for its low-calorie sweetening properties and dental benefits (27). These results emphasize *Kluyveromyces* spp. as a promising microbial platform for sustainable biotechnological production of xylitol from lignocellulosic biomass hydrolysates.

### 4.3 Ethanol Fermentation

Ethanol production was highest in *Saccharomyces cerevisiae*-fermented sugarcane bagasse residue, yielding 4.26 g/L with a conversion efficiency of 69.40%. This aligns with previous studies showing that *S. cerevisiae* preferentially ferments glucose over xylose, making sugarcane bagasse characterized by relatively higher glucose content, a more suitable substrate for ethanol production (29, 30). The lower ethanol titers in wheat straw and corn cob hydrolysates are likely due to their lower initial glucose levels and the presence of inhibitory compounds such as phenolics and furfurals, which may persist despite detoxification efforts (31, 32). To enhance ethanol yields from mixed sugar hydrolysates, future research should focus on adaptive evolution or genetic engineering of yeast strains capable of efficient co-fermentation of pentoses and hexoses, overcoming current substrate limitations (29, 31). This evidence highlights the potential of S. cerevisiae for bioethanol production from sugarcane bagasse while pointing to challenges inherent in fermenting substrates with diverse sugar profiles.

### 4.4 Substrate Comparisons and Industrial Relevance

Sugarcane bagasse emerged as the most promising feedstock for combined Xylitol and ethanol production, offering high sugar content, better fermentability, and superior product yields. Its suitability is supported by studies demonstrating efficient xylose and glucose utilization in sugarcane bagasse hydrolysates, resulting in high xylitol and ethanol yields, making it an attractive option for biorefineries focused on valorizing agricultural residues (33, 34). Wheat straw, despite having lower sugar content compared to bagasse, is more suitable for targeted Xylitol production due to its relatively higher pentose sugar content. This suggests the feasibility of a two-stage bioprocess where pentoses from wheat straw hydrolysate are first converted to Xylitol, while hexoses are subsequently fermented to ethanol, supporting process optimization and product specificity (35).

Corn cob, which demonstrated comparatively poorer performance, may require additional detoxification methods such as over liming or ion exchange and/or nutrient supplementation to improve fermentation outcomes, addressing challenges related to fermentation inhibitors present in this substrate (36).

These comparisons highlight the importance of substrate selection and tailored pretreatment/fermentation strategies for industrial bioprocesses aimed at efficient and sustainable production of biofuels and biochemicals.

### 4.5 Process Implications

The integration of acid/alkali pretreatment with enzymatic hydrolysis represents a scalable and sustainable approach for biomass valorization. This method combines chemical disruption of lignin and hemicellulose with biological depolymerization of cellulose and hemicellulose to optimize sugar release. The relatively high xylitol and ethanol titers obtained in this study

demonstrate the feasibility of co-producing value-added chemicals and biofuels from agricultural residues, supporting circular bioeconomy initiatives by converting waste biomass into valuable commodities (37, 38). Further process optimization, including fed-batch fermentation, scale-up studies, and techno-economic analysis, is recommended to assess industrial viability and economic sustainability. The use of genetically engineered microbial strains with enhanced inhibitor tolerance and co-fermentation capabilities can further improve process efficiency and reduce production costs, making biomass conversion more competitive and attractive for large-scale implementation (39, 40). Overall, the combination of chemical pretreatment and enzymatic hydrolysis forms the foundation for efficient biorefineries, supporting sustainable biofuel and biochemical production while contributing to environmental conservation goals. This study demonstrates several strengths, including the use of abundantly available, low-cost agricultural residues (sugarcane bagasse, wheat straw, and corn cob) and the successful integration of alkali pretreatment with enzymatic saccharification, which significantly enhanced fermentable sugar recovery. The dual production of xylitol and ethanol illustrates a viable biorefinery approach, while the high xylitol yields (up to 121.29 %) and relatively high titers achieved under laboratory conditions highlight the efficiency of the process. Furthermore, effective detoxification minimized fermentation inhibitors, improving microbial performance. However, certain limitations must be acknowledged: ethanol titers remained comparatively low relative to industrial benchmarks, and experiments were conducted only at a small laboratory scale, leaving process scalability and economic feasibility unaddressed. Additionally, the study focused on only two microbial strains and did not fully overcome the poor fermentability of corn cob hydrolysates. Future work should include strain engineering, fed-batch or continuous fermentation strategies, and techno-economic analysis to assess industrial applicability.

## 4. Conclusion

The integrated approach of alkali pretreatment and enzymatic saccharification applied to sugarcane bagasse, wheat straw, and corn cob effectively enhanced fermentable sugar release, enabling efficient microbial conversion to xylitol and ethanol. Sugarcane bagasse proved to be the most suitable feedstock for dual-product bioprocessing due to its higher fermentable sugar content and ethanol yield, whereas wheat straw's pentose-rich profile favored xylitol production at exceptionally high yields (up to 121.29 %). Fermentation conditions, particularly pH, and substrate composition were pivotal in achieving optimal yields and productivity. The effective combination of pretreatment strategies and detoxification protocols minimized the impact of inhibitors, confirming process feasibility and robustness. This study highlights the potential of integrating chemical and enzymatic saccharification as a scalable strategy for the valorization of lignocellulosic residues. Although promising, challenges remain in scaling up the process, improving ethanol titers to industrially relevant levels, and enhancing the fermentability of corn cob hydrolysates. Future work should focus on process intensification, fed-batch or continuous fermentation strategies, and metabolic engineering of yeast strains for simultaneous pentose-hexose co-fermentation. Comprehensive techno-economic and life-cycle analyses are also required to evaluate the industrial viability and environmental benefits. Overall, this research provides a strong foundation for developing integrated biorefineries that transform agricultural waste into commercially valuable products, supporting circular economy initiatives and the transition to a sustainable bioeconomy.

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**Data Availability Statement:** The data supporting the findings of this study are available in the Lab manual at Department of Biosciences, Grand Asian University Sialkot, Pakistan; and Department of Chemistry, University of Baltistan Skardu, Gilgit-Baltistan.

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**Conflicts of Interest:** Authors declare there is no conflict of interest.

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