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# Integrated Alkali–Enzyme Pretreatment Enhances Xylitol and Ethanol Production from Agricultural Residues Using *Kluyveromyces spp.* and *Saccharomyces cerevisiae*

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## Abstract

Lignocellulosic agricultural residues are abundant, underused feedstocks whose open burning contributes to environmental pollution. This study evaluated sugarcane bagasse, wheat straw, and corn cob for integrated xylitol and ethanol production. Biomass was subjected to acid or alkali pretreatment followed by enzymatic saccharification, and the resulting liquor and residual fractions were fermented with *Kluyveromyces spp.* and *Saccharomyces cerevisiae*. Alkali–enzyme-treated sugarcane bagasse liquor produced the highest xylose concentration (36.27 g/L), whereas its residue contained 6.21 g/L glucose. *Kluyveromyces spp.* produced 28.54 g/L xylitol from sugarcane bagasse liquor, corresponding to a reported yield of 78.68% and volumetric productivity of 0.016 g/L/h. *S. cerevisiae* produced 4.26 g/L ethanol from the bagasse residue, with a reported yield of 69.40% and productivity of 0.236 g/L/h. Wheat straw hydrolysate showed the highest reported apparent xylitol yield (121.29%) at pH 6, indicating a need to verify the yield basis while confirming the strong influence of substrate composition and pH. Overall, combining alkali pretreatment with enzymatic saccharification improved fermentable-sugar recovery and supported dual-product valorization of agricultural residues. Sugarcane bagasse was the most promising feedstock for integrated xylitol and ethanol production, although scale-up, inhibitor control, and techno-economic evaluation remain necessary.

**Keywords:** Lignocellulosic biomass; sugarcane bagasse; xylitol; ethanol; alkali pretreatment; enzymatic saccharification; yeast fermentation

## 1. Introduction

Lignocellulosic biomass is the most abundant renewable carbon source on Earth, accounting for nearly 57–60% of global biogenic carbon and representing approximately 181.5 billion tonnes of biomass annually, of which only a small fraction is currently utilized [1,2]. Major agricultural residues such as wheat straw, sugarcane bagasse, and corn cob are produced in large quantities in agricultural economies, including Pakistan, where substantial amounts are discarded or openly burned, contributing to environmental pollution and greenhouse-gas emissions [3].

Efficient utilization of lignocellulosic agricultural residues is important for advancing sustainable bioeconomy goals and mitigating climate change. Pakistan's residues, including wheat straw, rice husks, corn stalks, and sugarcane bagasse, have potential for renewable fuels and platform products that can reduce dependence on fossil resources [4]. Integrated biorefineries can convert these residues into biofuels, bioplastics, and other value-added bioproducts, supporting a circular bioeconomy while reducing waste [5].

Lignocellulosic biomass consists principally of cellulose, hemicellulose, and lignin, which form a recalcitrant structure resistant to microbial and enzymatic degradation [6,7]. Cellulose generally represents 40–50% of the biomass, hemicellulose 20–30%, and lignin 10–25%. Pretreatment is therefore required to disrupt lignin–carbohydrate associations, increase polysaccharide accessibility, and release fermentable sugars such as xylose and glucose. These sugars can be converted into xylitol, a low-calorie sweetener, and ethanol, a renewable fuel and industrial platform chemical [2].

Acid pretreatment efficiently depolymerizes hemicellulose but can generate fermentation inhibitors, including furfural and hydroxymethylfurfural. Alkali pretreatment removes lignin and increases cellulose accessibility, whereas enzymatic hydrolysis provides high specificity under mild conditions. Nevertheless, integrated evaluation of chemical pretreatment, enzymatic saccharification, and fermentation of both hydrolysate and residual fractions remains comparatively limited [6,7].

This study evaluated sugarcane bagasse, wheat straw, and corn cob as lignocellulosic feedstocks for integrated xylitol and ethanol production. Acid and alkali pretreatments, followed by enzymatic saccharification, were compared for xylose and glucose recovery; *Kluyveromyces spp.* and *Saccharomyces cerevisiae* were then used for conversion of pentose- and hexose-rich fractions, respectively. The effects of substrate type and pH on product concentration, reported yield, and volumetric productivity were also examined.

## 2. Materials and Methods

### 2.1 Raw Materials and Sample Preparation

Agricultural residues, including sugarcane bagasse, wheat straw, and corn cob, were collected from local farms in Faisalabad, Pakistan, and from industrial sources, including Rafhan Maize Products (Pvt.) Ltd. for corn cob and Chanar Sugar Mills (Pvt.) Ltd. for sugarcane bagasse. Samples were washed with tap water, oven-dried at 55 °C for 24 h to constant mass, milled to a particle size of 2–5 mm using a Wiley mill, and stored in airtight polyethylene bags under dry conditions until use [8,9].

### 2.2 Acid Pretreatment

For each biomass type, 200 g dry weight was treated with 1% (v/v) sulfuric acid ($H_2SO_4$), prepared from 72% concentrated sulfuric acid, 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 min. 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 [$Ca(OH)_2$], precipitated calcium sulfate was removed by vacuum filtration, and the clarified liquor was treated with 1% (w/v) activated charcoal before filtration through Whatman No. 1 filter paper [10,11].

### 2.3 Alkali Pretreatment

For alkali pretreatment, 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 and autoclaved at 121 °C for 90 min. After cooling, the pH was adjusted to 6.0–6.5 using concentrated hydrochloric acid, and lignin precipitates were removed by filtration. The liquor and residual solids were washed with distilled water to neutral pH and stored at 4 °C for subsequent saccharification [12–14].

### 2.4 Enzymatic Saccharification

Enzymatic hydrolysis was performed on acid- and alkali-pretreated liquor and residual solids. Commercial cellulase (≥10 FPU/mL) and recombinant xylanase from *Pichia pastoris* (≥20 U/mL) were obtained from the Industrial Biotechnology Division, NIBGE, Faisalabad. Saccharification was conducted 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 at 50 °C and 120 rpm for 24 h. Samples were collected every 6 h, and reducing sugars were quantified using the 3,5-dinitrosalicylic acid (DNS) assay at 540 nm. Reactions were stopped by heating at 95 °C for 5 min, followed by centrifugation at 10,000 × g for 10 min [15].

### 2.5 Microorganisms and Inoculum Development

*Kluyveromyces spp.* and *Saccharomyces cerevisiae* were obtained from the culture collection of the Industrial Biotechnology Laboratory, NIBGE, Faisalabad. Strains were maintained on yeast extract–peptone–dextrose agar slants at 4 °C and subcultured monthly. Single colonies were transferred to 500 mL Erlenmeyer flasks containing 200 mL 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 and 120 rpm for 12 h to an $OD_{600}$ of 1.3–1.5 (approximately $10^6$ cells/mL), centrifuged at 5,000 × g for 5 min, washed twice with sterile saline, and resuspended in sterile distilled water [16,17].

### 2.6 Fermentation Setup

Fermentation was conducted in 250 mL Erlenmeyer flasks containing 100 mL hydrolysate liquor or resuspended residue. Flasks were inoculated with 10% (v/v) yeast inoculum to a final concentration of $10^5$–$10^6$ cells/mL and incubated at 30 °C and 120 rpm for 72 h. Each condition was tested in duplicate and the experiment was repeated twice. Samples (5 mL) were collected at 0, 4, 8, 12, 24, 48, and 72 h for analysis. The pH was monitored and maintained at 5.5–6.0 using 0.1 M phosphate buffer when required [18–20].

### 2.7 Analytical Methods

Reducing sugars were quantified using the DNS assay at 540 nm [21,22]. Monomeric sugars, including xylose, glucose, and cellobiose, were quantified by high-performance liquid chromatography using a PerkinElmer system equipped with a Bio-Rad Aminex HPX-87H column and guard column. The mobile phase was 0.001 N sulfuric acid at 0.6 mL/min, the column temperature was 65–75 °C, and detection was performed at 210 nm. Calibration curves for sugars and fermentation products, including xylitol, ethanol, glycerol, and acetic acid, showed $R^2 \geq 0.99$.

### 2.8 Fermentation Calculations

Xylitol or ethanol yield (%) was calculated as (product formed/theoretical yield) × 100. Volumetric productivity (Qp) was calculated as product concentration divided by fermentation time (g/L/h). Specific sugar consumption rate (Qs) was defined as sugar consumed per unit cell mass per hour.

### 2.9 Statistical Analysis

Mean values and standard deviations were reported for duplicate experiments. One-way analysis of variance was used, and $p < 0.05$ was considered statistically significant. The statistical software and version were not specified in the source manuscript and should be confirmed before publication.

## 3. Results

### 3.1 Carbohydrate Recovery from Pretreated Biomass

Acid and alkali pretreatments released fermentable sugars from all three biomass types. Sugarcane bagasse produced the highest xylose concentrations, reaching 34.10 g/L after alkali hydrolysis and 33.21 g/L after acid hydrolysis (Table 1). The highest glucose concentration (6.19 g/L) was also obtained from alkali-treated

sugarcane bagasse liquor. Across the tested biomasses, alkali pretreatment produced slightly higher sugar concentrations than acid pretreatment.

**Table 1.** Carbohydrate composition 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 |
| **Sugarcane bagasse** | Alkali | 34.105 | 6.19 | 0.550 |
| **Wheat straw** | Acid | 25.183 | 1.98 | 0.458 |
| **Wheat straw** | Alkali | 26.890 | 2.79 | 0.810 |
| **Corn cob** | Acid | 24.339 | 2.43 | 0.414 |
| **Corn cob** | Alkali | 25.870 | 2.61 | 0.706 |

## 3.2 Effect of Enzymatic Saccharification

Enzymatic saccharification with cellulase and xylanase further increased sugar release from pretreated biomass. Alkali–enzyme-treated sugarcane bagasse liquor contained 36.27 g/L xylose, approximately 6% more than after alkali pretreatment alone, whereas the corresponding residue contained 6.21 g/L glucose. Figure 1 compares glucose and xylose concentrations after acid and alkali hydrolysis and shows the additional sugar release obtained from sugarcane bagasse fractions after enzymatic treatment.

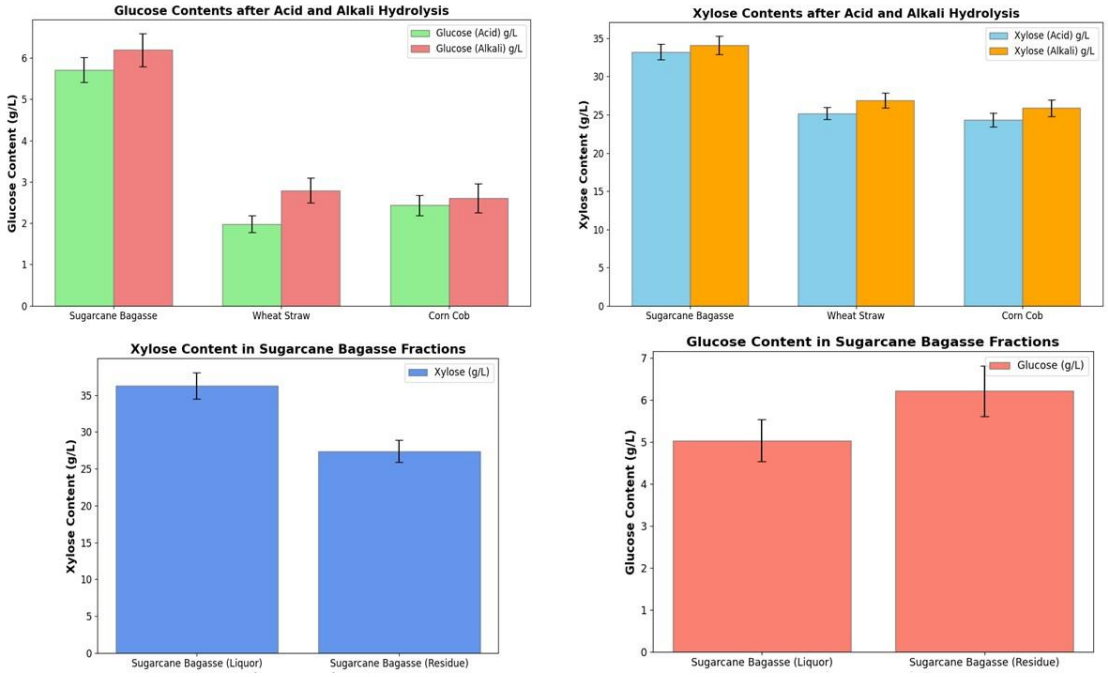


**Figure 1.** Carbohydrate concentrations after acid and alkali hydrolysis of sugarcane bagasse, wheat straw, and corn cob, and after combined alkali–enzyme treatment of sugarcane bagasse liquor and residue.

## 3.3 Xylitol Production

*Kluyveromyces spp.* converted xylose to xylitol in the tested hydrolysates. The highest xylitol concentration (28.54 g/L) was obtained from alkali–enzyme-treated sugarcane bagasse liquor, with a reported yield of 78.68% and volumetric productivity of 0.016 g/L/h. Wheat straw residue produced 24.59 g/L xylitol and a reported apparent yield of 121.29% at pH 6 (Table 2). Small quantities of glycerol and acetic acid were detected as secondary metabolites.

## 3.4 Ethanol Production

Ethanol production was higher in *Saccharomyces cerevisiae* fermentations than in *Kluyveromyces spp.* fermentations. The highest ethanol concentration (4.26 g/L) was obtained from the alkali–enzyme-treated sugarcane bagasse residue, with a reported yield of 69.40% and productivity of 0.236 g/L/h. Wheat straw and corn cob hydrolysates produced less than 1.3 g/L ethanol.

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

| Substrate and fraction | pH | Yeast | Xylose (g/L) | Xylitol (g/L) | Xylitol yield (%) | Ethanol (g/L) | Ethanol yield (%) |
|---|---|---|---|---|---|---|---|
| **Sugarcane bagasse liquor** | 6 | *Kluyveromyces spp.* | 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 spp.* | 20.27 | 24.59 | 121.29 | 0.81 | 36.74 |
| **Corn cob liquor** | 6 | *Kluyveromyces spp.* | 26.11 | 3.38 | 12.95 | 0.18 | 53.82 |

*Note: Values are reproduced from the source manuscript. Because the reported xylitol yield for wheat straw exceeds 100%, the authors should verify the calculation basis, substrate-consumption denominator, and analytical measurements before publication.*

### 3.5 Comparative Substrate Performance

Sugarcane bagasse showed the highest overall sugar release, xylitol concentration, and ethanol production, supporting its use as the preferred feedstock for dual-product conversion. Wheat straw contained less total sugar but produced the highest reported apparent xylitol yield at pH 6. Corn cob showed comparatively low product formation, indicating that additional detoxification or nutrient supplementation may be required.

### 3.6 HPLC Analysis

High-performance liquid chromatography indicated the presence of xylose, glucose, xylitol, ethanol, glycerol, and acetic acid in the fermentation broth. Only small furfural and hydroxymethylfurfural peaks were reported, suggesting that pH adjustment and activated-charcoal treatment reduced the concentration of fermentation inhibitors. The chromatogram images referenced in the source manuscript were not embedded in the uploaded file.

## 4. Discussion

The findings show that chemical pretreatment followed by enzymatic saccharification improved fermentable-sugar recovery and enabled microbial production of xylitol and ethanol. Sugarcane bagasse consistently produced the highest xylose and glucose concentrations and the highest ethanol titer, whereas wheat straw was more favorable for xylitol production on the basis of the reported yield values.

### 4.1 Carbohydrate Recovery and Pretreatment Efficiency

The slightly higher sugar concentrations after alkali treatment than after acid hydrolysis are consistent with more effective delignification and improved cellulose accessibility [23,24]. Alkali pretreatment disrupts lignin–carbohydrate complexes, swells cellulose fibers, reduces crystallinity, and increases the surface area available for enzymatic action. It may also reduce sugar degradation to furfural and hydroxymethylfurfural relative to severe acid conditions [24,25]. The approximately 6% increase in xylose concentration after enzymatic saccharification further supports the use of sequential chemical and enzymatic processing [25,26].

### 4.2 Xylitol Fermentation

*Kluyveromyces spp.* produced 28.54 g/L xylitol from sugarcane bagasse liquor, supporting its potential for conversion of xylose-rich lignocellulosic hydrolysates [27,28]. Wheat straw produced 24.59 g/L xylitol at pH 6; however, the corresponding reported yield of 121.29% is greater than the theoretical maximum implied by the stated calculation. This value should therefore be treated as an apparent yield until the denominator, sugar-consumption data, and analytical calculations are verified. Subject to that verification, the results suggest that pentose-rich wheat straw fractions may be suitable for targeted xylitol production.

### 4.3 Ethanol Fermentation

*Saccharomyces cerevisiae* produced the highest ethanol concentration from sugarcane bagasse residue (4.26 g/L; reported yield 69.40%). This agrees with the preference of conventional *S. cerevisiae* strains for glucose-rich substrates [29,30]. Lower ethanol production from wheat straw and corn cob may reflect their lower

glucose concentrations and residual inhibitory compounds [31,32]. Future work should evaluate inhibitor-tolerant or engineered strains capable of efficient pentose–hexose co-fermentation [29,31].

### 4.4 Substrate Selection and Industrial Relevance

Sugarcane bagasse was the most promising feedstock for combined xylitol and ethanol production because of its higher fermentable-sugar concentrations and product titers [33,34]. Wheat straw may be more appropriate for processes focused on xylitol, potentially within a two-stage biorefinery in which pentoses are converted to xylitol and hexoses are subsequently fermented to ethanol [35]. Corn cob may require additional detoxification, ion-exchange treatment, overliming, or nutrient supplementation to improve fermentation performance [36].

### 4.5 Strengths, Limitations, and Future Work

The study integrates low-cost agricultural residues, chemical pretreatment, enzymatic saccharification, and microbial fermentation within a dual-product biorefinery concept [37,38]. Its main strengths are the comparison of three feedstocks and the evaluation of both liquor and residual fractions. Important limitations include the small laboratory scale, low ethanol titers relative to industrial targets, use of only two yeast types, absence of complete statistical outputs, and the need to verify the xylitol-yield calculation. In addition, the HPLC chromatograms cited in the source manuscript were not supplied. Future work should include mass-balance validation, inhibitor quantification, strain engineering, fed-batch or continuous fermentation, scale-up, and techno-economic and life-cycle assessment [39,40].

## 5. Conclusions

Sequential alkali pretreatment and enzymatic saccharification increased fermentable-sugar recovery from sugarcane bagasse, wheat straw, and corn cob and supported microbial production of xylitol and ethanol. Sugarcane bagasse was the strongest feedstock for dual-product conversion, yielding 28.54 g/L xylitol from the liquor and 4.26 g/L ethanol from the residue, whereas wheat straw produced 24.59 g/L xylitol at pH 6. The reported 121.29% xylitol yield for wheat straw requires verification before publication. Process intensification, improved strains, complete mass balances, and techno-economic assessment are needed to establish industrial feasibility.

## Declarations

**Author contributions:** Muhammad Noman: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, and writing—original draft preparation. Malik Alamgir: Methodology, validation, formal analysis, and writing—review and editing. Irum Badshah Saleem: Supervision, resources, validation, interpretation of results, and writing—review and editing. Muhammad Noman: Project administration and correspondence. All authors have read and approved the final version of the manuscript.

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**Informed consent statement:** Not applicable.

**Data availability:** Data supporting the findings are retained in the laboratory records of the Department of Biosciences, Grand Asian University Sialkot, Pakistan, and the Department of Chemistry, University of Baltistan, Skardu, Pakistan, and may be requested from the corresponding author.

**Conflicts of interest:** The authors declare no conflict of interest.

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