

Original Article

Sustainable Bioethanol Production from Lignocellulosic Plant Wastes: A Renewable Solution to Energy Challenges

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"Cite this Article" | Received: 06 January 2026; Accepted: 11 May 2026; Published: 23 June 2026.

ABSTRACT

Background: Lignocellulosic agricultural residues are increasingly investigated as renewable feedstocks for second-generation bioethanol production because they support waste valorization and reduce dependence on food-based substrates. Sugarcane bagasse is a cellulose-rich agro-industrial residue, but its conversion into ethanol is restricted by lignin-associated biomass recalcitrance. **Objective:** This study evaluated bioethanol production from sugarcane bagasse using sodium hydroxide pretreatment, enzymatic saccharification, *Saccharomyces cerevisiae* fermentation, and ethanol recovery. **Methods:** Sugarcane bagasse was pretreated with 2.5% sodium hydroxide using autoclave-assisted treatment for 30, 60, and 90 min and microwave-assisted treatment for 10 and 20 min. Pretreated biomass was analyzed for cellulose and lignin content. Enzymatic saccharification was performed using cellulase from *Trichoderma reesei*, followed by batch fermentation using *Saccharomyces cerevisiae*. Ethanol was recovered by distillation and estimated spectrophotometrically using acidified potassium dichromate. **Results:** Autoclave-assisted pretreatment increased cellulose from 58.0% to 68.0% and reduced lignin from 30.7% to 11.9%. Microwave-assisted pretreatment produced the highest cellulose content of 71.0% and lowest lignin content of 8.0%. Saccharification absorbance was higher in the autoclaved sample than in the microwave-treated sample, 1.40 versus 1.20, and ethanol production was 2.0% versus 1.7%, respectively. **Conclusion:** Sugarcane bagasse supported laboratory-scale ethanol production after alkaline pretreatment, but the strongest delignification did not produce the highest ethanol concentration. Optimization of hydrolysis, fermentation, and ethanol yield assessment is required. **Keywords:** Bioethanol, Sugarcane Bagasse, Lignocellulosic Biomass, Alkaline Pretreatment, Saccharification, Fermentation, Renewable Energy.

EDITORIAL INFORMATION

Author Contributions: Concept: TI, ZA; Design: TI, SM; Data Collection: SM, MA, ZM; Analysis: TI, ZA, ZM; Drafting: TI, SM, MA, ZM.**Ethical Approval:** Department of Epidemiology, Universitas Prima, Indonesia.**Informed Consent:** Written informed consent was obtained from all participants**Conflict of Interest:** The authors declare no conflict of interest; **Funding:** No external funding; **Data Availability:** Available from the corresponding author on reasonable request; **Acknowledgments:** N/A.

INTRODUCTION

The increasing global demand for sustainable energy has intensified the search for renewable fuels that can reduce dependence on fossil resources while supporting environmental and economic sustainability. Bioethanol derived from lignocellulosic biomass has gained particular attention because it can be produced from abundant non-food plant residues, supports circular bioeconomy principles, and contributes to the valorization of agricultural and agro-industrial wastes that are often underutilized in developing countries (1,2). Unlike first-generation bioethanol, which depends largely on sugar- and starch-

based crops, second-generation bioethanol uses lignocellulosic feedstocks and therefore offers a more sustainable pathway by reducing competition with food resources and expanding the usable biomass base for renewable fuel production (3,4).

Lignocellulosic biomass is composed mainly of cellulose, hemicellulose, and lignin, and its conversion into ethanol requires the sequential disruption of biomass structure, hydrolysis of carbohydrate polymers into fermentable sugars, microbial fermentation, and ethanol recovery. Cellulose serves as the major source of glucose for fermentation, whereas hemicellulose contributes additional sugars depending on the feedstock and hydrolysis conditions. Lignin, however, forms a complex protective matrix around carbohydrate polymers and limits enzymatic access, thereby reducing saccharification efficiency and overall ethanol yield (5,6). This structural recalcitrance remains one of the central technical barriers in lignocellulosic bioethanol production and makes pretreatment a decisive step in the conversion process (7).

Agricultural residues are among the most promising lignocellulosic feedstocks because they are widely available, renewable, low-cost, and compatible with waste valorization strategies. Sugarcane bagasse is especially important in sugarcane-producing countries because it is generated in large quantities after juice extraction and contains substantial carbohydrate fractions suitable for biochemical conversion. Reviews on lignocellulosic bioethanol consistently identify agricultural residues as practical feedstocks for biofuel production, particularly where integrated biorefinery models can convert waste biomass into ethanol and additional value-added products (8,9). In this context, sugarcane bagasse represents a relevant substrate for laboratory-scale and applied bioethanol research because it links renewable energy production with agro-industrial waste management.

Pretreatment technologies are used to reduce biomass recalcitrance by altering the lignocellulosic architecture, removing or modifying lignin, increasing cellulose accessibility, and improving enzymatic digestibility. Physical, thermal, chemical, physicochemical, and biological pretreatment methods have been investigated, but their efficiency varies according to feedstock type, chemical severity, residence time, energy input, inhibitor formation, and downstream fermentation compatibility (10,11). Alkaline pretreatment is widely used because it can disrupt ester and ether linkages in the lignin-carbohydrate complex, promote swelling of cellulose fibers, and improve enzyme penetration into the biomass structure. Recent reviews have emphasized that pretreatment selection must be evaluated not only by lignin reduction but also by subsequent sugar release, fermentability, process cost, and environmental sustainability (12,13).

Microwave-assisted pretreatment has emerged as an intensification strategy for lignocellulosic biomass processing because it can provide rapid heating, reduce treatment duration, and enhance structural disruption when combined with chemical agents. However, improved cellulose exposure or lignin removal does not always guarantee higher ethanol production, because the final ethanol yield depends on the complete conversion chain, including hydrolysis efficiency, fermentable sugar availability, yeast performance, inhibitor generation, and downstream recovery (14,15). Therefore, comparative evaluation of pretreatment approaches should include both compositional changes and downstream ethanol production rather than relying only on cellulose enrichment or lignin reduction.

Despite extensive literature on lignocellulosic bioethanol, practical laboratory studies remain important for identifying how locally available agricultural residues behave under specific pretreatment, saccharification, and fermentation conditions. Current reviews emphasize that bioethanol production from lignocellulosic biomass still faces challenges related to pretreatment optimization, enzyme efficiency, fermentation performance, process integration, and techno-economic feasibility (16,17). These challenges justify experimental assessment of sugarcane bagasse under alkaline pretreatment conditions, particularly where conventional autoclave-assisted treatment and microwave-assisted treatment may produce different compositional and ethanol outcomes.

The present study evaluated sugarcane bagasse as a lignocellulosic feedstock for bioethanol production using sodium hydroxide pretreatment, enzymatic saccharification, *Saccharomyces cerevisiae*

fermentation, and ethanol recovery. The study compared autoclave-assisted and microwave-assisted alkaline pretreatment in terms of cellulose content, lignin reduction, saccharification response, and ethanol concentration. The objective was to determine whether alkaline pretreatment could improve the compositional suitability of sugarcane bagasse for bioethanol production and whether the pretreatment condition producing the greatest cellulose exposure and lignin reduction also resulted in higher ethanol recovery.

MATERIALS AND METHODS

This laboratory-based experimental study was conducted to evaluate bioethanol production from sugarcane bagasse through a sequential biochemical conversion process consisting of alkaline pretreatment, enzymatic saccharification, yeast fermentation, and ethanol estimation. The experimental design was aligned with the established second-generation bioethanol production pathway in which lignocellulosic biomass is first pretreated to reduce structural recalcitrance, then hydrolyzed to release fermentable sugars, and subsequently fermented to ethanol (3,5,18). Sugarcane bagasse was selected as the substrate because agricultural residues are recognized as sustainable lignocellulosic feedstocks for renewable fuel production and waste valorization (8,9).

Sugarcane bagasse was collected as an agro-industrial lignocellulosic residue, chopped into small pieces, air-dried for 2–3 days, and stored in polyethylene bags before processing. The dried biomass was ground to approximately 2 mm particle size to increase surface area and improve interaction between biomass particles and the pretreatment solution. A 2.5% sodium hydroxide solution was prepared by dissolving 25 g sodium hydroxide in 1000 mL distilled water. For conventional alkaline pretreatment, the ground biomass was soaked in sodium hydroxide solution for 60 min and then autoclaved at 121°C for 30, 60, and 90 min. After pretreatment, the biomass was washed repeatedly with distilled water until neutral pH was achieved, oven-dried, and stored for compositional analysis and subsequent hydrolysis. This procedure was used to assess the effect of increasing autoclave-assisted alkaline treatment duration on cellulose exposure and lignin reduction.

For microwave-assisted alkaline pretreatment, ground sugarcane bagasse was soaked in 2.5% sodium hydroxide solution at a 1:10 solid-to-liquid ratio and subjected to microwave treatment for 10 and 20 min. The treated biomass was then washed thoroughly with distilled water to remove residual alkali, neutralized to approximately pH 7, dried, and stored under clean laboratory conditions. Microwave-assisted alkaline treatment was included because recent bioethanol literature identifies intensified physical and chemical pretreatment approaches as potentially useful for improving biomass digestibility, reducing processing time, and enhancing lignocellulosic disruption (10,11,19).

The pretreated biomass samples were analyzed for cellulose and lignin content using acid digestion and gravimetric analytical procedures. Cellulose and lignin were expressed as percentages of treated dry biomass. These variables were selected as primary compositional indicators because cellulose enrichment reflects greater availability of carbohydrate substrate for hydrolysis, whereas lignin reduction reflects partial removal of a major barrier to enzymatic accessibility. The cellulose-to-lignin relationship was also considered analytically important because lignin limits enzymatic hydrolysis and may reduce the efficiency of downstream ethanol production (5,6).

Enzymatic saccharification was performed using cellulase obtained from *Trichoderma reesei*. Pretreated sugarcane bagasse was suspended in 0.05 M sodium citrate buffer at pH 5 and incubated with cellulase at 50°C for 2–3 days to hydrolyze cellulose into soluble reducing sugars. After incubation, the hydrolysate was separated from residual solids and assessed by spectrophotometric absorbance at 540 nm. Blank and standard readings were included in the assay process. The saccharification stage was included because enzymatic hydrolysis is a key conversion step linking pretreatment-induced structural modification with fermentable sugar availability for ethanol production (16,18).

The hydrolysate obtained after saccharification was used for fermentation. Batch fermentation was performed by inoculating the hydrolysate with baker's yeast, *Saccharomyces cerevisiae*, and incubating

the fermentation mixture at 30°C and pH 5 for 72 h. The fermentation medium contained yeast extract, magnesium sulfate, potassium dihydrogen phosphate, and calcium chloride to support yeast growth and metabolic activity during ethanol production. *Saccharomyces cerevisiae* was used because yeast-based fermentation remains one of the most established biological routes for converting fermentable sugars into ethanol in lignocellulosic bioethanol systems (3,20).

Following fermentation, the fermented broth was distilled at 78°C to recover ethanol-enriched distillate. Ethanol concentration was estimated using acidified potassium dichromate reagent followed by spectrophotometric analysis at 600 nm. The principal outcomes were cellulose percentage, lignin percentage, saccharification absorbance at 540 nm, ethanol absorbance at 600 nm, and ethanol percentage. The results were summarized descriptively using the directly observed experimental values. Treatment comparisons were interpreted cautiously because the available dataset consisted of aggregate laboratory observations without replicate-level inferential statistical output.

Procedural consistency was maintained by applying the same sodium hydroxide concentration, biomass preparation process, washing and neutralization approach, hydrolysis buffer pH, hydrolysis temperature, fermentation temperature, fermentation duration, distillation temperature, and spectrophotometric wavelength conditions across comparable samples. Residual alkali was removed before hydrolysis to reduce interference with enzyme activity and fermentation. Data integrity was maintained by reporting only observed compositional values, absorbance readings, and ethanol percentages, without introducing simulated or assumed statistical estimates. As the study used plant-derived agricultural residue and did not involve human participants, animals, identifiable personal information, or clinical biological specimens, formal ethical approval was not required.

RESULTS

The study evaluated compositional changes in sugarcane bagasse after alkaline pretreatment and assessed downstream saccharification and ethanol production. Autoclave-assisted sodium hydroxide pretreatment was evaluated at 30, 60, and 90 min, while microwave-assisted sodium hydroxide pretreatment was evaluated at 10 and 20 min. The outcomes included cellulose content, lignin content, saccharification absorbance at 540 nm, ethanol absorbance at 600 nm, and ethanol percentage after fermentation.

Table 1. Cellulose and Lignin Composition After Autoclave-Assisted Sodium Hydroxide Pretreatment

Pretreatment Time (min)	Cellulose (%)	Lignin (%)	Cellulose Change From 30 min	Lignin Change From 30 min
30	58.0	30.7	0.0	0.0
60	60.0	15.6	2.0	-15.1
90	68.0	11.9	10.0	-18.8

Autoclave-assisted sodium hydroxide pretreatment showed progressive compositional modification of sugarcane bagasse. Cellulose content increased from 58.0% at 30 min to 60.0% at 60 min and 68.0% at 90 min, giving an absolute increase of 10.0 percentage points from 30 to 90 min. Lignin content decreased from 30.7% at 30 min to 15.6% at 60 min and 11.9% at 90 min, corresponding to an absolute reduction of 18.8 percentage points over the same treatment interval.

Table 2. Cellulose and Lignin Composition After Microwave-Assisted Sodium Hydroxide Pretreatment

Microwave Treatment Time (min)	Cellulose (%)	Lignin (%)	Cellulose Change From 10 min	Lignin Change From 10 min
10	68.5	11.0	0.0	0.0
20	71.0	8.0	2.5	-3.0

Microwave-assisted sodium hydroxide pretreatment increased cellulose content from 68.5% after 10 min to 71.0% after 20 min, with an absolute increase of 2.5 percentage points. Lignin content decreased from 11.0% after 10 min to 8.0% after 20 min, giving an absolute reduction of 3.0 percentage points. The 20-min microwave-assisted treatment produced the highest cellulose content and the lowest lignin content among all reported pretreatment conditions.

The cellulose-to-lignin ratio increased across the pretreatment conditions, from 1.89 after 30-min autoclave-assisted treatment to 5.71 after 90-min autoclave-assisted treatment. Microwave-assisted

pretreatment produced higher ratios than the autoclave-assisted conditions, reaching 6.23 after 10 min and 8.88 after 20 min. This profile indicates greater relative cellulose enrichment and lignin reduction in the microwave-treated biomass, particularly after 20 min.

Table 3. Comparative Cellulose-to-Lignin Profile Across Pretreatment Conditions

Pretreatment Condition	Treatment Time (min)	Cellulose (%)	Lignin (%)	Cellulose-to-Lignin Ratio
Autoclave-assisted NaOH	30	58.0	30.7	1.89
Autoclave-assisted NaOH	60	60.0	15.6	3.85
Autoclave-assisted NaOH	90	68.0	11.9	5.71
Microwave-assisted NaOH	10	68.5	11.0	6.23
Microwave-assisted NaOH	20	71.0	8.0	8.88

Table 4. Best-Condition Comparison of Autoclave-Assisted and Microwave-Assisted Pretreatment

Pretreatment Condition	Treatment Time (min)	Cellulose (%)	Lignin (%)	Cellulose Difference	Lignin Difference
Autoclave-assisted NaOH	90	68.0	11.9	0.0	0.0
Microwave-assisted NaOH	20	71.0	8.0	3.0	-3.9

Comparison of the best-performing conditions from each pretreatment approach showed that 20-min microwave-assisted pretreatment produced 71.0% cellulose and 8.0% lignin, while 90-min autoclave-assisted pretreatment produced 68.0% cellulose and 11.9% lignin. Relative to the 90-min autoclave-assisted condition, the 20-min microwave-assisted condition showed 3.0 percentage points higher cellulose content and 3.9 percentage points lower lignin content.

Table 5. Saccharification Response After Enzymatic Hydrolysis

Sample	Absorbance at 540 nm	Difference From Blank	Difference From Standard
Blank	0.00	0.00	-0.35
Standard	0.35	0.35	0.00
Autoclaved sample	1.40	1.40	1.05
Microwave-treated sample	1.20	1.20	0.85

After enzymatic saccharification, the autoclaved sample showed an absorbance of 1.40 at 540 nm, while the microwave-treated sample showed an absorbance of 1.20. The autoclaved sample exceeded the standard absorbance by 1.05 units, and the microwave-treated sample exceeded the standard absorbance by 0.85 units. The autoclaved sample showed a 0.20-unit higher saccharification absorbance than the microwave-treated sample.

Table 6. Ethanol Production After Fermentation

Sample	Absorbance at 600 nm	Ethanol (%)	Ethanol Difference
Autoclaved sample	0.41	2.0	0.0
Microwave-treated sample	0.35	1.7	-0.3

Following fermentation and ethanol estimation, the autoclaved sample showed an absorbance of 0.41 at 600 nm and ethanol production of 2.0%. The microwave-treated sample showed an absorbance of 0.35 at 600 nm and ethanol production of 1.7%. The ethanol percentage in the autoclaved sample was 0.3 percentage points higher than that of the microwave-treated sample.

Table 7. Integrated Process-Level Comparison of Selected Pretreatment Conditions

Sample/Condition	Cellulose (%)	Lignin (%)	Cellulose-to-Lignin Ratio	Absorbance at 540 nm	Absorbance at 600 nm	Ethanol (%)
Autoclave-assisted NaOH, 90 min	68.0	11.9	5.71	1.40	0.41	2.0
Microwave-assisted NaOH, 20 min	71.0	8.0	8.88	1.20	0.35	1.7

The integrated comparison showed different patterns across pretreatment composition and downstream ethanol production. The 20-min microwave-assisted condition had the higher cellulose-to-lignin ratio, with 71.0% cellulose, 8.0% lignin, and a ratio of 8.88. The 90-min autoclave-assisted condition had lower cellulose content and higher lignin content, with 68.0% cellulose, 11.9% lignin, and a ratio of 5.71. However, the autoclave-assisted condition showed higher saccharification absorbance at 540 nm and higher ethanol percentage after fermentation, with values of 1.40 and 2.0%, respectively, compared with 1.20 and 1.7% in the microwave-assisted condition.

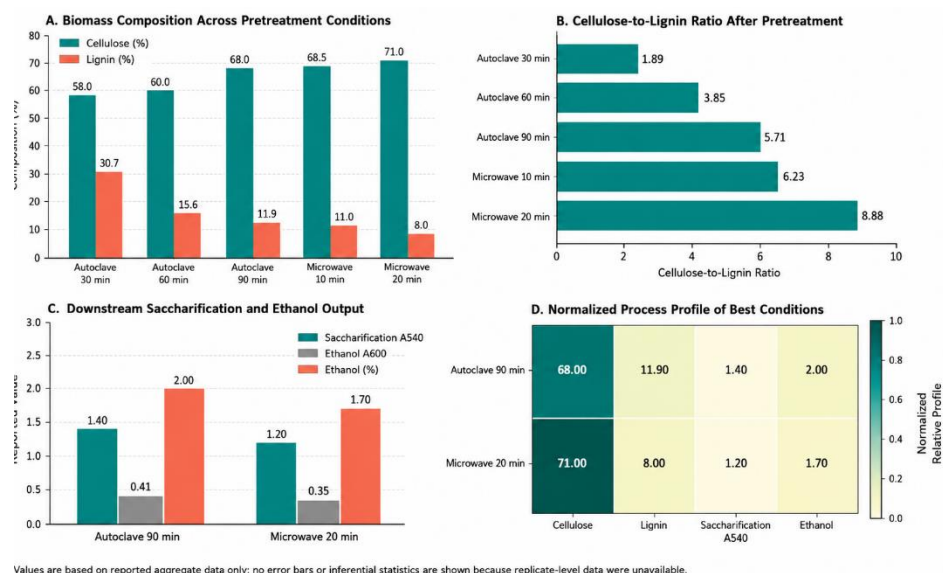


Figure 1 Pretreatment-linked biomass composition and bioethanol output from sugarcane bagasse. The panelled figure shows that progressive alkaline pretreatment increased cellulose exposure and reduced lignin content across the reported conditions. Autoclave-assisted sodium hydroxide pretreatment increased cellulose from 58.0% at 30 min to 68.0% at 90 min, while lignin decreased from 30.7% to 11.9%. Microwave-assisted sodium hydroxide pretreatment produced the most favorable compositional profile, with cellulose increasing to 71.0% and lignin decreasing to 8.0% after 20 min, giving the highest cellulose-to-lignin ratio of 8.88. However, downstream performance did not follow the same pattern: the autoclave-treated sample showed higher saccharification absorbance at 540 nm, 1.40 versus 1.20, and higher ethanol production, 2.0% versus 1.7%, compared with the microwave-treated sample. This indicates that improved delignification and cellulose enrichment alone did not directly translate into the highest ethanol recovery under the reported laboratory conditions, supporting the need for optimization of enzymatic hydrolysis, fermentation parameters, and ethanol recovery efficiency.

DISCUSSION

The present study evaluated sugarcane bagasse as a lignocellulosic substrate for laboratory-scale bioethanol production through alkaline pretreatment, enzymatic saccharification, yeast fermentation, and ethanol recovery. The results showed that sodium hydroxide pretreatment improved the compositional profile of sugarcane bagasse by increasing the relative cellulose content and reducing lignin content. In the autoclave-assisted pretreatment sequence, cellulose increased from 58.0% after 30 min to 68.0% after 90 min, while lignin decreased from 30.7% to 11.9%. This pattern is consistent with the established role of pretreatment in reducing biomass recalcitrance and improving access to carbohydrate polymers within lignocellulosic feedstocks (3,5,7). Because lignin restricts enzymatic penetration and reduces hydrolysis efficiency, its reduction is an important preparatory step in second-generation ethanol production from agricultural residues (6,8).

Microwave-assisted sodium hydroxide pretreatment produced the most favorable compositional profile among the reported experimental conditions. Cellulose increased to 71.0% and lignin decreased to 8.0% after 20 min of microwave-assisted treatment, giving a cellulose-to-lignin ratio of 8.88. This was higher than the ratio of 5.71 observed after 90-min autoclave-assisted pretreatment. These findings support the broader evidence that physicochemical pretreatment strategies can improve lignocellulosic digestibility by disrupting the plant cell wall matrix, enhancing cellulose exposure, and reducing the lignin barrier (10-13). Microwave-assisted approaches may offer process advantages because rapid heating and intensified interaction between alkali and biomass can shorten treatment duration while improving structural modification, although the overall value of such approaches depends on downstream hydrolysis and fermentation performance rather than compositional changes alone (11,13,19).

A key finding of the present study was that the pretreatment condition producing the strongest cellulose enrichment and lignin reduction did not produce the highest ethanol concentration. Although the 20-min microwave-assisted condition had higher cellulose content and lower lignin content than the 90-min autoclave-assisted condition, the autoclaved sample produced higher saccharification absorbance at 540

nm and higher ethanol concentration after fermentation. The autoclaved sample showed saccharification absorbance of 1.40 and ethanol production of 2.0%, compared with 1.20 and 1.7% in the microwave-treated sample. This distinction is important because lignocellulosic ethanol production is a multi-step process in which pretreatment, enzymatic hydrolysis, fermentable sugar release, microbial fermentation, and ethanol recovery collectively determine the final output (3,15,20). Therefore, cellulose exposure and lignin reduction should be interpreted as intermediate indicators of pretreatment performance rather than direct surrogates for ethanol yield.

The higher ethanol concentration in the autoclaved sample may reflect better compatibility between the pretreatment condition and the downstream saccharification-fermentation sequence used in this experiment. Pretreatment severity can affect not only lignin removal but also cellulose structure, hemicellulose solubilization, fermentable sugar availability, and formation of inhibitory compounds that may impair enzyme activity or yeast metabolism (5,7,12). The lower ethanol value in the microwave-treated sample, despite its superior cellulose-to-lignin ratio, suggests that the most effective delignification condition may not necessarily be the most effective ethanol-producing condition under fixed hydrolysis and fermentation parameters. Similar concerns have been emphasized in recent reviews, which indicate that pretreatment methods must be evaluated through integrated process outcomes, including sugar recovery, fermentability, downstream processing, cost, and environmental sustainability (2,14,16).

The saccharification findings showed that both pretreated samples generated absorbance values above the blank and standard readings, indicating measurable hydrolytic response after enzymatic treatment. However, the autoclaved sample produced a higher absorbance value than the microwave-treated sample. This pattern suggests that enzyme-accessible carbohydrate conversion under the reported conditions was greater in the autoclaved biomass, even though the microwave-treated biomass had a more favorable cellulose–lignin composition. Enzymatic hydrolysis is influenced by several substrate and process factors, including cellulose crystallinity, residual lignin characteristics, available surface area, enzyme loading, reaction time, pH, temperature, and the presence of compounds that interfere with cellulase activity (6,18). The present findings therefore reinforce the need to optimize saccharification conditions in parallel with pretreatment conditions rather than assuming that improved compositional indices alone will maximize sugar release.

The ethanol concentrations obtained in this study, 2.0% for the autoclaved sample and 1.7% for the microwave-treated sample, demonstrate laboratory-scale conversion of sugarcane bagasse into ethanol but also indicate that process efficiency remains modest. Current literature on lignocellulosic bioethanol emphasizes that commercial and high-yield production requires coordinated optimization of feedstock preparation, pretreatment technology, enzyme systems, fermentation organisms, inhibitor control, and downstream recovery (14,15,17). The present results are therefore best interpreted as proof-of-concept evidence for the conversion potential of sugarcane bagasse rather than as a fully optimized ethanol production process. This interpretation is particularly relevant for developing-country contexts, where underutilized lignocellulosic wastes may provide renewable feedstock options but require efficient, low-cost, and scalable conversion strategies (1,2).

The findings also have practical relevance for circular bioeconomy and agricultural waste valorization. Sugarcane bagasse is generated in large quantities in sugarcane-producing regions, and its conversion into bioethanol may reduce waste burden while contributing to renewable energy production. Reviews of agricultural residue-based bioethanol production highlight the importance of transforming low-value biomass into fuels and value-added products through integrated biorefinery approaches (8,9). However, the present results also show that the choice of pretreatment should be guided by complete process performance. A pretreatment method that maximizes cellulose content and minimizes lignin content may still require further adjustment of enzyme loading, hydrolysis duration, fermentation conditions, or detoxification strategy to achieve higher ethanol recovery.

The study has limitations that should be considered when interpreting the findings. The results were based on aggregate laboratory observations and did not include replicate-level variability, untreated biomass

controls, reducing sugar concentration, ethanol yield per gram of dry biomass, fermentation efficiency, or statistical comparison across treatment conditions. The absence of these parameters limits direct comparison with optimized lignocellulosic ethanol systems and prevents determination of the precision of observed differences. Nevertheless, the available data provide a coherent experimental pattern showing that alkaline pretreatment improved sugarcane bagasse composition, microwave-assisted treatment produced the strongest cellulose–lignin profile, and autoclave-assisted treatment produced the highest ethanol concentration under the reported conditions. Future studies should integrate compositional analysis with calibrated sugar quantification, ethanol yield normalization, inhibitor profiling, replicate-based statistical analysis, and process optimization to identify conditions that maximize both pretreatment efficiency and ethanol productivity.

CONCLUSION

Sugarcane bagasse demonstrated potential as a lignocellulosic feedstock for laboratory-scale bioethanol production after sodium hydroxide pretreatment, enzymatic saccharification, and *Saccharomyces cerevisiae* fermentation. Autoclave-assisted alkaline pretreatment increased cellulose content from 58.0% to 68.0% and reduced lignin from 30.7% to 11.9%, while microwave-assisted pretreatment produced the strongest compositional modification, with cellulose reaching 71.0% and lignin decreasing to 8.0%. Despite this superior cellulose-to-lignin profile, the autoclaved sample produced higher saccharification absorbance and ethanol concentration than the microwave-treated sample, yielding 2.0% ethanol compared with 1.7%. These findings indicate that improved delignification and cellulose exposure do not independently guarantee maximum ethanol recovery and that the complete pretreatment–hydrolysis–fermentation pathway must be optimized. The study supports the use of sugarcane bagasse as a renewable agricultural residue for second-generation ethanol production and highlights the need for calibrated sugar analysis, ethanol yield normalization, replicate-based validation, and process optimization to improve conversion efficiency.

REFERENCES

1. Adewuyi A. Underutilized lignocellulosic waste as sources of feedstock for biofuel production in developing countries. *Front Energy Res.* 2022. doi:10.3389/fenrg.2022.741570.
2. Afedzi AEK, Afrakomah GS, Gyan K, Khan J, Seidu R, Baidoo T, Sultan IN, Tareen AK, Parakulsuksatid P. Enhancing economic and environmental sustainability in lignocellulosic bioethanol production: key factors, innovative technologies, policy frameworks, and social considerations. *Sustainability.* 2025. doi:10.3390/su17020499.
3. Beluhan S, Mihajlovski K, Šantek B, Šantek MI. The production of bioethanol from lignocellulosic biomass: pretreatment methods, fermentation, and downstream processing. *Energies.* 2023. doi:10.3390/en16197003.
4. Broda M, Yelle D, Serwańska K. Bioethanol production from lignocellulosic biomass—challenges and solutions. *Molecules.* 2022. doi:10.3390/molecules27248717.
5. Cheah WY, Sankaran R, Show PL, Ibrahim TNBT, Chew KW, Culaba A, Chang JS. Pretreatment methods for lignocellulosic biofuels production: current advances, challenges and future prospects. *Biofuel Res J.* 2020. doi:10.18331/brj2020.7.1.4.
6. Devi A, Bajar S, Kour H, Kothari R, Pant D, Singh A. Lignocellulosic biomass valorization for bioethanol production: a circular bioeconomy approach. *Bioenergy Res.* 2022. doi:10.1007/s12155-022-10401-9.
7. Igwebuikwe C, Awad S, Andrès Y. Renewable energy potential: second-generation biomass as feedstock for bioethanol production. *Molecules.* 2024. doi:10.3390/molecules29071619.
8. Jayakumar M, Gindaba GT, Gebeyehu KB, Periyasamy S, Jabesa A, Baskar G, John BI, Pugazhendhi A. Bioethanol production from agricultural residues as lignocellulosic biomass feedstock's waste

- valorization approach: a comprehensive review. *Sci Total Environ.* 2023. doi:10.1016/j.scitotenv.2023.163158.
9. Kumar A, Rapoport A, Kunze G, Kumar S, Singh D, Singh B. Multifarious pretreatment strategies for the lignocellulosic substrates for the generation of renewable and sustainable biofuels: a review. *Renew Energy.* 2020. doi:10.1016/j.renene.2020.07.031.
 10. Lamichhane G, Acharya A, Poudel DK, Aryal B, Gyawali N, Niraula P, Phuyal S, Budhathoki P, Bk G, Parajuli N. Recent advances in bioethanol production from lignocellulosic biomass. *Int J Green Energy.* 2021. doi:10.1080/15435075.2021.1880910.
 11. Patel A, Shah A. Integrated lignocellulosic biorefinery: gateway for production of second generation ethanol and value added products. *J Bioresour Bioprod.* 2021. doi:10.1016/j.jobab.2021.02.001.
 12. Prasanthan A, Prasad RK, Balu M. Recent advancements and challenges in the lignocellulosic and algal biomass-based bioethanol production: a review. *Biofuels.* 2024. doi:10.1080/17597269.2024.2432148.
 13. Raj T, Chandrasekhar K, Kumar AN, Banu JR, Yoon JJ, Bhatia SK, Yang YH, Varjani S, Kim SH. Recent advances in commercial biorefineries for lignocellulosic ethanol production: current status, challenges and future perspectives. *Bioresour Technol.* 2021. doi:10.1016/j.biortech.2021.126292.
 14. Rezania S, Oryani B, Cho J, Talaiekhosani A, Sabbagh F, Hashemi B, Rupani PF, Mohammadi A. Different pretreatment technologies of lignocellulosic biomass for bioethanol production: an overview. *Energy.* 2020. doi:10.1016/j.energy.2020.117457.
 15. Robak K, Balcerak M. Current state-of-the-art in ethanol production from lignocellulosic feedstocks. *Microbiol Res.* 2020. doi:10.1016/j.micres.2020.126534.
 16. Samantaray B, Mohapatra S, Mishra R, Behera B, Thatoi H. Bioethanol production from agro-wastes: a comprehensive review with a focus on pretreatment, enzymatic hydrolysis, and fermentation. *Int J Green Energy.* 2023. doi:10.1080/15435075.2023.2253871.
 17. Toor M, Kumar SS, Malyan SK, Bishnoi N, Mathimani T, Rajendran K, Pugazhendhi A. An overview on bioethanol production from lignocellulosic feedstocks. *Chemosphere.* 2020. doi:10.1016/j.chemosphere.2019.125080.
 18. Vasić K, Knez Ž, Leitgeb M. Bioethanol production by enzymatic hydrolysis from different lignocellulosic sources. *Molecules.* 2021. doi:10.3390/molecules26030753.
 19. Woźniak A, Kuligowski K, Świerczek L, Cenian A. Review of lignocellulosic biomass pretreatment using physical, thermal and chemical methods for higher yields in bioethanol production. *Sustainability.* 2025;17(1):287. doi:10.3390/su17010287.
 20. Zhao L, Sun Z, Zhang CC, Nan J, Ren N, Lee DJ, Chen C. Advances in pretreatment of lignocellulosic biomass for bioenergy production: challenges and perspectives. *Bioresour Technol.* 2021. doi:10.1016/j.biortech.2021.126123.