

Bioprocessing of coconut husk to produce second-generation bioethanol using *Zymomonas mobilis* ZM4

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Title - Bioprocessing of coconut husk to produce second-generation bioethanol using
***Zymomonas mobilis* ZM4**

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Abstract

Coconut husk is an abundant lignocellulosic residue generated by the tropical coconut processing industry and remains largely underutilized despite its considerable carbohydrate content. This study developed an integrated strategy involving sequential alkaline, dilute-acid, and bleaching pretreatment, enzymatic hydrolysis, and fermentation with *Zymomonas mobilis* for the valorization of coconut husk into bioethanol. The sequential pretreatment enhanced cellulose accessibility and resulted in substantial removal of lignin and extractive components while retaining a considerable proportion of the carbohydrate fraction. Enzymatic hydrolysis using a cellulase–hemicellulase cocktail released approximately 230 mg glucose g⁻¹ and 161.25 mg xylose g⁻¹ of raw coconut husk, corresponding to overall sugar recovery efficiencies of approximately 50-60% following pretreatment and hydrolysis. Fermentation of the hydrolysate by *Z. mobilis* resulted in an ethanol yield of 0.0975 g g⁻¹ raw biomass, corresponding to approximately 83% of the theoretical ethanol yield based on the available fermentable sugars. Overall, these findings demonstrate the potential of coconut husk as a feedstock for second-generation bioethanol production and highlight the integrated approach as a promising strategy for efficient lignocellulosic biomass conversion and agricultural waste valorization.

Keywords: Coconut Husk, Lignocellulosic biomass, Pretreatment, Enzymatic Hydrolysis, Fermentation, Technoeconomic analysis.

1. Introduction

Several abundant plant-derived residues remain underutilized despite their potential as second-generation lignocellulosic feedstocks. Coconut (*Cocos nucifera* L.), widely cultivated across tropical regions, is one such resource that generates substantial lignocellulosic biomass. In this study, an integrated process was developed to convert coconut husk into bioethanol using *Zymomonas mobilis* ZM4 (*Z. mobilis*) as the fermentative microorganism.

Globally, annual coconut production exceeds 60 million tonne, generating more than 20 million tonne of husk residues (Satyanarayana, Arizaga and Wypych, 2009a; Stelte *et al.*, 2023; Vieira *et al.*, 2024) (FAO, 2025). Philippines (~15 million tonne), Indonesia (~16 million tonne), and India (~14 million tonne) are the leading producers, collectively accounting for nearly 75% of global coconut production (International Coconut Community, 2023; Satyanarayana, Arizaga, and Wypych, 2009b).

A coconut fruit contains outer skin (exocarp), fibrous husk (mesocarp), shell, seedcoat, solid and liquid endosperm, and embryo. The husk, comprising coir fiber and pith, accounts for approximately 35% of the fruit weight and is often discarded or underutilized. Coconut husk contains substantial amounts of cellulose (~26–33%), hemicellulose (~18–23%), and lignin (~32–36%), along with smaller quantities of extractives, including waxes, fats, oils, tannins, phenolics, pectins, pigments, proteins, soluble sugars, and ash (Bledzki and Gassan, 1999; Sun and Tomkinson, 2002; Klunklin *et al.*, 2023). However, its highly recalcitrant lignocellulosic structure limits carbohydrate accessibility and necessitates effective pretreatment. A few pretreatment strategies have been investigated for coconut residues and other lignocellulosic biomass (Putrino *et al.*, 2020), but harsh conditions can generate fermentation inhibitors. For example, Prado *et al.* (2014) reported furfural, 5-hydroxymethylfurfural (5-HMF), 4-hydroxybenzoic acid, and vanillin in pretreated coconut husk, with inhibitor formation generally increasing with pretreatment severity (Prado *et al.*,

2014). In this study, a previously optimized pretreatment strategy (Sangian and Widjaja, 2018) was employed to disrupt the lignocellulosic matrix and improve cellulose accessibility.

For microbial fermentation, *Z. mobilis* was selected because of its high ethanol-producing capacity through the Entner-Doudoroff (ED) pathway. Although its native substrate range is largely restricted to glucose, fructose, and sucrose, and does not include pentose sugars, *Z. mobilis* offers several advantages over conventional ethanologens. The ED pathway supports high ethanol yields with lower ATP generation and consequently lower biomass formation than the Embden-Meyerhof-Parnas (EMP) pathway used by *Saccharomyces cerevisiae* and *Escherichia coli* for glycolysis (Asefi et al., 2024; Mishra et al., 2025). In addition, *Z. mobilis* exhibits rapid glucose uptake, high ethanol productivity and tolerance, and relatively low by-product formation, making it an attractive platform for industrial bioethanol production (Wang et al., 2018). Despite the abundance of coconut husk globally and growing interest in advancing lignocellulosic biorefineries, systematic studies evaluating its conversion to ethanol using *Z. mobilis* remain limited.

This work addresses critical gaps by integrating carbohydrate extraction, combined pretreatment, enzymatic hydrolysis, fermentation with *Z. mobilis*, and downstream distillation process to produce ethanol. This study highlights the potential of coconut husk valorization for advancing circular bioeconomy frameworks in tropical agricultural economies such as South and Southeast Asia, where coconut residues are abundantly generated yet underutilized.

2. Materials and Methods

2.1 Feedstock Collection and Preparation

Coconut husk was obtained from Manipal, Udupi, Karnataka, India. The husks were thoroughly washed to remove adhering dirt, sun-dried to constant weight, and milled to a uniform particle size for subsequent pretreatment and enzymatic hydrolysis.

2.2 Pretreatment of Coconut Husk

Pretreatment of coconut husk biomass was carried out using 2 g of milled powder to enhance cellulose accessibility for downstream enzymatic saccharification. Alkaline delignification was performed by treating biomass with 100 mL of 1% (w/v) NaOH at 70°C for 2 h (Teck Y. Ding, Hii and Ong, 2012; Sangian and Widjaja, 2018). After each alkaline cycle, the residual solids were thoroughly washed with distilled water until neutral pH was achieved. The treatment was repeated three times to ensure effective lignin solubilization and disruption of lignin-carbohydrate complexes. The solid content was 1% (w/v) (Teck Y Ding, Hii and Ong, 2012). The delignified biomass was subsequently subjected to dilute acid pretreatment using 2% (v/v) H₂SO₄ at 121°C for 45 min to promote hemicellulose solubilization and partial depolymerization of structural polysaccharides (Talebnia, Karakashev and Angelidaki, 2010). To remove residual lignin chromophores and improve cellulose purity, the biomass was subsequently subjected to bleaching treatment using a mixture of 2% NaClO₂ and 2% CH₃COOH at 80°C for 2 h (Álvarez *et al.*, 2018; Imman *et al.*, 2021; Xu *et al.*, 2024a). The resulting cellulose-enriched solids were extensively washed, repeated for two cycles, and oven-dried at 60°C prior to hydrolysis. Following pretreatment, the recovered solid biomass was subjected to compositional characterization following applicable NREL laboratory analytical procedures. The biomass was analyzed for water extractives, ethanol extractives, ash, and acid-insoluble (Klason) lignin (Sluiter, Ruiz, *et al.*, 2008).

2.3 Enzymatic Hydrolysis of Pretreated Coconut Husk Biomass

Enzymatic hydrolysis of pretreated coconut husk biomass was performed under controlled saccharification conditions to maximize the release of fermentable sugars. A total of 1.7 g of cellulose-enriched pretreated biomass was suspended in 100 mL (2% w/v) of 50 mM sodium citrate buffer (pH 5.5). A 2% (w/v) solid loading was selected within the recommended range of 1-5% (w/v) for agricultural residues (de Araújo *et al.*, 2017), to ensure adequate enzyme accessibility while minimizing mass-transfer limitations. The slurry was thoroughly

homogenized to ensure uniform dispersion of solids prior to enzyme addition. Commercial cellulase derived from *Trichoderma reesei* (Sigma-Aldrich) was supplemented at an enzyme loading of 5 FPU g⁻¹ dry biomass, while hemicellulase from *Aspergillus niger* (Sigma-Aldrich) was added at 150 U g⁻¹ dry biomass to facilitate hemicellulose depolymerization and enhance overall saccharification efficiency. Hydrolysis reactions were conducted at 50°C in a water bath shaker incubator at 150 rpm for 72 h, as reported elsewhere (Lee, Kazlauskas and Park, 2017) for optimal fungal cellulase activity and lignocellulosic biomass saccharification. Upon completion of hydrolysis, the reaction mixture was centrifuged at 10,000 × g for 15 min at 4°C to separate residual solids from the liquid hydrolysate. The clarified supernatant was collected and analyzed for individual monosaccharides (glucose and xylose), which were quantified using HPLC equipped with a refractive index detector (Agilent 1260). Enzyme loading, buffer system, temperature, and incubation parameters were selected based on established saccharification protocols for lignocellulosic biomass (Ghose, 1987; Humbird *et al.*, 2002; Sun and Cheng, 2002a), ensuring methodological comparability with published hydrolysis studies.

2.4 Characterization of Biomass

A comprehensive characterization of coconut husk biomass was performed to evaluate its suitability as a lignocellulosic feedstock for bioethanol production. Moisture content was determined to assess storage stability and processing efficiency. Total carbohydrates and reducing sugars released during hydrolysis were quantified using colorimetric assays to evaluate saccharification performance. Individual fermentable sugars, specifically glucose and xylose, were measured using HPLC with a refractive index detector. In addition, structural changes induced by pretreatment were analyzed by Fourier Transform Infrared (FTIR) spectroscopy to confirm lignin removal and enhanced cellulose exposure. Together, these analyses provided critical insights into biomass composition, hydrolysis efficiency, and structural modifications essential for downstream fermentation processes.

2.4.1 Moisture Content Determination

Moisture content of coconut husk biomass was determined in accordance with ASTM E871-82 and NREL Laboratory Analytical Procedures (LAP) for total solids determination (Sluiter, Hames and Ruiz, 2008). In brief, samples were air-cleaned, size-reduced, and homogenized prior to analysis. An accurately weighed portion of raw biomass (2 g) was transferred into pre-weighed moisture dishes and dried in a hot air oven maintained at $105 \pm 2^\circ\text{C}$. Drying was continued until a constant weight was achieved, which occurred after 28 minutes. The dried samples were cooled in a desiccator to ambient temperature to avoid moisture reabsorption and then reweighed. Moisture content was calculated as the percentage loss in mass relative to the initial wet weight:

$$\text{Moisture (\%)} = ((W_i - W_f) / w_i) \times 100$$

Where W_i is the initial weight, and W_f is the final dry weight. All analyses were performed in triplicate, and average values were reported. This standardized approach ensured reliable quantification of moisture removal while preserving the integrity of structural carbohydrates, which is critical for subsequent pretreatment, enzymatic hydrolysis, and fermentation processes.

2.4.2 Determination of Total Carbohydrates and Reducing Sugars

Total carbohydrates, cellulose-derived sugars, and reducing sugars were quantified at each processing stage to enable a closed mass-balance analysis of coconut husk conversion from raw biomass to fermentable sugars. All measurements were normalized to the initial dry weight of coconut husk solids used at each step. Cellulose-associated carbohydrates retained in the solid fraction after pretreatment were quantified using the anthrone assay following the protocol mentioned elsewhere (Loewus, 2002; Leyva *et al.*, 2008). Total soluble carbohydrates in 100 mg of oven-dried biomass were determined using the phenol-sulfuric acid method based

on sugar dehydration to furfural derivatives and subsequent chromophore formation, with absorbance recorded at 485 nm and results expressed as mg sugar per g initial dry biomass (Masuko *et al.*, 2005). Reducing sugars contributing directly to fermentable carbon pools were qualitatively verified using Benedict's test (Stanley Benedict, 1909). Glucose standards (10-100 $\mu\text{g mL}^{-1}$) were used to create a standard curve for determining the actual concentration of total carbohydrates in both experiments (Fig S3).

2.4.3 Glucose & Xylose Quantification by HPLC-RI Detector

Glucose and xylose released during the enzymatic hydrolysis of pretreated coconut husk were quantified using High-Performance Liquid Chromatography (HPLC-RI Detector) (Agilent 1260) at ICGEB. Standard solutions of 2.5, 5.0, and 10.0 mg/mL of D-glucose and xylose (Sigma) were prepared in distilled water, and 10 μL of each was injected to generate a linear calibration curve ($R^2 \approx 0.999$) with retention times of ~ 5.9 -6.1 min. Hydrolysate samples were five-fold diluted, filtered through a 0.22 μm syringe filter, and 10 μL was injected per run. Chromatographic separation was performed using an Aminex HPX-87H, 300×7.8 mm column with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min and column temperature of 40°C , with detection by refractive index. Peaks were identified by retention time, and concentrations were calculated from the calibration curve with dilution factors applied. All standards and samples were analyzed in triplicate to ensure reproducibility, and quality control parameters, including peak symmetry, retention time stability, and calibration linearity, were monitored throughout the analysis.

2.4.4 Structural Characterization of Biomass after Pretreatment

Chemical and structural alterations in coconut husk biomass induced by pretreatment were characterized using Fourier Transform Infrared (FTIR) spectroscopy (IR Spirit-T, Shimadzu) at the Manipal-GoK Bioincubator. For FTIR analysis, untreated and pretreated biomass samples were oven-dried, finely ground, and mixed with spectroscopic-grade potassium

bromide (KBr) at a ratio of 1:100 (w/w), followed by palletization (Anand *et al.*, 2024; Du *et al.*, 2024). Infrared spectra were recorded using an FTIR spectrometer over the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans per sample. Characteristic absorption bands corresponding to hydroxyl ($-\text{OH}$), aliphatic C-H, carbonyl ($\text{C}=\text{O}$), aromatic skeletal vibrations, and polysaccharide C-O-C linkages were analyzed to assess lignin removal, hemicellulose solubilization, and cellulose exposure resulting from pretreatment (Sim *et al.*, 2012; Sharma, Sharma and Kuila, 2016).

2.5 Microbial Fermentation by *Zymomonas mobilis* ZM4

Microbial fermentation of coconut husk hydrolysate was carried out using *Z. mobilis* to evaluate ethanol production efficiency. Fermentation experiments were performed in a temperature-controlled orbital water bath shaker (SV Scientific, India Pvt Ltd) maintained at 30°C with agitation at 200 rpm for 72 h (Irfan, Nadeem and Syed, 2014). The fermentation medium consisted of yeast extract (10 g L^{-1}) and KH_2PO_4 (1 g L^{-1}) prepared in sterile distilled water, and the medium was sterilized by autoclaving at 121°C for 15 min and cooled to room temperature prior to use. Subsequently, 100 mL of hydrolysate was aseptically added to the sterile medium. The culture was inoculated with an actively growing overnight culture of *Z. mobilis* adjusted to $\text{OD}_{600} \approx 0.5$, corresponding to cells in the exponential growth phase, which was verified using a multimode reader (Varioskan™ Lux, Thermo Fisher Scientific). Anaerobic conditions were maintained by continuous purging with a gas mixture of 95% N_2 :5% CO_2 , and the fermentation pH was maintained at 6.0. After completion of fermentation, microbial cells were removed by centrifugation at $8,000 \times g$ for 15 min at 4°C. The clarified supernatant was analyzed for ethanol concentration using HPLC-RID. The fermented broth was subsequently subjected to further purity assessment.

2.6 Distillation

Following fermentation, ethanol was recovered from the clarified supernatant broth by fractional distillation based on the boiling point difference between ethanol and water. The cell-free broth obtained after centrifugation was transferred into a round-bottom flask of a rotavaporator (Heidolph Hei-VAP Advantage) at Manipal-GoK Bioincubator, and subjected to gradual heating, with the distillation bath temperature maintained between 75-80°C to align with the boiling point of ethanol (78°C). Distillate was continuously collected until the head temperature exceeded 85°C, ensuring efficient separation of ethanol from the broth. The collected distillate was determined by FTIR. To further enhance ethanol concentration and overcome the azeotropic behavior of the ethanol-water mixture, a second-stage vacuum distillation was performed. This vacuum distillation protocol yielded ethanol fractions of high purity suitable for downstream analytical and application studies.

2.7 Statistical analysis

Experiments were performed using three independent experimental replicates ($n = 3$), and the results are presented as mean $\pm$ standard deviation (SD). Differences between raw and pretreated coconut husk were evaluated using an unpaired, two-tailed Student's *t*-test. A *p*-value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Biomass Processing and Pretreatment Efficiency

In this study, raw coconut husk was sun-dried and then milled into a uniform powder to facilitate efficient biomass processing. The dried coconut husk fibers were mechanically ground into a fine and homogeneous powder using a laboratory grinder to ensure consistent particle size and reproducible downstream processing. Size reduction partially disrupted the rigid fibrous structure of the biomass, increasing cellulose exposure and enabling more uniform enzymatic accessibility (Sun and Cheng, 2002b).

Sequential pretreatment using alkaline, acid, and bleaching steps induced significant chemical and structural modifications in the biomass. Alkaline treatment resulted in noticeable darkening of the material, reflecting lignin solubilization and disruption of lignin-carbohydrate complexes. Acid treatment subsequently produced a yellowish slurry, indicating hemicellulose hydrolysis and release of soluble sugars. The final bleaching step yielded a white fibrous substrate, suggesting extensive delignification and enrichment of the cellulose fraction (Figure 1).

FTIR spectroscopy was used to examine structural changes in the biomass before and after pretreatment (Figure 2). The FTIR spectrum of untreated coconut husk showed characteristic features of lignocellulosic biomass in accordance with the published literature (Zhang *et al.*, 2018; Awoyale and Lokhat, 2021). In brief, a broad absorption band around $\sim 3400\text{ cm}^{-1}$ corresponded to O-H stretching vibrations of hydroxyl groups present in cellulose, hemicellulose, and lignin. Peaks near $\sim 2920\text{ cm}^{-1}$ were attributed to C-H stretching of aliphatic groups. The absorption band in the $\sim 1730\text{-}1650\text{ cm}^{-1}$ region represented C=O stretching vibrations associated with lignin and hemicellulosic acetyl groups, while signals around $\sim 1600\text{-}1510\text{ cm}^{-1}$ corresponded to aromatic skeletal vibrations of lignin. A strong peak at $\sim 1050\text{-}1030\text{ cm}^{-1}$ indicated C-O-C stretching of polysaccharides, confirming the presence of cellulose and hemicellulose. Additional peaks below 900 cm^{-1} corresponded to β -glycosidic linkages and C-H deformation vibrations, reflecting the complex lignocellulosic structure of untreated coconut husk.

In contrast, the FTIR spectrum of pretreated coconut husk showed clear structural modifications. The broad O-H stretching band at $\sim 3300\text{-}3400\text{ cm}^{-1}$ remained prominent, indicating the presence of cellulose and residual hemicellulose. Peaks at $\sim 2920\text{-}2850\text{ cm}^{-1}$ corresponded to aliphatic C-H stretching vibrations. A noticeable reduction in absorption intensity within the $\sim 1730\text{-}1650\text{ cm}^{-1}$ region indicated partial removal of carbonyl groups

associated with lignin and acetylated hemicellulose. Similarly, decreased intensity of aromatic bands between $\sim 1600\text{-}1510\text{ cm}^{-1}$ suggested disruption of lignin structures during pretreatment. Strong absorption bands in the $\sim 1150\text{-}1030\text{ cm}^{-1}$ region corresponded to C-O-C and C-O stretching vibrations of polysaccharides, confirming increased exposure of cellulose. Peaks below 900 cm^{-1} corresponding to β -glycosidic linkages further indicated preservation of the cellulose backbone following pretreatment (Yu et al., 2020).

3.2 Compositional characterization of raw and pretreated coconut husk

The ash content of raw coconut husk was $2.84 \pm 0.24\%$, whereas the pretreated biomass exhibited an ash content of $2.33 \pm 1.53\%$ (Table 2). Statistical analysis showed no significant difference ($p > 0.05$) between the two groups. As discussed in the NREL protocol (Sluiter, Hames, Ruiz, Scarlata, Sluiter and Templeton, 2008), the relatively stable ash content suggests that the alkaline pretreatment primarily removed organic constituents such as lignin and extractives while having a limited effect on inorganic mineral components.

The extractive composition of coconut husk changed significantly following pretreatment (Table 3). Water extractives decreased from $9.88 \pm 1.91\%$ in the raw biomass to $0.96 \pm 0.50\%$ after pretreatment ($p < 0.01$), indicating substantial removal of water-soluble compounds. Consequently, the total extractives content decreased significantly from $11.20 \pm 2.49\%$ to $2.89 \pm 0.70\%$ ($p < 0.01$). In contrast, the ethanol extractives showed no statistically significant difference between raw ($1.32 \pm 0.79\%$) and pretreated biomass ($1.93 \pm 1.20\%$, $p > 0.05$).

The acid-insoluble (Klason) lignin content of raw and pretreated coconut husk was determined according to the NREL standard protocol. As shown in table 4, the raw biomass contained $46.41 \pm 2.68\%$ acid-insoluble lignin, whereas the lignin content significantly decreased to $17.44 \pm 2.06\%$ following pretreatment ($p < 0.05$). This corresponds to an approximate 62.4% reduction in lignin content, demonstrating the pretreatment's effectiveness

in removing lignin and improving cellulose accessibility for subsequent enzymatic saccharification.

3.3 Moisture analysis and Functional properties of the pretreated samples

The functional properties of pretreated coconut husk were evaluated and compared with commercial cellulose to assess its suitability as a feedstock for bioethanol production (Table 1). The bulk density of coconut husk (0.612 g mL^{-1}) was higher than that of commercial cellulose (0.405 g mL^{-1}), and a similar trend was observed for packed density (0.816 g mL^{-1} vs. 0.486 g mL^{-1}). These higher density values indicate improved compaction characteristics, which can facilitate efficient handling, storage, and transportation of the biomass during industrial processing. In contrast, the hydrated density of coconut husk (0.241 g mL^{-1}) was considerably lower than that of commercial cellulose (0.486 g mL^{-1}), suggesting a more porous structure that allows greater water absorption.

Consistent with this observation, the water retention capacity (WRC) of coconut husk (5.611 g g^{-1}) was substantially higher than that of commercial cellulose (3.735 g g^{-1}). The elevated WRC reflects the strong hydrophilic nature of the lignocellulosic matrix and indicates an enhanced ability to retain moisture during hydrolytic reactions (Himmel *et al.*, 2007). Such properties are beneficial for enzymatic saccharification because retained water supports enzyme mobility and promotes sustained interaction between enzymes and the cellulose substrate. The swelling and hydration behaviour of coconut husk fibers may therefore increase the accessibility of hydrolytic enzymes, ultimately improving sugar release during biomass conversion (Mosier *et al.*, 2005a).

Moisture analysis indicated that the raw biomass contained 10.5% moisture, approximately 0.21 g water in 2.00 g of material. Subsequent oven drying at 100°C reduced the moisture content to below 0.5%, yielding 1.79 g of dry solids that were used as the reference basis for

subsequent mass-balance calculations. Effective moisture removal is essential to prevent microbial deterioration during storage and to minimize energy losses during subsequent thermochemical or biochemical processing. At the same time, the low residual moisture helps maintain the structural integrity of cellulose fibers, which is critical for efficient enzymatic hydrolysis (Kim and Holtzapple, 2005; Mosier *et al.*, 2005b; Dutta *et al.*, 2015).

Overall, the physicochemical characteristics of coconut husk indicate that it is a promising substrate for biorefinery applications. Its high water-retention and swelling capacity provide advantages for enzymatic hydrolysis, while its relatively high bulk density supports efficient material handling and logistics.

3.3 Enzymatic hydrolysis and fermentable sugar production from pretreated coconut husk

As cellulose and hemicellulose are closely associated within the lignocellulosic matrix, where hemicellulose interacts with cellulose microfibrils and can restrict cellulase accessibility. Removal of residual hemicellulose is recommended to enhance cellulose exposure and improve enzymatic saccharification (Østby *et al.*, 2020). In this work, the pretreated samples were enzymatically hydrolyzed by cellulase and hemicellulase for 72 h according to the previously published methods (Kafle *et al.*, 2015; Sanchez and Demain, 2017). The reported glucose and xylose concentrations therefore represent endpoint sugar yields measured after 72 h of hydrolysis under standardized saccharification conditions. Quantitative carbohydrate analysis demonstrated substantial retention of structural polysaccharides during pretreatment and their effective conversion to fermentable sugars during enzymatic hydrolysis of coconut husk biomass. Phenol-sulfuric acid assays indicated that raw coconut husk contained approximately 450 mg g⁻¹ of total structural carbohydrates, consistent with the typical cellulose and hemicellulose composition reported for lignocellulosic coconut residues. Following sequential acid-alkaline-bleaching pretreatment, the carbohydrate content retained

in the solid fraction was estimated at $\sim 300 \text{ mg g}^{-1}$ of raw biomass, suggesting efficient removal of lignin while preserving a considerable portion of the cellulose fraction.

Subsequent enzymatic hydrolysis of the pretreated solids resulted in significant solubilization of structural carbohydrates. These results demonstrate effective enzymatic conversion of exposed polysaccharides and highlight the role of pretreatment in enhancing hydrolytic efficiency.

The composition of the released sugars was further characterized using HPLC. Glucose was identified as the dominant sugar, with a yield of 230 mg g^{-1} raw biomass, indicating efficient saccharification of cellulose (Figure 3). The cellulose (glucan equivalent) content in pretreated biomass was calculated by converting the measured glucose concentration in hydrolyzed sample using the anhydro correction factor of 0.90 (162/180), following the NREL procedure for structural carbohydrate analysis (Sluiter, Hames, Ruiz, Scarlata, Sluiter, Templeton, *et al.*, 2008). This correction accounts for the difference between the molecular mass of free glucose (180 g/mol) released during hydrolysis and the anhydro glucose unit (162 g/mol) incorporated in cellulose/glucan. Accordingly, cellulose (glucan equivalent) was calculated as glucose released (mg/g) $\times 0.90$. In the present study, the glucose released after enzymatic hydrolysis was 230 mg/g raw biomass; therefore, the corresponding cellulose (glucan equivalent) was calculated as $230 \times 0.90 = 207 \text{ mg/g}$ raw biomass.

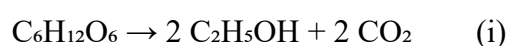
Although *Z. mobilis* does not naturally utilize xylose, its concentration was measured to determine the overall composition of potentially fermentable sugars in the hydrolysate. Xylose was detected at 161.25 mg g^{-1} of raw biomass, confirming effective hydrolysis of hemicellulosic components (Supplementary Figures 3 and 4). Glucose and xylose constituted the major soluble sugars released during enzymatic hydrolysis. Since xylose remains unutilized by wild-type *Z. mobilis*, engineering strains capable of glucose–xylose co-utilization could further improve overall carbon conversion and ethanol yield.

3.4 Ethanol synthesis via anerobic fermentation

The fermentability of the coconut husk hydrolysate was evaluated using *Z. mobilis*. Growth kinetics were compared in rich glucose medium (ZRMG) and coconut husk-derived hydrolysate medium (ZRMCH). Cell growth was monitored by measuring optical density at 600 nm (OD₆₀₀) over a 24-hour cultivation period. *Z. mobilis* exhibited robust growth in both glucose-rich medium and hydrolysate, indicating efficient utilization of fermentable sugars present in the hydrolysate. The calculated doubling time during the exponential phase was 70 ± 10 minutes in glucose medium and 105 ± 10 minutes in coconut husk hydrolysate (Figure 5). This suggests strong microbial compatibility with hydrolysate for downstream biofuel fermentation processes.

Ethanol production from coconut husk-derived hydrolysate fermented by *Z. mobilis* was quantified using HPLC equipped with a RI detector. The fermentation performance was evaluated by comparing the experimental ethanol yield with the theoretical yield based on glucose conversion.

According to the fermentation stoichiometry of *Z. mobilis*, one mole of glucose is theoretically converted into two moles of ethanol:



In this study, 0.46 g of glucose (0.00255 mol) recovered from coconut husk hydrolysate was available for fermentation. Based on this stoichiometry, the theoretical ethanol yield was 0.00510 mol (0.235 g). Experimentally, 0.00423 mol (0.195 g) of ethanol was produced, corresponding to approximately 83± 0.6% of the theoretical ethanol yield (Figure 6). The ethanol yield was 0.0975± 0.0007g g⁻¹ raw biomass.

Although *Z. mobilis* can achieve ethanol yields approaching 98% of the theoretical maximum under optimal conditions (Mishra *et al.*, 2025), the lower yield observed in this study may be associated with lignocellulose-derived inhibitory compounds present in the

hydrolysate. Further investigation is required to identify these inhibitors and determine their effects on fermentation efficiency.

To confirm the production of ethanol, the FTIR spectrum of the distilled fermentation sample was compared with that of the ethanol standard (Figure 7). Both spectra exhibited highly similar absorption bands corresponding to the characteristic functional groups of ethanol, indicating that the recovered distillate possessed the molecular fingerprint of ethanol. The broad absorption band observed at 3360 cm^{-1} in both spectra is attributed to the O–H stretching vibration of the hydroxyl group, arising from intermolecular hydrogen bonding, which is a characteristic feature of alcohols (Coates, 2000; Wong et al., 2015). The absorption bands at 2976 cm^{-1} in the ethanol standard and 2989 cm^{-1} in the distilled fermentation sample correspond to the C–H stretching vibrations of aliphatic methyl (CH_3) and methylene (CH_2) groups present in ethanol molecules (Stuart, 2005). The absorption bands at 1453 cm^{-1} and 1379 cm^{-1} in the ethanol standard, together with the corresponding bands at 1462 cm^{-1} and 1394 cm^{-1} in the distilled fermentation sample, are assigned to CH_2 bending and CH_3 bending vibrations, respectively. These bands are characteristic of ethanol and reflect deformation vibrations of the aliphatic hydrocarbon groups (Barwick *et al.*, 2001). In addition, the ethanol standard exhibited two prominent absorption bands at 1045 cm^{-1} and 1088 cm^{-1} , while the distilled fermentation sample displayed corresponding absorption bands at 1045 cm^{-1} and 1088 cm^{-1} , which are attributed to C–O stretching vibrations of the alcohol functional group. The 1045 cm^{-1} band represents the characteristic primary alcohol C–O stretching vibration, whereas the 1088 cm^{-1} band corresponds to an additional C–O stretching vibration of ethanol. These absorption bands are considered the most characteristic FTIR features of ethanol and provide strong evidence for the presence of alcohol in the distilled fermentation product (Silverstein, R.M., Webster F.X., Kiemle D.J. and Bryce D.L., 2015). Furthermore, the absorption bands observed at 880 cm^{-1} in the ethanol standard and 884 cm^{-1} in the distilled fermentation sample

correspond to the C-C-O skeletal vibration, further confirming the characteristic molecular structure of ethanol.

The close agreement between the FTIR spectra of the distilled fermentation sample and the ethanol standard confirms the successful production and recovery of ethanol following fermentation and distillation. The slight shifts observed in peak positions (typically within 5–15 cm^{-1}) are commonly attributed to differences in sample purity, intermolecular hydrogen bonding, residual moisture, and instrumental conditions, and do not alter the characteristic spectral fingerprint of ethanol. Similar FTIR absorption bands corresponding to O–H stretching ($\sim 3300\text{--}3400\text{ cm}^{-1}$), C–H stretching ($\sim 2970\text{--}2870\text{ cm}^{-1}$), CH_2/CH_3 bending ($\sim 1450\text{--}1380\text{ cm}^{-1}$), C–O stretching ($\sim 1045\text{--}1090\text{ cm}^{-1}$), and C–C–O skeletal vibration ($\sim 880\text{ cm}^{-1}$) have been reported for bioethanol produced from various lignocellulosic feedstocks, including sugarcane bagasse, corn stover, and coconut biomass (Garrigues *et al.*, 1997; Sim *et al.*, 2012; Mudalip *et al.*, 2013; Killian *et al.*, 2023).

These results demonstrate that *Z. mobilis* efficiently converted the available glucose into ethanol, achieving approximately 83% of the theoretical yield under the experimental conditions. To contextualize this performance, the ethanol conversion efficiency was compared with previously reported lignocellulosic bioethanol studies (Table 5).

The high conversion efficiency demonstrates the suitability of coconut husk hydrolysate as a substrate for ethanol production using *Z. mobilis*. Collectively, these findings highlight the potential of coconut husk as a lignocellulosic feedstock and *Z. mobilis* as an efficient microbial catalyst for second-generation bioethanol production.

4. Preliminary Techno-Economic Analysis of Coconut Husk-Based Bioethanol Production

A preliminary laboratory-scale economic assessment was conducted to estimate the major processing costs associated with bioethanol production from coconut husk using the integrated pretreatment, enzymatic hydrolysis, and fermentation process developed in this study. The objective of this assessment was to identify the principal cost contributors under laboratory-scale conditions rather than to establish the commercial feasibility of the process.

Coconut husk is an abundant lignocellulosic agricultural residue generated in large quantities in tropical countries and is generally regarded as a low-value waste biomass. Consequently, feedstock procurement costs were negligible, providing an economic advantage over conventional first-generation bioethanol feedstocks, in which raw material costs may account for approximately 40-60% of the total production cost (Fischer, Klein-Marcuschamer and Stephanopoulos, 2008; Humbird *et al.*, 2011). Furthermore, the utilization of coconut husk promotes agricultural waste valorization, reduces environmental burden associated with biomass disposal, avoids competition with food resources, and supports the development of sustainable lignocellulosic biorefineries within a circular bioeconomy framework (Sarkar *et al.*, 2022). Based on the experimental mass balance obtained in this study, 1 g of raw coconut husk produced approximately 0.0975 g of ethanol, corresponding to an ethanol productivity of 97.5 g kg⁻¹ biomass, or approximately 97.5 kg ethanol per metric ton of raw coconut husk under the laboratory-scale process conditions employed. A stepwise cost assessment was performed to estimate the contribution of each processing stage (Figure 8). Feedstock acquisition contributed only a negligible cost (approximately US\$0.0002 per 10 g biomass). Biomass preparation, including washing, drying, and milling, required approximately US\$0.09, primarily due to electricity and water consumption. Sequential pretreatment contributed approximately US\$0.19, reflecting the costs associated with chemical reagents, heating, and biomass neutralization. Among all process stages, enzymatic hydrolysis represented the largest cost component (approximately US\$1.18 per 10 g biomass) owing to

the use of commercial cellulase and hemicellulase required for efficient saccharification of the lignocellulosic substrate. Similar findings have been reported in previous techno-economic studies, where enzyme expenditure has consistently been identified as one of the major economic bottlenecks in second-generation bioethanol production (Humbird *et al.*, 2011; Klein-Marcuschamer *et al.*, 2012). Fermentation contributed a comparatively smaller cost (approximately US\$0.24), including inoculum preparation, culture media, and incubation. Downstream processing, consisting of centrifugation and fractional distillation for ethanol recovery, contributed approximately US\$0.41, reflecting the relatively high energy demand associated with ethanol separation and purification. Overall, the estimated laboratory-scale processing cost was approximately US\$2.11 per 10 g of coconut husk, with enzymatic hydrolysis and downstream ethanol recovery identified as the primary cost-driving steps. The fermentation process achieved an ethanol conversion efficiency of approximately 83% of the theoretical yield, demonstrating efficient conversion of the fermentable glucose generated during enzymatic hydrolysis into ethanol by *Z. mobilis* under the experimental conditions employed. The process requires several equipment and storage facilities, including an autoclave, water bath, shaking incubator, bioreactor or fermenter, rotary evaporator, and ethanol storage container.

Nevertheless, the present assessment provides a useful preliminary framework for identifying the major cost contributors associated with coconut husk-based bioethanol production. The results indicate that reducing enzyme consumption through enzyme recycling or low-cost enzyme production, optimizing pretreatment severity, implementing high solids saccharification, improving energy efficiency during ethanol recovery, and integrating process streams are likely to substantially improve process economics. Future studies should therefore focus on comprehensive techno-economic analysis incorporating process simulation,

sensitivity analysis, life-cycle assessment, and pilot-scale validation to establish the commercial viability of coconut husk-based bioethanol production.

5. Future Prospects

This study was performed at the laboratory scale, where processing costs are relatively high. However, scaling the process to industrial levels is expected to substantially reduce operational expenses through improved process efficiency, bulk chemical usage, and optimized energy consumption.

One important limitation is that *Z. mobilis* primarily utilizes glucose as a carbon source, whereas lignocellulosic biomass hydrolysates contain a mixture of fermentable sugars, including xylose and arabinose. Therefore, future work will employ metabolic engineering strategies in *Z. mobilis* to introduce efficient pathways for xylose and arabinose utilization and enable co-consumption of mixed sugars to improve substrate utilization efficiency and enhance overall ethanol yield. In parallel, systematic optimization of pretreatment severity, including chemical concentration, temperature, and treatment duration, will be required to maximize delignification and carbohydrate recovery while minimizing sugar degradation and inhibitor formation.

Although cellulase and hemicellulase enzymes were used in this study for biomass saccharification, the development and application of optimized enzyme cocktails could further improve hydrolysis efficiency while lowering enzyme-related processing costs. Advances in enzyme engineering, enzyme recycling strategies, and consolidated bioprocessing approaches may also contribute to improved economic feasibility. Future studies should also characterize pretreatment-derived inhibitors, including furfural, HMF, organic acids, and lignin-derived phenolics, and determine their effects on *Z. mobilis* growth and ethanol productivity. Integrating inhibitor profiling with pretreatment optimization, microbial tolerance engineering,

enzyme engineering, enzyme recycling, and consolidated bioprocessing could further improve the efficiency and economic feasibility of coconut husk-to-bioethanol conversion. Additionally, time-course fermentation studies with higher solid loading and with periodic sampling will be required to evaluate substrate consumption, ethanol production, and fermentation kinetics.

Furthermore, the integration of emerging technologies such as artificial intelligence (AI) and machine learning (ML) for process modeling and optimization offers promising opportunities. These tools can assist in predicting optimal pretreatment conditions, enzyme dosages, and fermentation parameters, thereby minimizing operational costs while maximizing bioethanol productivity. Collectively, such technological advancements could play a critical role in advancing sustainable and economically viable bioethanol production from coconut husk biomass.

6. Conclusion

Coconut husk is a sustainable and cost-effective lignocellulosic biomass with strong potential for bioethanol production. In this study, efficient pretreatment, cellulose extraction, and enzymatic hydrolysis enabled the release of fermentable sugars from the biomass. Subsequent fermentation using *Z. mobilis* demonstrated high ethanol conversion efficiency, confirming the suitability of this microorganism for lignocellulosic biofuel production.

Furthermore, physicochemical characterization and functional analysis of the extracted cellulose indicated that the material may have additional value beyond bioethanol synthesis, including potential applications in various industrial and biotechnological sectors. Overall, the findings highlight coconut husk as a promising renewable feedstock for sustainable biofuel production and integrated biorefinery applications.

Abbreviations

- 540 1. ATP - Adenosine triphosphate
541 2. HPLC - High-performance liquid chromatography
542 3. FTIR - Fourier Transform Infrared Spectroscopy
543 4. TEA - Techno-economic analysis
544 5. W/V - Weight by volume
545 6. NaClO₂ - Sodium chlorite
546 7. NaOH - Sodium hydroxide
547 8. CH₃COOH - Glacial acetic acid

548

549 **Declarations**

550 Data Availability Statement

551 All datasets generated for this study are included in the manuscript.

552 Ethics approval and consent to participate

553 Not applicable.

554 Consent for publication

555 All authors have agreed to this submission and are aware of its contents.

556 Availability of data and materials

557 All datasets generated and/or analyzed during the current study are included in this
558 published article [and its supplementary information file].

559 Competing interests

560 The authors declare that they have no competing interests.

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Author's contributions

VK and PL conceived the study. VK, PL, and SSY designed the experiments. VK performed the experiments, with SSY providing assistance with troubleshooting throughout the study. VK, SM, and PL prepared the initial draft of the manuscript. All authors (VK, PL, SM, B, and SY) contributed to data analysis, revision, and improvement of the manuscript. All authors reviewed and approved the final version of the manuscript.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

Figure 1. Experimental workflow for bioethanol production from coconut husk biomass. All experiments were performed with at least two independent biological replicates. Total carbohydrate content in raw and pretreated biomass was estimated using the anthrone and phenol-sulfuric acid colorimetric assays. Individual sugars in the enzymatic hydrolysate and fermentation samples were quantified using HPLC, providing greater sensitivity and specificity for sugar analysis.

Figure 2. Fourier Transform Infrared (FTIR) spectrum of untreated coconut husk biomass (A) and pretreated coconut husk (B).

Figure 3. High-performance liquid chromatography (HPLC) chromatograms of glucose standards and coconut husk hydrolysate analyzed using a refractive index (RI) detector. (A) Chromatogram of 2.5 mg mL⁻¹ glucose standard; (B) 5 mg mL⁻¹ glucose standard; (C) 10 mg mL⁻¹ glucose standard; and (D) coconut husk hydrolysate (10 µL of 5× diluted sample). All chromatograms show a prominent peak corresponding to glucose with a retention time of approximately 9.4-9.6 min. Additional minor peaks at other retention times likely represent soluble compounds or impurities present in the hydrolysate matrix. (E) Calibration curve of glucose standards generated by plotting HPLC peak area against glucose concentration (mg mL⁻¹) using the chromatographic data shown in Figure S1. Linear regression analysis using

the 2.5 and 5 mg mL⁻¹ standards produced a slope of 151,096 peak area units per mg mL⁻¹. Based on this calibration, the glucose concentration in the hydrolysate sample with a peak area of 278,625 was calculated to be 9.20 mg mL⁻¹. Considering the total hydrolysate volume (50 mL), the total glucose content corresponded to 460 mg per 2 g of coconut husk, equivalent to 230 mg glucose per gram of raw coconut husk biomass.

Figure 4. Growth profile and doubling time of *Z. mobilis* in glucose medium and coconut husk hydrolysate.

Figure 5. HPLC-RI chromatograms of ethanol standards and fermented sample analyzed. (A) 1 µg/mL ethanol; (B) 2.5 µg/mL ethanol; (C) 5 µg/mL ethanol; (D) 10 µg/mL ethanol; (E) 20 µg/mL ethanol; (F) 10 µL of fermented sample analyzed (five-fold diluted). All chromatograms showed a prominent peak corresponding to ethanol with retention times of a) 20.684; b) 20.656; c) 20.619; d) 20.560; e) 20.464; and f) 20.639 min, respectively, demonstrating high chromatographic consistency across the calibration range. The linear relationship between ethanol concentration and peak area was used to generate an external calibration curve for the quantitative determination of ethanol in fermented coconut husk hydrolysate samples, and; (G) Calibration curve of ethanol standards generated by plotting HPLC peak area against ethanol concentration using the chromatographic data shown in Figure 6. Ethanol in fermented coconut husk hydrolysate was detected at a retention time of approximately 20.6 min using HPLC equipped with a refractive index detector. The peak area obtained for the fermented sample was converted to concentration using the calibration equation after correcting for dilution. These results confirm efficient conversion of fermentable sugars by *Zymomonas mobilis* during lignocellulosic ethanol fermentation. Ethanol yield reached 0.0975 ± 0.0007 g g⁻¹ biomass (n = 2), corresponding to 83 ± 0.6 %

of the theoretical yield based on glucose availability, highlighting efficient bioconversion of coconut husk hydrolysate by *Zymomonas mobilis*.

Figure 6. Fourier-transform infrared (FTIR) spectra of (A) ethanol (standard) and (B) distilled fermentation sample. The FTIR spectra demonstrate the characteristic functional groups of ethanol in both the analytical standard and the distilled fermentation sample. The broad absorption band at 3360 cm^{-1} corresponds to O–H stretching of the hydroxyl group, while the absorption bands at 2976 cm^{-1} (standard) and 2989 cm^{-1} (distilled sample) are assigned to C–H stretching vibrations of aliphatic methyl and methylene groups. The bands observed at 1453 cm^{-1} and 1379 cm^{-1} in the ethanol standard, and 1462 cm^{-1} and 1394 cm^{-1} in the distilled fermentation sample, correspond to CH_2 bending and CH_3 bending, respectively. The characteristic absorption bands at 1045 cm^{-1} and 1088 cm^{-1} in the ethanol standard, and 1045 cm^{-1} and 1088 cm^{-1} in the distilled fermentation sample, are attributed to C–O stretching vibrations of the alcohol functional group, with the 1045 cm^{-1} band representing the primary alcohol C–O stretching vibration. The absorption bands at 880 cm^{-1} (standard) and 884 cm^{-1} (distilled fermentation sample) correspond to the C–C–O skeletal vibration of ethanol. The close agreement in the characteristic absorption bands between the distilled fermentation sample and the 99% ethanol standard confirms the successful production and recovery of ethanol following fermentation and distillation.

Figure 7. Techno-economic analysis of major process steps in coconut husk bioethanol production.

914 Table 1.

Property	Extracted Cellulose	Commercial Cellulose
Bulk Density (g/mL)	0.612 ± 0.003	0.404 ± 0.004
Packed Density (g/mL)	0.815 ± 0.005	0.484 ± 0.006
Hydrated Density (g/mL)	0.240 ± 0.003	0.486 ± 0.006
Water Retention (g/g)	5.611 ± 0.004	3.736 ± 0.005

915 Table 1. Physicochemical and functional properties of cellulose extracted from coconut husk
 916 compared with commercial cellulose. Bulk density, packed density, hydrated density, and
 917 water retention capacity were evaluated to characterize the structural and hydration properties
 918 of the extracted cellulose. Values are presented as mean ± standard deviation (SD).

919 Table 2.

Biomass	Ash content (%) (Mean ± SD)
Raw	2.84 ± 0.24
Pretreated	2.33 ± 1.53

920 Table 2. Ash content of raw and pretreated coconut husk. Ash content was determined
 921 according to the NREL protocol and expressed as percentage of dry biomass. Values are
 922 presented as mean ± standard deviation (SD).

923 Table 3.

Component	Raw coconut husk (%)	Pretreated biomass (%)	<i>p</i> -value
Water extractives	9.88 ± 1.91 ^a	0.96 ± 0.50 ^b	<0.01
Ethanol extractives	1.32 ± 0.79 ^a	1.93 ± 1.20 ^a	>0.05
Total extractives	11.20 ± 2.49 ^a	2.89 ± 0.70 ^b	<0.01

924 Table 3. Water-soluble, ethanol-soluble, and total extractives in raw and pretreated coconut
 925 husk. Extractive contents were determined according to the NREL protocol and expressed as
 926 percentage of dry biomass and presented as mean ± standard deviation (n = 3). Different
 927 superscript letters within the same row indicate statistically significant differences between raw
 928 and pretreated biomass (*p* < 0.05).

929

Table 4.

Biomass	Acid-insoluble lignin (%) (Mean $\pm$ SD)
Raw coconut husk	46.41 $\pm$ 2.68 ^a
Pretreated coconut husk	17.44 $\pm$ 2.06 ^b

Table 4. Acid-insoluble (Klason) lignin content of raw and pretreated coconut husk. Lignin content was determined according to the NREL protocol and expressed as percentage of dry biomass. Values are presented as mean $\pm$ standard deviation (n = 3). Different superscript letters indicate a statistically significant difference between raw and pretreated biomass ($p < 0.05$; unpaired two-tailed Student's t -test).

Table 5.

Feedstock	Pretreatment	Microorganism	Fermentation conditions	Ethanol yield/conversion	Reference
Coconut husk	Sequential acid-alkali-bleaching	<i>Zymomonas mobilis</i> ZM4	30°C, 72 h	83% theoretical yield	Present study
Coconut husk	Alkali pretreatment	<i>Saccharomyces cerevisiae</i>	30°C, 48–72 h	~59.6 - 81% conversion	(Cabral <i>et al.</i> , 2016)
Coconut coir	Alkaline pretreatment	<i>Saccharomyces cerevisiae</i>	35°C, 48 h	~51 - 87% efficiency	(Mariano <i>et al.</i> , 2021)
Coconut husk	Alkaline (KOH) + Acid (H ₂ SO ₄)	<i>Saccharomyces cerevisiae</i>	37°C, 264 h	5.72% (v/v) absolute yield	(Srinivasan <i>et al.</i> , 2025)
Coconut coir	Alkaline pretreatment (Ammonia)	<i>Saccharomyces cerevisiae</i>	30°C, 72 h	13.13 % of bioethanol content	(Jannah, Nurisman and Yandriani, 2023)
Mixed Lignocellulosic Biomass	Alkaline pretreatment	<i>S. cerevisiae</i>	30 °C 96 h	27.87 $\pm$ 0.11 g/L	(Amjad <i>et al.</i> , 2025)

Dairy manure	Dilute NaOH pretreatment	<i>Z. mobilis</i>	30 °C without shaking	71.91 %	(You <i>et al.</i> , 2017)
Rice bran	Alkaline pretreatment (KOH)	<i>Z. mobilis</i> ZM4	30 °C	69.51 %	(Todhanakas <i>em et al.</i> , 2015)
Corn stover	dilute acid pretreated	<i>Escherichia coli</i> FBR5	35 °C	0.31 g/g	(Avci <i>et al.</i> , 2013)
Wheat stover	dilute acid pretreated	<i>Escherichia coli</i> FBR5	35 °C	0.28 g/g	(Saha, Nichols and Cotta, 2011)
crude poplar	two-step pretreatment using sodium chlorite (SC)-dilute sulfuric acid (DSA)	<i>Saccharomyces cerevisiae</i>	30 °C	93.13% of the theoretical value	(Xu <i>et al.</i> , 2024b)

Table 5. Comparison of ethanol production from selected lignocellulosic feedstocks. Reported studies are compared based on feedstock type, pretreatment strategy, fermentative microorganism, fermentation conditions, and ethanol yield or conversion efficiency. The present coconut husk–*Zymomonas mobilis* ZM4 system is included for comparison with previously reported lignocellulosic bioethanol processes. Direct quantitative comparisons should be interpreted with caution because ethanol production is reported using different metrics across studies, including g L⁻¹, percentage conversion, percentage of theoretical yield, and % (v/v).

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