# Synergistic Saccharification of Vietnamese Sugarcane Bagasse Using Acetyl Esterase Combined with Cellulase to Enhance Fermentable Sugar Recovery for Bioethanol Production

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**Abstract.** In the work, analyzed the conversion of sugarcane bagasse into fermentable sugars and ethanol through an integrated chemical and enzymatic processing route. Investigated the effect of dilute NaOH pretreatment combined with a synergistic enzymatic system comprising Celluclast® 1.5 L and acetyl esterase on biomass digestibility. Identified substantial compositional modifications after pretreatment, including an increase in cellulose content to 49.8%, deacetylation efficiency of 83.3%, lignin removal of 55.2%, and limited cellulose loss of 11.6%. Studied the role of enzymatic deacetylation in enhancing cellulose accessibility and improving subsequent enzymatic hydrolysis performance. Determined glucan and xylan conversion yields of 71.8% and 63.4%, respectively, during enzymatic hydrolysis conducted at 5% (w/v) solids loading. Established hydrolysis kinetics demonstrating that the majority of fermentable sugars were released within the first 24 h of reaction. Formed a sugar-rich hydrolysate containing 23.5 g/L glucose and 12.0 g/L xylose suitable for downstream microbial fermentation. Developed an ethanolic fermentation process using *Saccharomyces cerevisiae* HIT, producing approximately 9 g/L ethanol after 24 h.

## 1 Introduction

Sugarcane bagasse (SB) is the most abundant lignocellulosic by-product of the Vietnamese sugar industry, with millions of tons generated annually, attributed to Vietnam's position among the world's leading sugarcane-producing countries [1]. The chemical composition of SB is relatively stable across major cultivation regions, being rich in cellulose (≈40–45%), hemicellulose (≈25–30%), and lignin (≈18–22%) [2]. Despite its high content of carbon-dense biopolymers that hold considerable value for the bio-based economy, SB in Vietnam and many countries in the region is still primarily utilized for boiler combustion, in-house power generation, or low-value materials such as composite boards and ceiling-pressed panels, under-exploiting its potential for biochemical conversion into fermentable sugars.

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Amid the paradigm shift toward second-generation biorefinery systems aimed at reducing emissions and displacing fossil-based fuels, bioethanol has emerged as the most intensively investigated product derived from SB, owing to its role as a key renewable liquid fuel that can be scaled and integrated into downstream energy supply chains, particularly within existing sugar-mill biorefinery configurations.

However, the complex structure between lignin, hemicellulose, and cellulose makes sugarcane bagasse resistant to biodegradation, especially the hemicellulose shell that protects the cellulose fibers and the acetyl groups attached to the xylan chain. Compared with other hemicellulose sources, xylan in sugarcane bagasse is often acetylated to a significant extent with strong ester bonds (–O–acetyl), which limits the ability to swell the fibers, reduces the exposed cellulose surface area and hinders the adsorption process as well as the activity of the cellulase enzyme complex [3]. At the same time, the acetyl group also increases the risk of generating free acetic acid and microbial inhibitors during harsh pretreatments such as steam explosion and dilute acid hydrolysis, which wastes energy, increases equipment corrosion, and causes sugar losses due to carbohydrate degradation. In this context, synergistic enzymatic saccharification is a sustainable approach, aiming at breaking down the hemicellulose shield without affecting the integrity of the polysaccharides.

Acetyl-xylan acetyl esterase commonly categorized within the broader group of acetyl esterases (AE) is a key accessory enzyme in lignocellulosic biomass bioconversion, specifically targeting the structural constraints of hemicellulose. AE catalyzes the selective hydrolysis of O-acetyl ester linkages on acetyl-xylan without cleaving the main polysaccharide backbone, resulting in a less crystalline and more amorphous xylan architecture. Enzymatic deacetylation mediated by AE reduces steric hindrance caused by acetyl substituents while enabling controlled acetate release under mild hydrolysis conditions, thereby increasing hemicellulose network porosity and fiber hydration. This structural relaxation expands cellulose surface exposure and improves substrate accessibility for cellulase, ultimately enhancing downstream enzymatic saccharification and fermentable sugar recovery [4].

When AE is integrated with multicomponent cellulase, the acetylation barrier on xylan is selectively removed under mild conditions, promoting improved fiber swelling, higher porosity, and enhanced hemicellulose hydration, which in turn increases cellulase accessibility to amorphous cellulose regions and boosts the release efficiency of soluble glucose and cellobiose without cleaving the core polysaccharide backbone. This AE enhanced saccharification represents a process-level upstream innovation that does not rely on genetic strain modification, thereby minimizing carbohydrate degradation and lowering the need for hydrolysate detoxification [5]. This approach is particularly well aligned with Vietnamese sugarcane bagasse (SB), a high-value lignocellulosic residue that has historically received limited process optimization using targeted acetyl-esterase-assisted enzymatic conversion strategies. The selective removal of acetyl substituents mitigates steric constraints within the hemicellulose matrix, reduces fermentable sugar losses, preserves polymeric carbon, and lowers thermal energy demand, while maintaining strong process compatibility with downstream fermentation by robust industrial yeasts such as S. cerevisiae. Importantly, AE-assisted cellulase hydrolysis represents a process-level innovation that restructures biomass architecture to unlock cellulose accessibility and improve overall carbon-to-ethanol conversion efficiency, without relying on genetic strain engineering [6]. When integrated into existing sugar-mill second-generation biorefinery configurations, this strategy could increase ethanol output per unit biomass, minimize auxiliary processing and hydrolysate detoxification costs, and shift SB utilization from inefficient combustion toward high-yield biochemical valorization, thereby supporting

Vietnam's circular bioeconomy development, sustainability targets, and long-term fossil-fuel displacement goals.

## 2 Materials and Methods

### 2.1 Preparation chemicals and substrates

All chemicals of analytical grade used in this study were obtained from commercial sources without further purification, unless otherwise stated. Celluclast® 1.5 L was purchased from Sigma-Aldrich (Merck, Darmstadt, Germany), is a cellulase enzyme from *Trichoderma reesei* that was used in the enzymatic hydrolysis. The acetyl esterase (17,6 U/mg) was provided by Giap [1]. Sugarcane bagasse was obtained from the Minh Khai market, Tay Tuu, Hanoi, Vietnam. The processed materials were washed twice with distilled water, air-dried in a shade, chopped into smaller size as 3–4 cm, and dried at 55 ± 2ºC to constant weights in a thermal oven. The dried materials were then ground into a fine powder, passed through a 1 mm sieve, and respectively stored in airtight containers. The *S. cerevisiae* HIT strain was provided by the microbial culture collection of the HaUI Institute of Technology, Hanoi University of Industry (Hanoi, Vietnam). The working culture and experimental inoculum were prepared under laboratory conditions prior to ethanol fermentation assays.

### 2.2 Pretreatment and Enzymatic Hydrolysis of Sugarcane Bagasse

Dilute NaOH pretreatment of sugarcane bagasse (SB) was performed at 1% (w/v) NaOH, 10:1 (v/w) liquid-to-solid ratio, 85°C for 60 min in a shaking water bath, followed by filtration. The alkaline liquor generated after NaOH pretreatment was collected and retained for lignin analysis, while the solid fraction was washed to pH 7.0, pressed, oven-dried at 60°C, and re-quantified on a dry matter (DM) basis to determine mass yield. Enzymatic saccharification was subsequently conducted at 5% (w/v, DM) solids loading in 100 mL citrate buffer (pH 5.0) in 500 mL Erlenmeyer flasks at 45°C, 150 rpm for 48 h, using a cellulase cocktail (Celluclast 1.5 L, 15 U/g) and acetyl esterase (AE, 50 U/g). The hydrolysis was evaluated using three independent experimental conditions: (1) DM + Celluclast® 1.5 L, (2) DM + AE, and (3) DM + Celluclast® 1.5 L + AE. All hydrolysate samples were centrifuged (10,000 rpm, 5 min), filtered (0.22 μm), and analyzed for reducing sugars, glucose or xylose released, glucan/xylan content, and dissolved lignin.

### 2.3 Fermentation

The yeast strain *S. cerevisiae* HIT was pre-cultured in a medium containing sucrose (20 g/L), yeast extract (2.5 g/L), urea (0.6 g/L), $K_2HPO_4$ (0.87 g/L), $MgSO_4 \cdot 7H_2O$ (0.49 g/L), $ZnSO_4 \cdot 7H_2O$ (0.029 g/L), and $MnSO_4 \cdot H_2O$ (0.017 g/L), with the pH adjusted to 5.0. The preculture was incubated at 30°C for 48 h. The experimental medium contained glucose at a concentration equivalent to that present in the hydrolysate. The hydrolysate was supplemented with the same components as the preculture medium at identical concentrations, except for fermentable sugars, and subsequently sterilized by autoclaving at 121°C for 20 min. After cooling to room temperature, the hydrolysate was aseptically inoculated with 100 mL of *S. cerevisiae* HIT. Fermentation was carried out at 30°C with agitation at 100 rpm, at pH 4.5, for 36 h. Samples were collected at regular intervals (0, 6, 12, 18, 24, 30, and 36 h) to monitor yeast growth, reducing sugar consumption, and ethanol

production. All experiments were conducted in triplicate, and the results are presented as mean values ± standard deviation.

### 2.4 Analytical methods

High-performance liquid chromatography (HPLC, Milford, MA, USA) was used to measure the amount of monomeric sugar (glucose, xylose, etc.) in the hydrolysate. This method involved a waters system with a waters RI detector and a BIO-RAD HPX87H column at 45°C that eluted at a rate of 0.6mL/min with 5mM sulfuric acid [11]. Triplicate runs were performed. Reducing sugar concentration was determined using the dinitrosalicylic acid (DNS) method using glucose as a standard. Glucan and xylan conversion yields during enzymatic hydrolysis were calculated as the proportion of glucose or xylose released relative to the initial glucan or xylan content in the pretreated bagasse, using the relevant stoichiometric conversion factors, as described by Tavares et al [7]. Ash content across all bagasse samples was carried out at 800ºC for 2 h according to Silva (1995). The soluble lignin in the filtrate was measured in a standard quartz UV cuvette (10 mm path length) at 205 nm using a Hitachi UH-5300 UV-Vis Spectrophotometer (Hitachi High-Tech, Japan). An absorptivity (extinction coefficient) of 105 L $g^{-1}$ $cm^{-1}$ was applied to calculate the concentration of acid-soluble lignin in the hydrolysate [8]. Acetyl esterase activity was determined spectrophotometrically by monitoring the release of p-nitrophenol from p-nitrophenyl acetate at 405 nm ($\varepsilon_{405}$ = 2.5 $mM^{-1}$ $cm^{-1}$) using a SpectraMax plate reader (NanoQuant, Tecan, Austria). The final substrate concentration was 1 mM in phosphate buffer (pH 6.8). The reaction was carried out at 37°C for 10 min. One unit of acetyl esterase activity was defined as the amount of enzyme required to release 1 µmol of p-nitrophenol per minute under these conditions [9]. Ethanol concentration was determined by gas chromatography according to the method described by Salakkam et al. Error and statistical analyses varied depending on the calibration method. All measurements were performed in at least triplicate, and the results are presented as mean ± standard deviation. Descriptive statistical analyses were conducted using the Data Analysis Tool in Microsoft Excel.

## 3 Results and Discussion

### 3.1 Effect of enzymatic treatments on sugarcane bagasse composition

Raw sugarcane bagasse contained approximately 61% structural carbohydrates, indicating its suitability as a lignocellulosic feedstock, although acetylated hemicellulose and lignin likely limited enzymatic accessibility. Dilute NaOH pretreatment preserved cellulose while partially removing lignin and hemicellulose substitutions. Treatment with Celluclast® 1.5 L alone increased the relative cellulose content to 44.6%, mainly due to partial removal of hemicellulose and lignin, whereas acetyl and arabinan contents were only slightly reduced, reflecting the limited ability of cellulase to overcome hemicellulose substitutions. In contrast, acetyl esterase (AE) alone induced substantial deacetylation without significant cellulose enrichment. The combined application of Celluclast® 1.5 L and AE produced the most pronounced compositional changes, increasing cellulose content to 49.8% and markedly reducing hemicellulose, acetyl, arabinan, and lignin contents. These results highlight a strong synergistic effect between cellulase and AE, underscoring the importance of deacetylation in enhancing cellulose accessibility in NaOH pretreated sugarcane bagasse (Table 1).

**Table 1.** Chemical composition of sugarcane bagasse after pretreatment and enzymatic treatment.

| Substrate | Cellulose (%) | Hemicellulose (%) | | | Lignin (%) | Ashes (%) |
|---|---|---|---|---|---|---|
| | | Total (%) | Acetyl (%) | Arabinan (%) | | |
| Sugarcane bagasse | 36.2 ± 1.6 | 24.9 ± 1.2 | 2.7 ± 0.3 | 2.9 ± 0.1 | 21.2 ± 0.8 | 4.9 ± 0.2 |
| NaOH 1% | 38.4 ± 1.6 | 23.5 ± 0.9 | 2.4 ± 0.1 | 2.6 ± 0.3 | 17.8 ± 0.8 | 4.6 ± 0.3 |
| Celluclast® 1.5 L | 44.6 ± 1.3 | 20.8 ± 0.7 | 2.1 ± 0.1 | 2.2 ± 0.2 | 16.5 ± 0.6 | 4.2 ± 0.1 |
| AE | 39.6 ± 1.4 | 21.9 ± 0.8 | 1.1 ± 0.1 | 1.6 ± 0.1 | 17.2 ± 0.7 | 4.4 ± 0.3 |
| Celluclast® 1.5 L and AE | 49.8 ± 1.1 | 17.2 ± 0.6 | 0.7 ± 0.1 | 1.0 ± 0.2 | 14.8 ± 0.5 | 3.9 ± 0.2 |

As detailed in the mass balance presented in Table 2, the applied pretreatment resulted in a solid recovery of 64.2%, corresponding to an overall mass loss of 35.8%. Notably, the cellulose fraction was largely preserved, with only 11.6% solubilized, indicating effective protection of structural carbohydrates under the applied conditions. In contrast, substantial removal of lignin was achieved, with 55.2% of the initial lignin fraction being solubilized, leading to a relative enrichment of cellulose in the solid residue (Table 2). The apparent carbohydrate enrichment can be attributed to the selective removal of non-cellulosic components, including ashes and hemicellulose-associated substituents. Approximately 39.2% of xylan was solubilized, accompanied by extensive removal of acetyl and arabinan groups, reaching 83.3% and 77.9%, respectively. This pronounced deacetylation and side-chain removal indicate effective disruption of hemicellulose substitutions and lignin–carbohydrate linkages, which contributes to the enhanced accessibility of the remaining cellulose-rich solid fraction.

**Table 2.** Mass balance (%) of main components after Celluclast® 1.5 L and AE treatment.

| Component | Sugarcane bagasse (%) | Pretreated bagasse (%) | Component loss (%) |
|---|---|---|---|
| Mass yield | 100 | 64.2 ± 1.0 | 35.8 |
| Cellulose | 36.2 ± 1.6 | 32.0 ± 1.0 | 11.6 |
| Xylan | 18.1 ± 1.2 | 11.0 ± 0.4 | 39.2 |
| Acetyl groups | 2.7 ± 0.3 | 0.45 ± 0.03 | 83.3 |
| Arabinan groups | 2.9 ± 0.1 | 0.64 ± 0.06 | 77.9 |
| Lignin | 21.2 ± 0.8 | 9.5 ± 0.3 | 55.2 |
| Ashes | 4.9 ± 0.2 | 2.5 ± 0.2 | 49.0 |
| Total | 86.0 | 56.1 | – |

The present results show extensive deacetylation (83.3% acetyl loss) and substantial removal of hemicellulose side groups (77.9% arabinan loss), accompanied by significant lignin reduction (55.2%) while largely preserving cellulose (loss 11.6%). These findings are consistent with previous reports indicating that acetylation of xylan/hemicellulose is a major contributor to biomass recalcitrance, limiting cellulase–xylanase accessibility and sugar release [10]. Supplementation with acetyl xylan esterase (AXE) has been shown to enhance xylan solubilization and indirectly improve cellulose hydrolysis, particularly when xylan and cellulose are hydrolyzed simultaneously. Similar effects have been reported for sugarcane-derived substrates, where AXE significantly improved saccharification efficiency compared to other esterases. Moreover, strong synergistic interactions between xylanase and AXE have been observed, leading to increased xylan conversion and concomitant improvements in glucan hydrolysis [11]. Overall, the high degree of

deacetylation achieved in this study, together with effective delignification and cellulose preservation, is consistent with the established mechanism whereby acetyl removal reduces steric hindrance and weakens lignin–hemicellulose associations, thereby facilitating more efficient cellulase action in mixed enzyme systems.

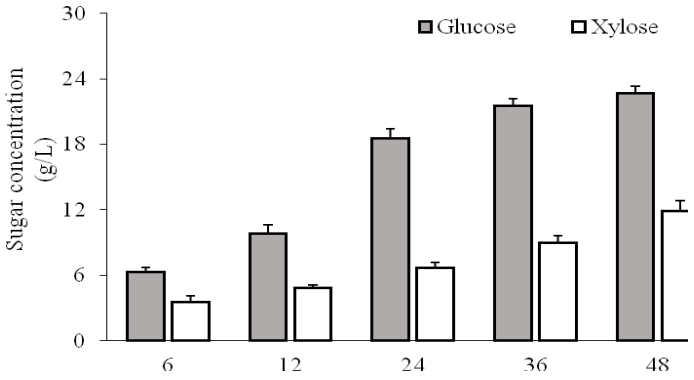


**Fig. 1.** Fermentable sugars released from pretreated bagasse by enzymatic hydrolysis using Celluclast® 1.5 L and AE.

Figure 1 shows the time-dependent release of glucose and xylose during enzymatic hydrolysis. Both sugars increased with hydrolysis time, with glucose concentrations consistently higher than those of xylose, indicating more efficient cellulose hydrolysis. In the early stage (6–12 h), glucose increased from approximately 6.2 to 9.8 g/L, while xylose rose more slowly from 3.5 to 5.0 g/L, suggesting preferential hydrolysis of the more accessible cellulose fractions. A marked increase in glucose concentration was observed at 24 h (≈18.5 g/L), accompanied by a moderate rise in xylose (≈6.5 g/L), reflecting improved substrate accessibility. At prolonged hydrolysis times (36–48 h), sugar release gradually slowed, with glucose and xylose reaching approximately 21.5–23.0 g/L and 8.5–12.0 g/L, respectively. In this study, a solids loading of 5% (w/v) was applied, leading to an intensive release of fermentable sugars during the early stage of enzymatic hydrolysis. This pronounced initial sugar liberation is likely a key factor underlying the marked decline in hydrolysis rate observed after 24 h, potentially due to product inhibition and reduced enzyme effectiveness. In addition, residual lignin not only restricts enzyme accessibility to cellulose but also promotes irreversible enzyme adsorption, thereby substantially impairing catalytic performance. The decline in hydrolysis efficiency at later stages can be attributed to increased cellulose crystallinity, in addition to product inhibition and lignin-associated constraints. During the initial stage, enzymatic hydrolysis selectively targets amorphous cellulose regions, leading to their depletion and a concomitant enrichment of the remaining substrate in more ordered, crystalline domains that are inherently less susceptible to enzymatic cleavage [12]. Therefore, 24 h was identified as the optimal hydrolysis time from both kinetic and efficiency perspectives, as it achieved high sugar release within a short reaction period, providing a favorable balance between process yield and operational cost. Extending the reaction time to 36–48 h resulted in only marginal additional sugar release and is likely to be economically unattractive for large-scale applications.

**Table 3.** Enzymatic conversion of glucan and xylan to fermentable sugars.

| Parameter | Glucan | Xylan | Cellobiose | Glucose | Xylose |
|---|---|---|---|---|---|
| Conversion yield (%) | 71.8 ± 2.3 | 63.4 ± 2.0 | – | – | – |
| Concentration (g/L) | – | – | 0.9 ± 0.1 | 23.5 ± 0.6 | 12.0 ± 0.7 |

Enzymatic hydrolysates were obtained after 48 h of hydrolysis using Celluclast® 1.5 L supplemented with acetyl esterase (AE), and the resulting sugar concentrations correspond to the final hydrolysate used for subsequent fermentation. As summarized in Table 3, enzymatic hydrolysis of the pretreated bagasse achieved high conversion yields for both glucan and xylan, reaching 71.8% and 63.4%, respectively. After 48 h, the hydrolysate contained 23.5 g/L glucose and 12.0 g/L xylose, while only trace amounts of cellobiose were detected, indicating efficient cellulose depolymerization and minimal product inhibition. Várnai et al. (2014) reported that the application of acetyl xylan esterase (AXE) to natural (non-pretreated) sugarcane fractions effectively removed acetyl and other acyl substitutions from hemicellulose, thereby enhancing cellulose and xylan accessibility. This enzymatic deacetylation disrupted hemicellulose–lignin associations and alleviated steric hindrance, ultimately leading to improved enzymatic hydrolysis of both polysaccharide fractions [13].

### 3.2 Ethanol Production from Enzymatic Hydrolysate of Sugarcane Bagasse

Figure 2 demonstrates the dynamic evolution of fermentable sugar consumption and ethanol production throughout the fermentation process. At the initial stage (0 h), the high concentration of fermentable sugar (~24 g/L) and the negligible ethanol level indicate the absence of active fermentation. During the first 6 h, fermentable sugar was rapidly consumed, accompanied by a sharp increase in ethanol concentration, reflecting intense microbial metabolic activity and efficient conversion of sugars into ethanol. Ethanol production continued to increase and reached a maximum value of approximately 9 g/L at 24 h, while residual fermentable sugar decreased to below 2 g/L, suggesting near-complete substrate utilization. This stage represents the optimal fermentation period, characterized by maximal ethanol yield and effective carbon conversion. After 24 h, a slight decline in ethanol concentration was observed despite minimal remaining sugar, which may be attributed to ethanol volatilization, metabolic reassimilation, or inhibitory effects caused by ethanol accumulation. Overall, the clear inverse relationship between fermentable sugar and ethanol concentration confirms efficient fermentation kinetics, identifying 24 h as the optimal fermentation time for maximizing ethanol production.

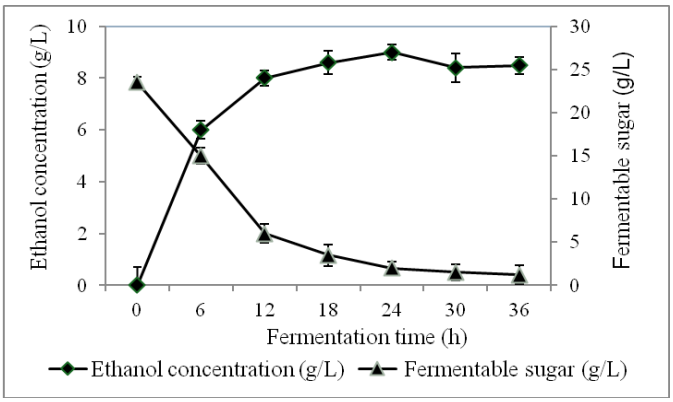


**Fig. 2.** Fermentation of *S. cerevisiae* HIT using sugars from sugarcane bagasse.

The ethanol concentration obtained in this study is comparable to values reported for batch fermentation of sugarcane bagasse hydrolysates under moderate sugar loading. Tavares et al. reported a maximum ethanol concentration of approximately 9.8 g/L during fermentation of alkaline-sulfite-pretreated sugarcane bagasse hydrolysate using *S. cerevisiae*, demonstrating similar fermentation performance to that observed in the present work [14]. Similarly, Martín et al. observed limited ethanol accumulation during early-stage fermentation of bagasse hydrolysates, which was attributed to the presence of inhibitory compounds generated during pretreatment [15].

In contrast to these batch systems, substantially higher ethanol concentrations have been achieved through intensified process configurations, such as simultaneous saccharification and fermentation (SSF) or simultaneous saccharification and co-fermentation (SSCF), where enhanced sugar availability and process integration play a decisive role. Accordingly, the moderate ethanol titer observed in this study can be mainly attributed to the limited fermentable sugar concentration available for fermentation, rather than intrinsic limitations of the yeast strain. Nevertheless, the efficient consumption of glucose and the clear inverse relationship between sugar depletion and ethanol formation indicate robust fermentation kinetics, with 24 h identified as the optimal fermentation time under the investigated conditions. These observations directly underscore the importance of improving the upstream enzymatic hydrolysis step to increase fermentable sugar availability, thereby providing a clear rationale for the future optimization strategies discussed below.

## 4 Conclusion

This study demonstrates that dilute NaOH pretreatment combined with synergistic enzymatic treatment using Celluclast® 1.5 L and acetyl esterase (AE) effectively enhances the enzymatic digestibility of sugarcane bagasse. The combined enzyme system significantly increased cellulose content (49.8%) while achieving extensive deacetylation (83.3%), removal of hemicellulose side chains, and substantial lignin reduction (55.2%), with minimal cellulose loss (11.6%). These structural modifications improved cellulose accessibility, resulting in high glucan and xylan conversion yields of 71.8% and 63.4%, respectively, and the release of 23.5 g/L glucose and 12.0 g/L xylose at 5% (w/v) solids loading after 48 h. However, most fermentable sugars were generated within the first 24 h. Accordingly, 24 h was identified as the optimal hydrolysis time, providing a favorable balance between sugar yield, reaction kinetics, and operational cost. Subsequent fermentation by *S. cerevisiae* HIT showed efficient sugar utilization and ethanol production, reaching a maximum ethanol concentration of approximately 9 g/L at 24 h. Although the obtained ethanol titer is limited by the low solids loading applied during enzymatic hydrolysis, the results clearly highlight the crucial role of enzymatic deacetylation in reducing biomass recalcitrance. Increasing solids loading, optimizing enzyme systems, or integrating this approach with existing first-generation ethanol processes represent promising strategies to further improve the industrial feasibility of lignocellulosic ethanol production.

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