Comparative Study of Sulfuric Acid and Cellulase Enzymatic Hydrolysis of Cassava Stem Cellulose for Bioethanol Production

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ABSTRACT

Bioethanol production from lignocellulosic biomass is influenced by the effectiveness of the hydrolysis process in producing fermentable sugars. This study aimed to compare the effects of acid hydrolysis and enzymatic hydrolysis on glucose conversion and bioethanol content produced from cassava stems. Acid hydrolysis was carried out using H_2SO_4 at concentrations of 1 M, 2 M, and 3 M, while enzymatic hydrolysis used varying enzyme dosages of 1 g, 2 g, and 3 g. The hydrolyzed sugars were fermented with Saccharomyces cerevisiae, and the bioethanol was separated by distillation and analyzed for its composition. All experiments were conducted in triplicate, and data were analyzed using Welch's independent t-test. The results showed that enzymatic hydrolysis produced higher glucose concentration and glucose conversion after fermentation than acid hydrolysis. However, acid hydrolysis produced a significantly higher bioethanol content (78–90±2%) than enzymatic hydrolysis (10–28±1%) ($p = 0.002$). These findings indicate that under the operating conditions used, acid hydrolysis is more effective at producing bioethanol, whereas enzymatic hydrolysis is more effective at producing fermentable sugars. This study concluded that the hydrolysis method influences glucose conversion and bioethanol yield. Further research is needed to optimize enzymatic hydrolysis, fermentation, and distillation conditions and evaluate the combination of acid pretreatment and enzymatic hydrolysis to increase bioethanol production.

1. Introduction

According to data from the Ministry of Energy and Mineral Resources (ESDM), fuel oil (BBM), especially the government-subsidized variety, has continued to increase annually. In 2020, the amount was 18.14 million kiloliters, and it grew to 29.68 million kiloliters in 2022. This could lead to a shortage of fossil fuels [1]. Therefore, Indonesia needs to switch to more environmentally friendly renewable energy sources, such as bioethanol, to reduce dependence on fossil fuels and promote sustainable development. Bioethanol is alcohol from plant-based materials or agricultural waste [2], [3]. These materials are fermented to produce bioethanol, which converts carbohydrates such as starch, sugar, and cellulose into alcohol [4]. Bioethanol has excellent development potential in Indonesia because the country has abundant raw materials for its production. Bioethanol can be made from several biomass products, including palm, cassava, corn stover, nipah, sago, sugarcane, cocoa husk waste, and sorghum [1], [5]. Although the production cost of bioethanol from agricultural raw materials may be higher than that of fossil fuels, government incentives and technological improvements can help reduce these costs. Applying appropriate technology can reduce the

production cost of bioethanol. This makes bioethanol an increasingly attractive option in the context of renewable energy [6].

According to 2019 BPS statistics, cassava production centers are spread across eight provinces in Indonesia, with the Special Region of Yogyakarta being the third largest after Lampung and Banten, with a percentage of 12.30% [7]. Cassava stem waste is also inefficient because only 10% of the stem height can be replanted, with the remaining 90% discarded. Cassava stems contain significant amounts of lignocellulose, including 56.82% α-cellulose, 21.72% lignin, and 21.45% Acid Detergent Fiber (ADF) [8]. The cellulose in cassava stem waste can be broken down into glucose, which can be used as a feedstock for bioethanol production. Hydrolysis is the process of breaking down molecules with water in the presence of acids or enzymes. Hydrolysis in an acidic environment breaks glycosidic bonds. Acid hydrolysis commonly uses sulfuric acid, acetic acid, phosphoric acid, and hydrochloric acid. Acid can alter the structure of lignocellulose and increase cellulose digestibility by increasing accessibility. Therefore, the sugar produced from the acid process can be higher [9]. Besides using acids, hydrolysis can also be carried out using cellulase enzymes [10].

Unlike chemically catalyzed acid hydrolysis, enzymatic hydrolysis uses biological catalysts to convert cellulose into glucose. Cellulase enzymes act on the β-1,4-glycosidic bonds in cellulose chains, enabling a more controlled conversion process. This method typically operates under milder, less extreme operating conditions, including moderate temperatures and neutral pH. Because it operates at a specific site, enzymatic hydrolysis generally produces fewer byproducts and inhibitors and produces sugars with higher purity [11]. Factors that significantly influence the success of enzymatic hydrolysis include the selection of microbial strains and substrate types [12].

Sulfuric acid hydrolysis and enzymatic hydrolysis differ significantly in operating conditions, reaction mechanisms, selectivity, and byproduct formation. Both hydrolysis methods have been widely used to convert lignocellulosic biomass into sugars for bioethanol production. From an operational perspective, acid hydrolysis is often considered faster and requires lower catalyst costs. However, the operating conditions used are harsher, the energy requirements are also higher, it has the potential to cause corrosion problems in equipment, and an additional step, namely neutralization, is required. On the other hand, enzymatic hydrolysis is more environmentally friendly and produces fewer inhibitor compounds or byproducts, although it generally requires a longer reaction time and is relatively more expensive.

Cassava stems have significant potential as a raw material for bioethanol production due to their properties and availability. Although acid and enzymatic hydrolysis have been widely investigated individually, direct comparisons of acid and enzymatic hydrolysis for cassava stems under identical conditions remain rare. This gap makes it difficult to determine which hydrolysis strategy provides superior glucose production and ethanol yield. This study aimed to investigate the impact of variations in acid and enzyme concentrations during the hydrolysis of cassava stem cellulose on glucose yield and bioethanol production. We hypothesize that enzymatic hydrolysis will produce higher glucose yields with fewer inhibitors, but acid hydrolysis will yield higher ethanol due to more complete sugar release.

2. Research Methodology

2.1. Materials

The materials used in this study included cassava stem waste from plantations in Gunung Kidul Regency, distilled water (CV. Sentra Teknosains), sulfuric acid, H_2SO_4 97-98% (P.A., Merck, Germany), citric acid, $C_6H_8O_7$ (P.A., Merck, Germany), cellulase enzyme (CV. Chatson Jaya Lab), sodium hydroxide, NaOH (P.A., Merck, Germany), buffer solution (CV. Sentra Teknosains), urea (P.A., Merck, Germany), and *Saccharomyces Cerevisiae* (yeast instant, S.I.L France).

2.2. Procedures

1) Preparation of Cassava Stem Samples

The cassava stems used in this research were sourced from Gunung Kidul Regency, Special Region of Yogyakarta. They were cut and dried for approximately five days in the sun. They were then dried again in an oven at 80°C until their weight was constant. The dried cassava stems were then cleaned and ground (reduced in size) using a disk mill, and sieved through a 20-mesh sieve to obtain the powder.

2) Pre-Treatment of Cassava Stem Sample

30 g of cassava stem waste powder was placed in a 500 mL Erlenmeyer flask and added to 330 mL of 4% NaOH solution. The mixture was heated on a hot plate at 100°C for 120 minutes, then cooled and filtered through a sieve to separate the solution from the solids. The resulting solids were washed with distilled water until the filtrate became clear, and the solid slurry was oven-dried at 70°C to remove moisture content.

3) The Process of Acid Hydrolysis of Cellulose into Glucose

Twenty grams of cassava stem cellulose powder was hydrolyzed with 200 mL of sulfuric acid at concentrations of 1.0, 2.0, and 3.0 M at 90°C for 30 minutes with stirring at 680 rpm. The hydrolysis product was cooled and filtered to obtain the filtrate, which was made neutral with NaOH. The reducing sugar content was analyzed using UV-Vis spectroscopy by determining the wavelength calibration curve.

4) The Process of Enzymatic Hydrolysis of Cellulose into Glucose

Twenty grams of cassava stem cellulose powder were placed in a beaker, and 200 mL of distilled water was added. The solution's pH was adjusted to 6 with citric acid, then a buffer solution was added to maintain a stable pH. The solution was then heated on a hot plate-magnetic stirrer to 50°C with stirring at 680 rpm. Next, cellulase enzymes were added at 1, 2, and 3 grams, and the hydrolysis was carried out for 90 minutes. The hydrolysis product was then cooled and filtered to obtain the filtrate. The reducing sugar content was analyzed using UV-Vis spectroscopy by determining the wavelength calibration curve.

5) Fermentation of Glucose into Bioethanol

Before the fermentation stage, a starter made from Saccharomyces cerevisiae is required. The acidity (pH) of the hydrolysis product is measured, and NaOH is added little by little to achieve a solution pH of 4.5-5.5. A total of 20 mL of the hydrolysis product is taken and placed in an Erlenmeyer flask, and 0.01 grams of urea is added. Saccharomyces cerevisiae yeast is added to the solution at a rate of 3 grams per liter. The solution is left to stand in a closed container for 24 hours. After that, filter paper filtration is performed, and the starter is ready. The fermentation process uses 200 mL of the hydrolysis product, added to the starter, along with 0.09 grams of urea. Fermentation is carried out at room temperature (30°C) for 3 days.

6) Distillation of Bioethanol

The fermentation liquid is then vacuum-distilled using a rotary evaporator at 60-70°C to separate the main product, ethanol, from the distillation residue, which is used as a by-product. The results of the distillation process were analyzed for their bioethanol content.

7) Analysis of Cellulose Yield

This analysis assesses the effectiveness of the pretreatment and helps optimize process parameters. High results indicate that the method successfully delignified the cellulose to its full potential. Cellulose yield can be calculated using the following equation:

$$cellulose\ yield\ = \frac{dry\ weight}{initial\ weight} x100\% \quad (1)$$

8) Analysis of Glucose Concentration by UV-Vis Spectroscopy

The Dinitrosalicylic acid (DNS) method was used to determine reducing sugar levels. A 1 mL clear sample was placed in a test tube, followed by 3 mL of DNS reagent, and homogenized by vortexing. The mixture was then heated in boiling water for 15 minutes, then cooled to room temperature. Absorbance was measured at a wavelength of 540 nm. The blank solution used distilled water, while a standard curve was prepared from glucose solutions with varying concentrations. The measurement data were analyzed using a regression method to determine reducing sugar levels. Glucose levels were tested both before and after fermentation.

9) Analysis of Bioethanol Concentration

Ethanol content is determined with an alcohol refractometer, which measures the concentration of ethanol in the resulting bioethanol solution. This ethanol content is used to assess the quality and purity of bioethanol and to evaluate the efficiency of the hydrolysis process, whether using acid or enzymes.

2.3. Statistical Analysis

All experiments were performed in triplicate. The results are expressed as the mean ± standard deviation (SD). Statistical significance was assessed using Welch's independent t-test, with $p < 0.05$ considered statistically significant.

3. Results and Discussion

3.1. Pretreatment and Hydrolysis

Pretreatment of cassava stem powder aims to remove lignin and hemicellulose; this process uses a 4% NaOH solution at 100°C for 120 minutes. The solution's color changes from clear to dark brown during the process (Fig. 1(a)), indicating lignin dissolution. This color change is consistent with the delignification mechanism (Fig. 1(b)), in which hydroxide ions cleave bonds in the lignin structure, forming sodium phenolate compounds that are soluble under alkaline conditions. Cellulose powder (Fig. 1(c)) was obtained after repeated washing and drying in an oven. This indicates that the non-cellulose fraction has been successfully separated. Quantitative data on cellulose yield from three experiments are presented in Table 1.

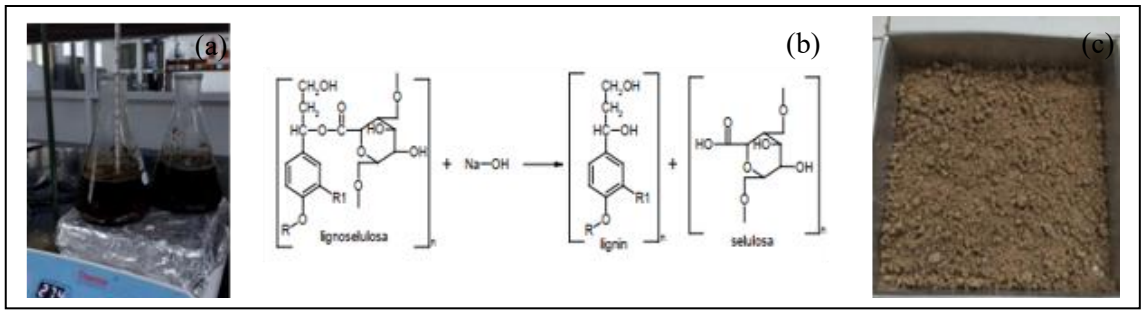

Fig. 1. (a) Chemical pretreatment, (b) Delignification reaction with NaOH [14], (c) Cellulose powder

Table 1. Results of the Pretreatment Process

Description	Pretreatment Process with 4% NaOH
Initial weight of cassava stem powder (g)	120
Cellulose yield (%)	23.84±3
Total cellulose weight (g)	52.14±2

The yield obtained in this study was 23.84±3%. This result is higher than the previous study [13], which was 7.2%. Both studies used the same NaOH concentration. However, this value is still lower than the 34% result reported by [14] using 12% NaOH. Microwave assistance has been shown to significantly increase the yield to 52.34% at the same NaOH concentration [15]. This difference in results indicates that alkali concentration, operating temperature, and biomass characteristics affect cellulose yield. Higher NaOH concentrations can accelerate delignification, but excessive use can cause cellulose degradation. The pretreatment process effectively isolated cellulose from cassava stems, yielding a satisfactory yield compared with previous studies. This cellulose fraction was then used as feedstock for hydrolysis in the next stage.

The glucose content in hydrolysates was determined using the 3,5-dinitrosalicylic acid (DNS) method, measured spectrophotometrically at 540 nm. Upon heating, DNS reacts with the aldehyde group of glucose, producing 3-amino-5-nitrosalicylic acid (ANS), which generates a dark brown color proportional to the glucose concentration Fig. 2.

Fig. 2 shows the glucose reduction reaction of 3,5-dinitrosalicylic acid (DNS). The DNS reagent, which contains a nitro group, reacts with the aldehyde group in glucose, reducing it to 3-amino-5-nitrosalicylic acid (ANS). This ANS product produces a dark brown to reddish color, with the color intensity directly proportional to the concentration of reducing sugar in the sample. The DNS method is widely used for quantitative analysis of reducing sugars because it is sensitive, simple, and can be applied to various substrates [16].

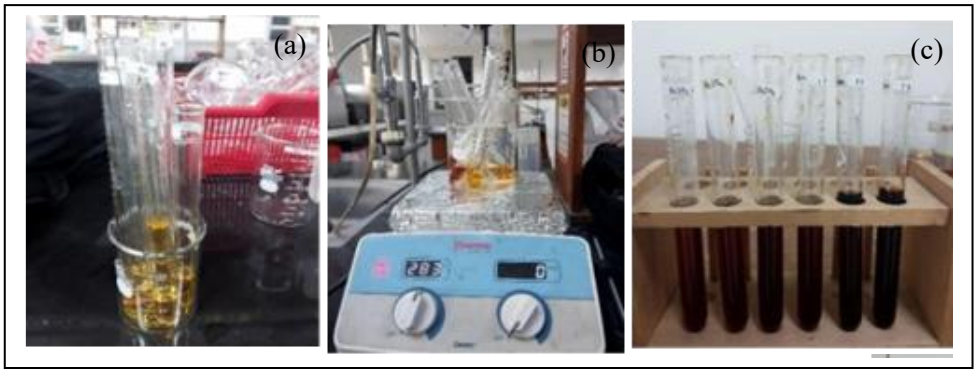

Fig. 2. (a) Before reaction, (b) Reaction process, (c) After reaction of DNS with glucose

The enzymatic method yields higher glucose concentrations at the same absorbance values, indicating that enzymatic hydrolysis converts the substrate into glucose more efficiently. Table 2 presents the glucose concentration obtained after hydrolysis. The highest glucose concentration is obtained from the enzymatic hydrolysis process (1450±136 mg/L). This result is higher than those reported in previous studies [17]. This confirms that enzymatic hydrolysis is preferable to acid hydrolysis because enzymes such as cellulase act specifically on the β-1,4-glycosidic bonds in cellulose. Due to this specificity, the cleavage of cellulose chains occurs in a controlled manner, resulting in higher-purity glucose [18].

Table 2. The Concentration of Glucose After Hydrolysis

Hydrolysis	Concentration	Glucose (mg/L)
Acid Hydrolysis	1 M	411±14
	2 M	589±95
	3 M	972±99
Enzymatic Hydrolysis	1 g	809±69
	2 g	1408±202
	3 g	1450±214

3.2. Glucose Conversion During Fermentation

Fig. 3. shows a graph comparing glucose levels in each variation before and after fermentation. In general, glucose levels decrease after fermentation, indicating that microorganisms (yeast) have consumed glucose as a carbon source in the metabolic process. However, the effectiveness of fermentation is determined not only by glucose consumption but also by the end products formed, such as ethanol and CO_2.

The concentration of sulfuric acid used in the fermentation process affects glucose conversion. Sulfuric acid with a concentration of 2 M produced the highest glucose conversion of 58±3%. The decrease in glucose conversion at sulfuric acid concentrations of 3 M and 1 M may be due to several factors, including the formation of inhibitory compounds. This is directly proportional to the research [19] This is directly proportional to the research, as the concentration of the solution increases, the number of free radical groups in the hydrolysis solution will also increase. However, low solution concentrations also reduce the water content of the hydrolysis solution. As a result, hydroxide ions are needed to bind free radicals, enabling the formation of inhibitor compounds.

In samples hydrolyzed using acid methods, it was observed that the higher the glucose concentration before fermentation, the higher the glucose concentration remaining after fermentation. This indicates that although fermentation occurred, the conversion of glucose into fermentation products did not proceed to completion, possibly due to the presence of compounds that inhibit acid hydrolysis and affect microbial activity. This is reinforced by research [20] and [21], low-concentration strong acid solutions have limitations because they produce by-products such as furfural and hydroxymethylfurfural (HMF). These compounds can inhibit the fermentation process. Reducing the formation of inhibitory compounds during the pretreatment stage [22], [23] is a detoxification strategy to mitigate the adverse effects of inhibitors.

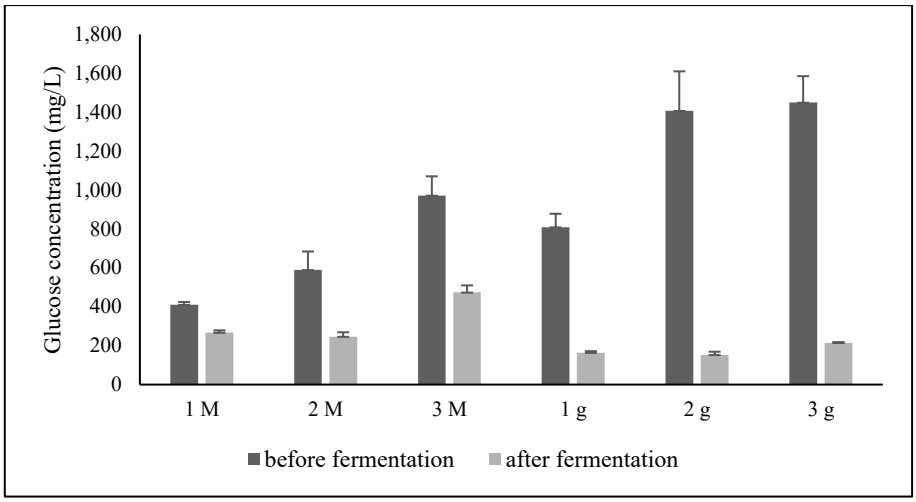

Fig. 3. Comparison of glucose levels in each variation before and after fermentation

In contrast, samples hydrolyzed using the enzyme method showed a greater difference in glucose levels before and after fermentation. For example, the 3 g enzyme sample had the highest initial glucose level; after fermentation, the glucose level decreased drastically to 214.7±4 mg/L. The decrease in glucose in the enzymatic sample relative to the acid sample indicates that the glucose produced by enzymatic hydrolysis has higher purity and is more readily consumed by microorganisms during fermentation [24]. The sugars produced can be directly consumed by microorganisms, thereby rapidly decreasing the glucose concentration in the medium during fermentation [25]. These results are consistent with research [26], which states that enzymatic hydrolysis can lead to rapid fermentation and high glucose levels. The catalytic properties of enzymes can activate other compounds, thereby accelerating the reaction.

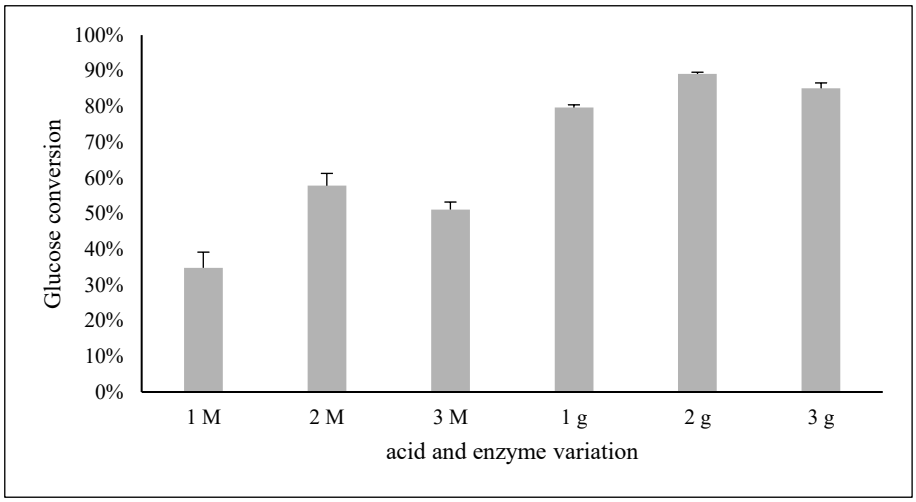

Fig. 4. Effect of sulfuric acid concentration and enzyme loading on glucose conversion

Fig. 4 shows that increasing the dose of either acid or enzyme does not linearly increase glucose conversion. At higher doses, conversion actually decreases. This indicates that there is an optimal condition. In this study, the optimal condition was achieved at a medium concentration of 2 M acid and 2 grams of enzyme. Overall, glucose conversion via enzymatic hydrolysis was higher than acid hydrolysis. The increased conversion at the beginning of enzyme addition indicates the enzyme's important role in accelerating the reaction, as more substrate can bind to the active site simultaneously. This phenomenon is explained by Michaelis–Menten kinetics, in which increasing enzyme concentration at constant substrate concentration increases Vmax because more enzyme–substrate complexes form.

The decrease in conversion at 3 g of enzyme indicates the presence of limiting factors in the system. Excess enzyme leaves many active sites unoccupied, thus decreasing reaction efficiency. Furthermore,

glucose accumulation can inhibit enzyme activity, and mass-transfer limitations can arise from increased viscosity, which hinders enzyme-substrate contact. These results align with research by [27], which confirmed that although increasing enzyme can increase conversion to the optimum, excess enzyme actually decreases efficiency due to kinetic and transport limitations.

3.3. Ethanol Concentration

The higher the concentration of sulfuric acid used, the higher the concentration of bioethanol. Research conducted by [28] found that increasing the concentration can increase the efficiency of glucose fermentation into ethanol.

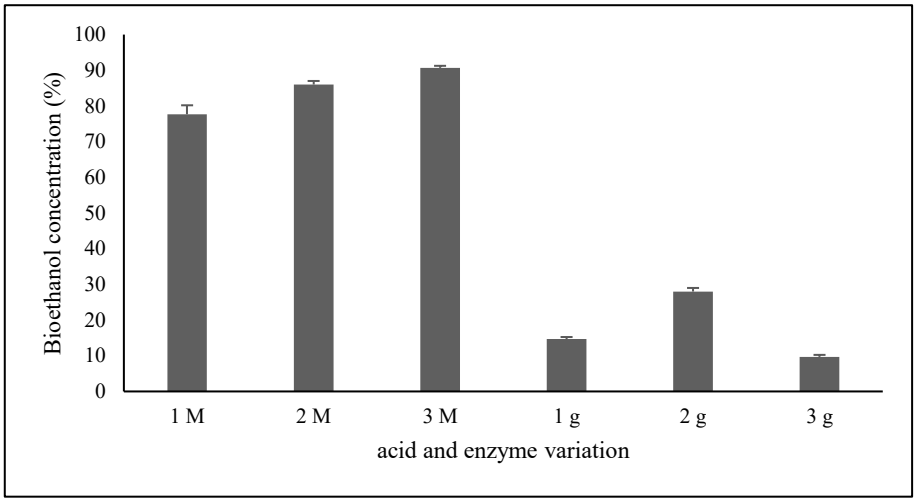

Fig. 5. Bioethanol concentration under acid and enzymatic hydrolysis

Increasing the sulfuric acid concentration increased bioethanol from 77±3% to 90±1% (Fig. 5), indicating that higher acid concentrations accelerate hydrolysis and produce more sugars for fermentation [29]. Enzymatic hydrolysis yield increased at 2 g; however, it remained lower than that of the acid method. This is due to differences in fermentation pathways: the enzymatic method can be limited by the availability of NADH/NADPH, thus reducing ethanol production [30]. Acid hydrolysates may contain more fermentable sugars (including xylose) that yeast can use, while enzymatic hydrolysates are primarily glucose, which may lead to different fermentation kinetics.

Furthermore, the fermentation products from enzymatic hydrolysis were more difficult to distill than those from acid hydrolysis. Fermentation media that still contained biomass solids, enzymes, and microbial biomass were thought to increase solution viscosity, thereby inhibiting heat transfer and reducing the efficiency of ethanol separation. As a result, ethanol recovery during distillation was lower, which also contributed to the low bioethanol content obtained from the enzymatic hydrolysis method. Therefore, under the conditions of this study, acid hydrolysis was more effective than enzymatic hydrolysis for producing bioethanol.

An independent Welch's t-test revealed a statistically significant difference in bioethanol concentration between the acid hydrolysis and enzymatic hydrolysis methods ($p = 0.002$). The mean bioethanol concentration obtained from acid hydrolysis was significantly higher than that obtained from enzymatic hydrolysis.

4. Conclusion

This study shows that the hydrolysis method affects glucose conversion and the resulting bioethanol content. Enzymatic hydrolysis yielded higher glucose concentrations and conversions after fermentation, indicating that this method is more effective at hydrolyzing lignocellulosic biomass into fermentable sugars. However, acid hydrolysis produced significantly higher bioethanol content than enzymatic hydrolysis ($p = 0.002$). These results indicate that under the operating conditions used in this study, acid hydrolysis is more effective in producing bioethanol than enzymatic hydrolysis. This study has several limitations, including the use of only one operating condition across the hydrolysis, fermentation, and distillation stages. It also failed to evaluate the formation of inhibitory compounds such as furfural and 5-hydroxymethylfurfural (HMF), fermentation efficiency, and ethanol recovery

during distillation. These factors are suspected to contribute to the low bioethanol content produced by enzymatic hydrolysis despite its higher glucose conversion. Therefore, further research is needed to optimize enzymatic hydrolysis conditions, including enzyme dosage, hydrolysis time, pH, and temperature, and to evaluate fermentation conditions and distillation efficiency. Furthermore, studies that combine acid pretreatment with enzymatic hydrolysis, along with analyses of inhibitory compounds and ethanol recovery, are needed to improve the utilization of fermentable sugars and achieve higher bioethanol yields.

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References

[1] J. L. Holechek, H. M. E. Geli, M. N. Sawalhah, and R. Valdez, "A global assessment: Can renewable energy replace fossil fuels by 2050?," *Sustainability*, vol. 14, no. 8, Apr 2022, doi: 10.3390/su14084792.

[2] M. Johnson, "Bioethanol production from agricultural wastes using cogeneration plant's residual thermal energy in distillation phase," *Irrigation & Drainage Systems Engineering*, vol. 13, no. 4, pp. 445, 2024, doi: 10.37421/2168-9768.2024.13.445.

[3] A. P. Ingle, J. Rathod, R. Pandit, S. S. da Silva, and M. Rai, "Comparative evaluation of free and immobilized cellulase for enzymatic hydrolysis of lignocellulosic biomass for sustainable bioethanol production," *Cellulose*, vol. 24, no. 12, pp. 5529–5540, Dec 2017, doi: 10.1007/S10570-017-1517-1.

[4] A. Sharma, R. Singh, P. Kumar, and S. K. Gupta, "Recent advances in bioethanol production from lignocellulosic biomass: Pretreatment, hydrolysis, and fermentation strategies," *Biocatalysis and Agricultural Biotechnology*, vol. 59, Jun 2024, doi: 10.1016/j.bcab.2024.103155.

[5] K. Vasić, Ž. Knez, and M. Leitgeb, "Bioethanol production by enzymatic hydrolysis from different lignocellulosic sources," *Molecules*, vol. 26, no. 3, pp. 753, Feb 2021, doi: 10.3390/MOLECULES26030753.

[6] Y. Zheng, H. H. Ngo, H. Luo, R. Wang, C. Li, C. Zhang, and X. Wang, "Production of cost-competitive bioethanol and value-added co-products from distillers' grains: Techno-economic evaluation and environmental impact analysis," *Bioresource Technology*, vol. 397, Apr 2024, doi: 10.1016/j.biortech.2024.130470.

[7] Pusat Data dan Sistem Informasi Pertanian, Kementerian Pertanian RI, *Outlook Komoditas Tanaman Pangan Ubi Kayu Tahun 2020*, Jakarta, Indonesia: Kementerian Pertanian RI, 2020.

[8] L. Lismeri, A. P. Karina, T. Taharuddin, Y. Darni, A. Azhar, A. Arfiana, and S. Sudibyo, "Production of hydroxypropyl methylcellulose (HPMC) from α-cellulose derived from cassava stems," in *Proceedings of the 1st International Conference on Industry Science Technology and Sustainability (IConISTS 2023)*, *Advances in Engineering Research*, vol. 235, pp. 68–80, Atlantis Press, 2024, doi: 10.2991/978-94-6463-475-4_8.

[9] N. Ilić, M. Milić, S. Beluhan, and S. Dimitrijević-Branković, "Cellulases: From lignocellulosic biomass to improved production," *Energies*, vol. 16, no. 8, Apr 2023, doi: 10.3390/en16083598.

[10] F. Dimawarnita, P. D. Indriyantini, Y. Faramitha, and U. Perwitasari, "Isolation and characterization α-cellulose from cocoa pod husk using peracetic acid," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 1187, no. 1, May 2023, doi: 10.1088/1755-1315/1187/1/012043.

[11] P. Basera, S. Chakraborty, and N. Sharma, "Lignocellulosic biomass: Insights into enzymatic hydrolysis, influential factors, and economic viability," *Discover Sustainability*, vol. 5, no. 311, Oct 2024, doi: 10.1007/s43621-024-00543-5.

[12] T. Kreetachat, N. Suriyachai, P. Khongchamnan, K. Suwannahong, S. Wongcharee, C. Sakulthaew, C. Chokejaroenret, and S. Imman, "Optimization of acid-catalyzed hydrolysis and simultaneous saccharification and fermentation for enhanced ethanol production from sweet stalk sorghum," *Catalysts*, vol. 15, no. 4, pp. 379, Apr 2025, doi: 10.3390/catal15040379.

[13] H. Julianto, M. Farid, and A. Rasyide, "Ekstraksi nanoselulosa dengan metode hidrolisis asam sebagai penguat komposit absorpsi suara," *J. Tek. ITS*, vol. 6, no. 223, pp. 42–45, 2017, doi: 10.12962/j23373539.v6i2.24259.

[14] D. P. Dewanti, "Potensi selulosa dari limbah tandan kosong kelapa sawit untuk bahan baku bioplastik ramah lingkungan," *J. Teknol. Lingkung.*, vol. 19, no. 1, pp. 81–88, Mar 2018, doi: 10.29122/jtl.v19i1.2644.

[15] A. Kamalini, Shanmugaprakash Muthusamy, R. Ramapriya, Balajii Muthusamy, Arivalagan Pugazhendhi, "Optimization of sugar recovery efficiency using microwave assisted alkaline pretreatment of cassava stem using response surface methodology and its structural characterization," *Journal of Molecular Liquids*, vol. 254, Mar 2018, doi: 10.1016/j.molliq.2018.01.091.

[16] N. N. Deshavath, G. Mukherjee, V. V. Goud, V. D. Veeranki, and C. V. Sastri, "Pitfalls in the 3, 5-dinitrosalicylic acid (DNS) assay for the reducing sugars: Interference of furfural and 5-hydroxymethylfurfural," *Int. J. Biol. Macromol.*, vol. 156, pp. 180–185, Aug 2020, doi: 10.1016/J.IJBIOMAC.2020.04.045.

[17] H. Guo, Y. Zhao, J. Chang, and D. Lee, "Inhibitor formation and detoxification during lignocellulose biorefinery: A review," *Bioresource Technology*, vol. 361, Oct 2022, doi: 10.1016/j.biortech.2022.127666.

[18] G. Balasundaram, R. Banu, S. Varjani, A.A. Kazmi, and V. K. Tyagi, "Recalcitrant compounds formation, their toxicity, and mitigation: Key issues in biomass pretreatment and anaerobic digestion," *Chemosphere*, vol. 291, part 3, Mar 2022, doi: 10.1016/j.chemosphere.2021.132930.

[19] J. Du, J. Liang, X. Zhang, J. Wang, W. Li, P. Song, and X. Feng, "Identifying the negative cooperation between major inhibitors of cellulase activity and minimizing their inhibitory potential during hydrolysis of acid-pretreated corn stover," *Bioresource Technology*, vol. 343, Jan 2022, doi: 10.1016/j.biortech.2021.126113.

[20] H. Sun, L. Liu, W. Liu, Z. Zheng, Y. Fan, and J. Ouyang, "Removal of inhibitory furan aldehydes in lignocellulosic hydrolysates via chitosan-chitin nanofiber hybrid hydrogel beads," *Bioresource Technology*, vol. 346, Feb 2022, doi: 10.1016/j.biortech.2021.126563.

[21] S. K. Soni, A. Sharma, and R. Soni, "Microbial enzyme systems in the production of second generation bioethanol," *Sustainability*, vol. 15, no. 4, Feb 2023, doi: 10.3390/su15043590.

[22] D. Santos, J.G. W. Siqueira, M. G. L. da Silva, M. Donato, G. da Silva, B. Pratto, A.A. Albuquerque, E. D. Dutra, and J. L. S. Sonego, "Enzymatic hydrolysis of lignocellulose biomass: structural features, process aspects, kinetics, and computational tools," *Biomass*, vol. 6, no. 1, Feb 2026, doi: 10.3390/biomass6010013.

[23] S. Zhao, H. Li, T. Sumpradit, and A. Khan, "Enhancing biomass conservation and enzymatic hydrolysis of sweet sorghum bagasse by combining pretreatment with ensiling and NaOH," *Frontiers Microbiology*, vol. 15, Mar 2024, doi: 10.3389/fmicb.2024.1370686.

[24] N. A. Arifiyanti, D. N. A. Kartini, and M. Billah, "Bioetanol dari biji nangka dengan proses likuifaksi dan fermentasi menggunakan *Saccharomyces cerevisiae*," *Chempro*, vol. 1, no. 1, pp. 51–55, Nov 2023, doi: 10.33005/chempro.v1i01.47.

[25] C. Ramayanti and K. R. Giasmara, "Bioethanol production from waste paper using separate hydrolysis and fermentation," *J. Chem. Res*, vol. 5, no. 1, pp. 429–433, 2017.

[26] R. Sarwono, E. Triwahyuni, Y. Aristiawan, H. H. Kurniawan, and T. Anindyawati, "Konversi selulosa tandan kosong sawit (Tks) menjadi etanol," *J. Selulosa*, vol. 4, no. 01, May 2016, doi: 10.25269/jsel.v4i01.50.

[27] S. M. D. Kolo and E. Edi, "Hidrolisis ampas biji sorgum dengan microwave untuk produksi gula pereduksi sebagai bahan baku bioetanol," *J. Saintek Lahan Kering*, vol. 1, no. 2, pp. 22–23, Dec 2018, doi: 10.32938/slk.v1i2.596.

[28] R. Safitri, I. Dwi Anggita, F. M. Safitri, D. A. Agung, and I. Ratnadewi, "Pengaruh konsentrasi asam sulfat dalam proses hidrolisis selulosa dari kulit buah naga merah (*Hylocereus costaricensis*) untuk produksi bioetanol," *Ind. Res. Work. Natl. Semin.*, vol. 9, pp. 1–5, 2018.

[29] T. Widyaningrum and A. A. Rizqiyah, "Pengaruh Rasio crude enzim selulase trichoderma reesei dan aspergillus niger terhadap kadar gula dan bioetanol fermentasi kulit kacang tanah (*Arachis hypogaea L.*) menggunakan *Zymomonas* mobilis," *Biosci. J. Ilm. Biol.*, vol. 11, no. 2, pp. 1615, Dec 2023, doi: 10.33394/bioscientist.v11i2.9487.

[30] M. Gitta Pawitra, "Studi Modifikasi jalur metabolik *Saccharomyces Cerevisiae* dalam proses pembuatan etanol modification study of metabolic pathways of *Saccharomyces cerevisiae* in ethanol production," *J. Ilm. Tek. Kim. UNPAM*, vol. 3, no. 1, 2019.