Production of bioethanol from sugarcane bagasse using cellulase enzyme

Sri Rulianah[1*], *Shabrina* Adani Putri[1], *Prayitno*[1], *Listiyana* Candra Dewi[1], and *Ahmad* Zaky Aldillah[1]

[1]Department of Chemical Engineering Politeknik Negeri Malang Soekarno-Hatta Street No. 9, Malang 65141, Indonesia

Abstract. Sugarcane bagasse has a high lignocellulose content, namely cellulose content 32–55%, hemicellulose 26.7–32%, and lignin 19–24%. With this content, sugarcane bagasse has good potential to be used as raw material for bioethanol production. In this study, commercial cellulase enzymes were used for the bagasse hydrolysis process. The purpose of this study was to determine the best conditions from variations in the addition of cellulase enzymes and fermentation time on the levels and yield of bioethanol using SSF process in a fermentor. This research was conducted with the following stages: pre-treatment; delignification; analysis of lignocellulose; SSF process; analysis of ethanol content. In this study, the varied factors were the inclusion of 3 and 3,5% (v/v) of cellulase enzymes and the duration of fermentation, from 3, 4, 5, 6, and 7 days. The results of this study showed that the addition of 3.5% (v/v) enzyme and fermentation for 7 days were the best conditions, the ethanol content obtained was 2.64%, and the yield was 14.78%. In this study, the hydrolysis process only used a single cellulase enzyme, and it turned out to produce a lower product when compared to using crude cellulase enzymes using *Phanerochaete chrysosporium*.

1 Introduction

More extensive research is being done to provide alternative energy sources to replace fossil fuels due to the annual increase in energy usage and demand. Fossil fuels can be replaced by plant-based materials that have been processed into biofuels. One kind of biofuel that could take the place of gasoline is bioethanol. The fermentation of sugars from biomass including carbs (wheat, corn), sucrose (sugarcane), or lignocellulosic materials (bagasse from sugarcane, empty fruit bunches of palm oil, etc.) can produce bioethanol [1]. The fact that lignocellulosic materials, like sugarcane waste, are abundant, inexpensive, and not intended for human use makes them attractive as ethanol raw materials [2]. The large number of plant-based resources in Indonesia supports the development potential of bioethanol as a renewable energy source. Bagasse is a byproduct of processing sugarcane that has a large potential for producing bioethanol. About half of the bagasse produced in sugar mills is used as boiler fuel [3]. The remaining bagasse is frequently abandoned because it is thought to have no

* Corresponding author: sri.rulianah@polinema.ac.id

economic value. The amount of sugarcane bagasse produced by sugar mills in Indonesia can produce 614,827 kL of ethanol annually [4]. This suggests that there is still a long way to go in the production of bioethanol from sugarcane bagasse. The bioethanol production process is influenced by several factors, such as raw material type, raw material size, delignification process, hydrolysis process, and fermentation process. Sugarcane bagasse has the advantage of producing high ethanol content. This is evidenced by reference [5] who compared ethanol content in sugarcane bagasse, wheat straw, rice straw, ragi straw, and water hyacinth in bioethanol production. Maximum ethanol was obtained from sugarcane bagasse at about 11.90 g/L, followed by wheat straw at 9.56 g/L, rice straw at 8.84 g/L, and water hyacinth at 6.19 g/L ethanol in fermentation. This indicates that sugarcane bagasse has superior potential for producing ethanol with the highest content among these lignocellulosic materials [5]. The possibility of producing bioethanol from bagasse is relatively high. In Indonesia, 62 sugar mills could potentially produce 614,827 kL of bioethanol from 2,991,114 tons of bagasse. Sugarcane bagasse can be used to produce bioethanol because of its composition, which includes cellulose 32–55%, hemicellulose 19–24%, and lignin 19–24% [6-9]. The cellulose and hemicellulose act as complex sugars in this process [10].

Research on the effect of variations in cellulase enzyme concentration has been conducted by reference [11] on bioethanol production from corn cob waste. The pretreatment process begins by grinding corn cobs into small pieces, drying them, and grinding them to a particle size of 20 to 40 mesh. Reference [11] found that using enzyme concentrations of 3, 5, 7, 9, and 11% showed that variations in enzyme addition could affect the levels of bioethanol obtained. The more enzymes added, the higher the levels of bioethanol produced, because more lignocellulose is converted into glucose, which will be converted into bioethanol by Saccharomyces cerevisiae. The highest bioethanol concentration was 8% with the addition of 11% v/v cellulose enzyme with a fermentation time of 72 hours [11]. However, this study does not show whether the enzyme is pure (commercial) or self-produced, because if it is self-produced, the enzyme produced is likely to still be a crude enzyme, consisting of a mixture of several enzymes depending on the substrate.

Reference [12] analyzed the effect of yeast (*Saccharomyces cerevisiae*) percentage and fermentation time on the process using duckweed (Lemna minor) for bioethanol production. Bioethanol was produced through carbohydrate conversion to glucose via acid hydrolysis using HCl. The hydrolysate was then fermented with added yeast to produce bioethanol. The study varied yeast amounts at 5%, 15%, and 25% and fermentation times of 5, 6, and 7 days. Results showed that both fermentation duration and yeast percentage impacted the ethanol content. Optimal ethanol content was achieved with 25% yeast and 7 days of fermentation, reaching 3.81% with an optimal density of 0.9438 g/cm³ [12].

Research on the effect of fermentation time variations on bioethanol production, specifically from sugarcane bagasse and using the Simultaneous Saccharification and Fermentation (SSF) method, has been conducted by reference [13] focused on improving efficiency in the simultaneous saccharification and fermentation process by utilizing cellulase enzymes for second-generation bioethanol production from sugarcane bagasse. The pretreatment process involved removing lignin by refluxing and heating in 1 N NaOH solution for 2 hours. SSF was performed using a combination of *Saccharomyces cerevisiae*, cellulase enzyme from *Trichoderma reesei*, and nutrients for 3, 5, and 7 days as incubation time variables. The optimal result was achieved at 5 days of incubation with an ethanol content of 0.7485% and reducing sugar concentration of 0.4418 mg/mL. This indicates that incubation time affects ethanol content and reducing sugar concentration; longer incubation leads to higher ethanol content and reduced sugar concentration [13].

Reference [9] studied the effect of fermentation time and the addition of crude cellulase on the concentration of bioethanol from sugarcane bagasse using the SSF method. The study included several stages, namely preparation of raw materials for sugarcane bagasse,

preparation of crude cellulase, fermentation implementation, and analysis of bioethanol products. The study focused on fermentation time with three levels (48 hours, 72 hours, and 96 hours) and the addition of crude cellulase from *Phanerochaete chrysosporium* with variations (5%, 10%, 20%, 30%, 40%, and 50% (v/v)). The results showed that increasing the fermentation time to 96 hours and the concentration of crude cellulase to 50% significantly increased the content and yield of bioethanol. The best conditions were achieved at a crude cellulase concentration of 50% and a fermentation time of 96 hours, resulting in an ethanol content of 9.22% and an ethanol yield of 10.4%. In other words, the longer the fermentation and the addition of crude cellulase, the greater the concentration and yield of bioethanol produced [9].

Reference [14] continued his research using fermented sugarcane bagasse with *Phanerochaete chrysosporium* to produce crude cellulase. The crude enzyme results obtained were used to produce bioethanol with sugarcane bagasse raw materials using the SSF method, with variations in fermentation time of 96, 120, and 144 hours and the addition of crude cellulose at 10, 20, 30, 40, and 50% (v/v), the best process conditions were obtained at a fermentation time of 144 hours, and the addition of crude cellulase 50% v/v, the ethanol content in the fermentor was 11.04%, and the yield was 16.24%. These results are good and in accordance with the levels of bioethanol in fermenters carried out in industry, but there are still shortcomings, namely the use of too much enzyme so that it is less effective, so further research is needed to increase the enzyme concentration.

Reference [15], continued the research on bioethanol production using sugarcane bagasse as raw material with variable changes in the addition of *Saccharomyces cerevisiae* 1, 2, 3, 4, and 5% (w/v), and the addition of crude cellulose 40, 50, and 60% (v/v). In this research, the best conditions were obtained by adding 4% *Saccharomyces cerevisiae* and 60% crude cellulase. Bioethanol was obtained with a concentration of 9.18% and a yield of 16.79%.

Based on previous research, the author conducted a follow-up study on the production of bioethanol using sugarcane bagasse as the raw material, but utilizing commercial cellulase enzymes to see if commercial cellulase enzymes are capable of hydrolyzing lignocellulosic components like the crude cellulase enzymes produced by *Phanerochaete chrysosporium* and can produce a sufficiently high ethanol concentration similar to the previous study that used crude cellulase enzymes from *Phanerochaete chrysosporium*? This study will examine the changes in enzyme addition variables and fermentation duration with the hope of obtaining bioethanol products in a fermentor with concentrations approaching those found in industrial fermenters (8-12%). In this research, variations in cellulase enzyme concentration will be conducted according to theoretical enzyme requirements (3% and 3.5% v/v) and fermentation durations (3, 4, 5, 6, and 7 days) based on previous studies. The analysis conducted includes the composition of lignocellulose (lignin, hemicellulose, cellulose) before and after delignification, ethanol yield, and ethanol content.

2 Material and Methods

2.1 Material and Equipment

Material. The materials used in this research include sugarcane bagasse, glucose, cellulase enzyme, technical concentrated H_2SO_4 (95-98%), CTAB (Cetyltrimethylammonium Bromide), Sodium Lauryl Sulphate, EDTA, Sodium Borate Decahydrate, Disodium Hydrogen Phosphate, 2-Ethoxy Ethanol, ADS (Acid Detergent Soluble) solution, NDS (Neutral Detergent Soluble) solution, DNS (Dinitro salicylic Acid), Ka-Na Tartrate, CMC (Carboxymethyl Cellulose), alcohol, 2M NaOH, 12% NaOH, $(NH_4)_2PO_4$, $MgSO_4 \cdot 7H_2O$, yeast (*Saccharomyces cerevisiae)*, distilled water, and ACN (Acetonitrile).

The equipment used: beaker glass, measuring cup, Erlenmeyer, Erlenmeyer flask, stirring rod, burette, bulb pipette, dropper, pycnometer, glass funnel, thermometer, oven, furnace, desiccator, analytical balance, sieve, blender, spatula, mashed glass, watch glass, reflux condenser, two-neck flask, heating mantle, Fermenter capacity 10 L Major Science Bench Top MS-F1, boiling flask, adapter, Erlenmeyer flask, hot plate, Liebig type condenser, stand, clamp, distilling head, hose, vacuum pump, vortex, autoclave, Vis spectrophotometer, gas chromatography (GC-FID), filter cloth, pH paper, aluminium foil.

2.2 Methods

2.2.1 Pretreatment of Sugarcane Bagasse

The sugarcane bagasse was cleaned with water to remove impurities, sun-dried for 3-5 days until dry, then ground into powder and sieved using a screening tool with a size of 100 mesh.

2.2.2 Determination of ADF (Acid Detergent Fiber) Content

Approximately 0.4 grams of the sample was weighed and placed into a beaker glass. Then, 40 mL of ADF solution was added, and the beaker glass was covered. The mixture was boiled in a water bath at 100˚C for 1 hour, with occasional shaking. It was then filtered using a vacuum pump and washed with approximately 100 mL of hot distilled water and 50 mL of 70% ethanol using the vacuum pump. The washed residue was oven-dried at 105˚C for 8 hours, cooled in a desiccator for approximately ½ hour, and then weighed (b grams).

2.2.3 Determination of NDF (Neutral Detergent Fiber) Content

Approximately 0.2 grams of the sample was weighed and placed into a beaker glass. Then, 30 mL of NDF solution was added, and the beaker glass was covered. The mixture was boiled in a water bath at 100˚C for 1 hour, with occasional shaking. The residue was filtered using a pre-weighed glass crucible (a grams) equipped with a vacuum pump and washed with approximately 100 mL of hot distilled water and 50 mL of 70% ethanol using the vacuum pump. The residue was oven-dried at 105˚C for 8 hours, cooled in a desiccator for approximately ½ hour, and then weighed (b grams).

2.2.4 Determination of Lignin and Cellulose Content

The ADF-containing glass crucible was placed in a beaker glass, and 20 mL of 72% H2SO4 was added to the glass crucible and homogenized. The mixture was left for 2 hours in a fume hood. The solid was oven-dried for 8 hours at 105˚C, placed in a desiccator, and then weighed (c grams). The sample was then placed in a porcelain crucible and asked in a furnace at 500˚C for 2 hours, then cooled in a desiccator for approximately ½ hour and weighed (d grams).

2.2.5 Delignification Process

A total of 50 grams of sugarcane bagasse powder was soaked in a 12% NaOH solution, with a ratio of sugarcane bagasse mass to NaOH volume of 1:10. The solution was heated in an autoclave at 121°C for 1 hour. The solution was then cooled and filtered using a filter cloth. The precipitate was collected, washed with distilled water until neutral, and dried in an oven for 6-7 hours at 105˚C.

2.2.6 Cellulase Enzyme Activity Test

A total of 1.8 mL of 1% CMC substrate and 0.2 mL of cellulase enzyme were mixed in a test tube. The mixture was homogenized using a vortex mixer, heated in water at 50°C for 60 minutes, then 2 mL of DNS was added and homogenized again, and the mixture was heated in boiling water at 100°C for 5 minutes. The absorbance value of the mixture was measured at a wavelength of 540 nm using a UV-VIS spectrophotometer, followed by plotting a graph.

2.2.7 Simultaneous Saccharification and Fermentation (SSF) Process

The SSF medium was prepared first, consisting of 7% (b/v) sugarcane bagasse powder and 4000 mL of nutrient medium, and sterilized in an autoclave at 121°C for 15 minutes. After cooling, the SSF medium was placed into a fermenter. Yeast at 4% (b/v) and cellulase enzyme with varying concentrations of 3% and 3.5% were added to the fermenter. The enzyme concentration used was based on theoretical enzyme requirements. The media was incubated at 30°C, stirring at 100 rpm for 8 hours per day, with varying incubation times of 3, 4, 5, 6, and 7 days based on previous research results. Samples were taken from the fermentation results at each incubation time variable. Process fermentation of SSF Process is shown in Figure 1.

Fig. 1. Fermenter capacity 10 L.

3 Results and Discussion

The Van Soest method was used in this study to analyze the composition of sugarcane bagasse. Measurements of ADF (Acid Detergent Fiber), NDF (Neutral Detergent Fiber), cellulose, hemicellulose, lignin, and insoluble ash content were made, and the values of these components were compared before and after delignification. A 12% (w/v) NaOH concentration was used for delignification. Using NaOH solution during the delignification process increases the cellulose content, with an optimal cellulose content of 50% at a NaOH concentration of 12% (w/v) [16].

ADF is a nutrient component that is insoluble in acid detergent and is made up of cellulose, lignin, and silica. Comprising the components of the ADF, cellulose is the most easily digested, whereas lignin's double linkages make it more difficult to digest. Low digestibility coefficients in feed can be caused by a high lignin content. NDF is a nutritional component that plays a significant role in the construction of plant cells and is insoluble in neutral detergent. It is made up of fiber protein, lignin, cellulose, hemicellulose, and silica [17].

Table 1. The effect of delignification for ADF, NDF, cellulose, hemicellulose and lignin content.

Component	Before Delignification (%)	After Delignification (%)
ADF	62,5	57,5
NDF	85	70
Cellulose	37,5	47,5
Hemicellulose	22,5	10
Lignin	20	7,5
Ash	5	2,5

The delignification process using 12% NaOH at a temperature of 121°C for 1 hour was able to reduce the lignin content by 62.5% and increase the cellulose content by 27%. This delignification process not only reduced the lignin content but also decreased the hemicellulose content by 55.56%, as hemicellulose compounds are easily affected by 12% NaOH, even though hemicellulose is also needed to produce simple sugars. Similarly, the results from ADF and NDF also decreased. This outcome is better than using Na_2CO_3 (also an alkaline pretreatment), which only increases cellulose content by 8.6% [18]. The dissolving of lignin and hemicellulose as well as the deesterification (saponification) of ester linkages between molecules are the primary processes in the alkaline pretreatment process. Alkaline pretreatment causes the hemicellulose, lignin, and silica to dissolve; uranic and acetate esters to hydrolyze; and cellulose to expand, all of which contribute to the breakdown of the cell wall [19].

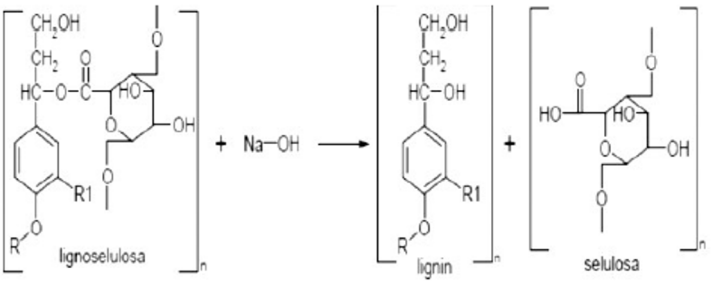

Fig. 2. Mechanism of Breaking the Bonds between Lignin and Cellulose Using NaOH [20].

The polyionic characteristics of lignocellulose are improved by pretreatment with NaOH, which is linked to the diffusion of sodium ions (Na) into the lignocellulosic structure. As a carboxylate ion exchange agent, NaOH causes the material to swell significantly. Consequently, the amount of lignin drops, and cellulose content increases in lignocellulosic substrates. NaOH can dissolve the bonds that bind cellulose, hemicellulose, and even the lignin itself. The procedure includes cleaving the ether and ester bonds that bind lignin to hemicellulose and to cellulose as well as the hydrogen bonds that bind lignin to hemicellulose. NaOH can also break down lignin complexes with carbon and ether linkages into smaller lignin fragments. Additionally, the reaction with NaOH disrupts bonds in lignin compounds, which causes lignin molecules to degrade. When hydroxide ions (OH-) from NaOH attack phenolic OH groups, lignin begins to degrade. The solution gets darker, turning a deep black color, which indicates that lignin has disintegrated. This color change signifies the breakdown of compounds containing chromophore groups, which have conjugated double bonds. The results of reference [21] and reference [22], who performed delignification utilizing an alkaline pretreatment with NaOH to extract cellulose and hemicellulose and break down lignin structures in biomass, agree with this study. Increased cellulose content and decreased hemicellulose and lignin content are the outcomes of this delignification procedure.

To assess the effectiveness and performance of the enzymes engaged in biochemical reactions, this study used CMC (carboxyl methyl cellulose) as a substrate and the DNS method to analyze enzyme activity. According to reference [21], the best substrate for inducing the synthesis of extracellular cellulolytic enzymes is carboxymethyl cellulose (CMC), with a concentration of 1% CMC being ideal for cellulase production. This test works on the basis that cellulase degrades CMC into its monomers, which include reducing sugars such cello dextrin. Enzyme activity and the quantity of reducing sugars generated are often correlated; the greater the enzyme activity, the greater the amount of reducing sugars produced. The amount of reducing sugars produced during the reaction was measured using dinitro salicylic acid (DNS) at a wavelength of 540 nm [23].

The Simultaneous Saccharification and Fermentation (SSF) method, a biotechnology approach that combines two crucial phases in the synthesis of bioethanol or other fermentation products in a single stage, was employed in this study's fermentation process. Enzymes like amylase or cellulase are utilized in the initial stage to convert complex materials like cellulose or starch into simple sugars like glucose. Because it creates sugars that microorganisms can ferment, this stage is known as saccharification. During the second stage, known as fermentation, sugars created during saccharification are consumed by microorganisms such as yeast (*Saccharomyces cerevisiae*) and transformed into desired products like bioethanol. With SSF, both phases take place concurrently in a single reactor tank, providing benefits including shortened production times and improved process efficiency [9].

Table 2. The effect of variation of time fermentation and enzyme cellulose concentration for ethanol content.

Enzyme Celullose (%)	Time Fermentation (day)	Ethanol Content (%)	Yield of Ethanol (%)
3 %	3	1,94	9,48
	4	2,02	10,16
	5	2,05	10,90
	6	2,14	11,63
	7	2,17	13,58
3,5%	3	2,45	11,96
	4	2,53	12,93
	5	2,55	14,02
	6	2,62	14,65
	7	2,64	14,78

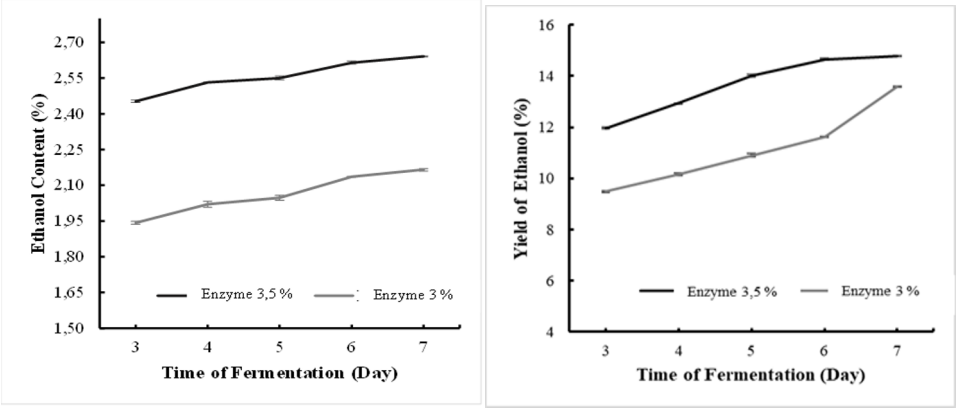

Fig. 3. The Effect of Variation in Enzyme Concentration and Time of Fermentation.

(a). Ethanol Content. (b). Ethanol Yield.

Table 2 shows that the addition of 3% (v/v) enzyme produces less ethanol than the addition of 3.5% (v/v), but the increase in the average ethanol concentration from the third to the seventh day shows that the addition of 3% (v/v) enzyme is more effective than the addition of 3.5% (v/v). Specifically, the increase in enzyme of 3% resulted in an average increase in ethanol concentration of about 2.76%, while the addition of 3.5% (v/v) enzyme resulted in an average increase in ethanol concentration of about 1.87%. The amount of ethanol produced shows that the amount of ethanol produced increases with the concentration of the enzyme added during the SSF process. Based on reference [23], there is an increase in glucose produced using cellulase enzymes to hydrolyze cellulose during the SSF process. Because Saccharomyces cerevisiae has more glucose available for fermentation, the fermentation process will produce a higher concentration of bioethanol [23]. Therefore, the concentration of bioethanol produced increases with the addition of enzymes. This study supports the findings of reference [11], who claim that the amount of bioethanol generated is influenced using enzymes. The research conducted involved corn cob as the raw material and variations in the addition of cellulase enzyme at concentrations of 3, 5, 7, 9, and 11% (v/v). From this study, the highest bioethanol yield of 8% was obtained with the addition of 11% (v/v) cellulase enzyme substrate after a fermentation time of 72 hours. The amount of bioethanol tends to increase with the increase in the number of enzymes used, as more glucose will be converted into bioethanol through that reaction [11].

In addition to enzyme concentration, fermentation time also affects the ethanol content produced. From the data presented, there was an increase in bioethanol content from the third to the seventh day. The longer the fermentation time tends to increase the bioethanol content because *Saccharomyces cerevisiae* (yeast) has more time to convert the substrate (sugar) into ethanol. From the results of the ethanol concentration, the results obtained are higher compared to the comparative results [12] which produced a bioethanol concentration of 0.7485% using cellulose enzymes from *Trichoderma reesei* with an incubation time of 5 days [12]. However, the bioethanol concentration in this study was lower when compared to the references [9],[14], [15] which made bioethanol from bagasse raw materials using crude cellulase from bagasse raw materials using *Phanerochaete chrysporium*, which obtained a higher bioethanol content than the bioethanol content in these results. This is because commercial cellulase enzymes only contain cellulase enzymes and are only able to break down cellulose components. Commercial cellulase enzymes are unable to break down hemicellulose which is also contained in bagasse. This is different from crude cellulase from *Phanerochaete chrysosporium* which contains several types of enzymes other than cellulase, such as xylanase which can break down xylan in bagasse into simple sugars that can be converted into bioethanol [9], [14], [15].

The bioethanol yields described in Table 1 show that the amount of bioethanol yield depends on the duration of fermentation and enzyme concentration; the longer the fermentation period and the higher the enzyme concentration to its maximum limit, the more bioethanol is produced. This is related to the amount of ethanol produced. Because the bioethanol yield and bioethanol concentration are directly correlated, if the bioethanol content in the fermentor is high, the yield obtained is also high. The results of this study are also in accordance with the results of previous studies including references [9], [14], [15].

From several research findings, it can be determined that the factors influencing ethanol yield are enzyme efficiency, which is obtained from enzyme concentration, fermentation time, substrate quality (sugarcane bagasse), and enzyme activity. The concentration of cellulase enzymes has a significant impact on the rate of conversion of cellulose into simple sugars. A concentration of enzymes that is too low may not be sufficient to effectively hydrolyze cellulose, while a concentration that is too high can lead to decreased efficiency due to additional costs and the potential inactivation of the enzymes. The fermentation time affects the amount of ethanol produced. A fermentation period that is too short may not

provide enough time for maximum conversion, while fermentation that is too long can lead to reduced yields because of byproducts or decreased enzyme activity. The quality of the substrate, such as the content of cellulose, hemicellulose, and lignin, affects the yield of ethanol. Enzyme activity is influenced by pH and reaction temperature. By considering these factors and optimizing process conditions, the industry can maximize bioethanol yields from sugarcane bagasse and enhance the efficiency and sustainability of the production process.

4 Conclusion

The concentration and yield of bioethanol produced are influenced by the addition of cellulase enzyme and fermentation time, where the addition of 3.5% enzyme and 7 days fermentation time can produce a concentration of 2.64% and a yield of 14.78%.

For further research, if a single cellulase enzyme is used, the delignification process needs to be varied so that the reduction in lignin content is above 90% so that cellulose degradation can occur optimally, which will have an impact on increasing the bioethanol content in the product. To be applicable in the industry, the concentration of bioethanol produced in the fermentor must reach levels like those in industrial bioethanol fermenters using molasses as raw material, which is around 8-12% by volume or equivalent to 6.4-9.5% by mass.

The authors would like to acknowledge the State Polytechnic of Malang for providing financial support through Industrial Collaborative Research 2024. We also extend our appreciation to all staff in the Chemical Engineering Department for their encouragement and support during this research.

References

1. B. Mohanty and I. I. Abdullahi, Biosciences Biotechnology Research Asia. **13**(2), 1153-1161 (2016)
2. R, Mukhtam, S. K. Bhargava, S. Bankupalli, and A. S. Ball, Journal of Sustainable Bioenergy System. **6**, 72-92 (2016)
3. A. S. D. S. N. Hidayati, S. Kurniawan, N. W. Restu, and B. Ismuyanto, NATURAL B. **3**(4), 311-317 (2016)
4. S. M. R. Khattab and T. Watanabe, Bioethanol Production from Food Crops (Academic Press), 187-212 (2019)
5. C. T. Puttaswamy, B. R. Sagar, U. Simha, S. Manjappa, and C. S. V. Kumar, Indian Journal of Advances in Chemical Science. **S1**, 239-244 (2016)
6. S. Tyagi, K.-J. Lee, S. I. Mulla, N. Garg, and J.-C. Chae, Applied Microbiology and Bioengineering, 21-42 (2019)
7. R. A. Wahyuono, M. N. Hakim, and S. A. Santoso, Energy Procedia. **65**, 324–330 (2015)
8. N. Idiawati, E. M. Harfinda, and L. Arianie, J. Natur Indones. **16**(1), 1-9 (2014)
9. S. Rulianah, Prayitno, N. Hendrawati, and K. N. Nafisa, Production of Bioethanol from Bagasse with A Simultaneous Saccarification and Fermentation (SSF) Process Using Crude Cellulase from Phanerocaete chrysosporium, in AIP Conference Proceedings, Semarang, Indonesia, September 25-26 (2019)
10. M. I. J. Ibrahim, S. M. Sapuan,, E. S. Zainudin, and M. Y. M. Zuhri, Journal of Materials Research & Technology. **9**(1), 200-211 (2020)
11. Z. F. Khaira, E. Yenie, and S. R. Muria, JOM FTEKNIK, **2**(2), 1-8 (2015)
12. S. Khodijah and A. Abtokhi, Jurnal Neutrino. E. **7**(2), 71-76 (2015)
13. S. K. Wahono, V. T. Rosyida, C. Darsih, D. Pratiwi, A. Frediansyah, and Hernawan, Energy Procedia. **65**, 331-336 (2015)

14. S. Rulianah, P. Prayitno, A. Indiastari, and D. Fatmawati, The Effect of fermentation time and addition of crude cellulose to concentration of Bioethanol in bagasse fermentation, IOP Conference Series: Materials Science and Engineering, ATASEC, (2021)
15. S. Rulianah, Prayitno, and C. A. Maulidina, ICOLIB, 398-498 (2023)
16. P. J. V. Soest, Nutritional Ecology of the Ruminant (Commstock Publishing Associates, A Devision of Cornell University Press, Ithaca and London, 1982)
17. D. C. Church and W. G. Pond, Digestive Animal Physiologi and Nutrition 2nd (Prentice Hell a Devision of Simon and Schuster Englewood Clief, New York, 1986)
18. M. J. Nosratpour, K. Karimi, and M. Sadeghi, Journal of Environmental Management. **226**, 329-339 (2018)
19. M. H. Nasution, S. Lelinasari, and M. G. S. Kelana, A review of sugarcane bagasse pretreatment for Bioethanol Production, in International Bioprocessing Association Subject Conference IBASC 2021, IOP Conference Series: Earth and Environmental Science. 953, (2022), 012014
20. S. S. Handayani, Amrullah, H. Fatimah, and R. Seftiani, Journal of Physics: Conference Series. **1280**, 2 (2018)
21. I. Irwan and L. A. Salim, International Journal of Transdisciplinary Knowledge. **2**(2), 1-15 (2021)
22. T. Sriana, T. Dianpalupidewi, S. M .P. Ukhrawi, and I. F. Nata, Buletin Profesi Insinyur. **4**(2), 49-52 (2021)
23. A. L. Lehninger, Dasar-Dasar Biokimia jilid 1 (Erlangga, Jakarta, 2022)