International Journal on Advanced Science Engineering Information Technology

Vol.14 (2024) No. 1
ISSN: 2088-5334

Sustainable Biorefinery: Effect of Time Fermentation on Hidrolisis Product from Cocoa Pod Husk

Desniorita [a,*], Novizar Nazir [b], Rita Youfa [a], M. Taufik Eka Prasada [c], Elda Pelita [c]

[a] *Department of Renewable Energy Bioprocess Engineering Technology, ATI Padang Polytechnic, 25171, Indonesia*
[b] *Faculty of Agricultural Technology, Andalas University, 25163, Indonesia*
[c] *Department of Chemical Analysis, ATI Padang Polytechnic, 25171, Indonesia*
Corresponding author: [*]desniorita@gmail.com

***Abstract*— Cocoa-producing nations, including Indonesia, play a pivotal role in global cocoa production, with cocoa beans as a primary agricultural commodity. Despite this, cocoa pod husks (CPH) have historically been dismissed as household waste. This study investigates the untapped potential of CPH for bioethanol production, focusing on the influence of fermentation duration on hydrolysis products. Using 1.5 N sulfuric acid, cocoa pods underwent a 120-minute hydrolysis with a 1.9 g/mL concentration/solvent ratio. Subsequent fermentation involved a 10% *Saccharomyces cerevisiae* starter culture at 30°C and pH 5 under anaerobic conditions. Mass chromatography spectroscopy revealed a progressive ethanol increase, peaking at 4.21% volume on the seventh day, and a substantial reduction in remaining sugar content from 18.45% to 5.53%. These findings underscore the correlation between prolonged fermentation and enhanced ethanol production. The study highlights CPH's potential as a valuable resource for sustainable bioethanol production, reducing waste and offering a renewable energy source. Emphasizing resource optimization and environmental stewardship in agriculture, this research aligns with the global demand for eco-friendly alternatives in the energy sector.**

***Keywords*— Biorefinery; time fermentation; cocoa pod husk; *Saccharomyces cerevisiae*; gas chromatography.**

Manuscript received 8 Nov. 2022; revised 3 Oct. 2023; accepted 9 Jan. 2024. Date of publication 29 Feb. 2024.

I. INTRODUCTION

Indonesia was the largest cocoa-producing Asian country in the world besides Malaysia. Cocoa (Theobroma cacao l.) is a plant that is widely consumed globally. This plant is most widely cultivated in rural areas [1], [2]. The cocoa rind is a waste product (cocoa fruit waste), which is quite a lot compared to cocoa beans. The largest waste from cocoa bean processing is the cocoa husk or cocoa pod, which accounts for 75% of the total fruit [3]. Many cocoa pod husks will be advantageous if used as raw material to synthesize ethanol or biogas [4], [5]. The most significant component of the cocoa rind is carbohydrates. The carbohydrates from cocoa pod husk waste can be used as animal feed nutrients. Along with advances in science and technology, cocoa pod husks can now be fermented into bioethanol with the help of *Saccharomyces cerevisiae* [6].

Indonesia was the largest cocoa-producing Asian country in the world besides Malaysia. Cocoa *(Theobroma cacao l.)* is a plant that is widely consumed globally. This plant is most widely cultivated in rural areas [], [2]. The cocoa rind is a waste product (cocoa fruit waste), which is quite a lot compared to cocoa beans. Cocoa pod husks have not been used for a long time and are only disposed of as organic waste or used as animal feed. Many cocoa pod husks will be advantageous if used as raw material to synthesize ethanol or biogas [4], [5]. The most significant component of the cocoa rind is carbohydrates. The carbohydrates from cocoa pod husk waste can be used as animal feed nutrients. Along with advances in science and technology, cocoa pod husks can now be fermented into bioethanol with the help of *Saccharomyces cerevisiae* [6].

Fermentation is the process of producing energy in cells under anaerobic conditions (without oxygen). Fermentation is a process that does not require oxygen to break down glucose and is used by various microorganisms and cells [7], [8]. Glycolysis with one or two other reactions will be the only way fermentation produces energy [9]. In general, the fermentation process is the process of converting sugar into ethanol. In the conversion of sugar to ethanol, *Saccharomyces cerevisiae* (yeast) can help [10]–[12]. The fermentation process usually occurs at a temperature of 30°C and a pH of

4-5. The fermentation process will also produce ethanol and CO_2 [13]. *Saccharomyces cerevisiae* can ferment lignocellulosic biomass from cellulose compounds, namely hexose and glucose, into ethanol. *Saccharomyces cerevisiae* for fermentation gave better results because this yeast can work well under anaerobic conditions [14]. Bioethanol was ethanol whose main ingredients were plant origin and was commonly used in pharmaceutical processes. Therefore, Indonesia still needs a more effective source of bioethanol as fuel [15]. Ethanol, or ethyl alcohol, is a clear, colorless liquid, soluble in water, ether, acetone, benzene, and all organic solvents. It has a characteristic odor of alcohol, is biodegradable, has low toxicity, and does not cause major air pollution if it leaks [16]. The raw materials used in the production of yeast-based ethanol, mainly in China, America, and Brazil, usually use corn and sugarcane as raw materials [17]–[19]. In addition, raw materials derived from dates can also produce bioethanol [20].

Biorefinery is stated as a solution to handling bioeconomics [21]. Technological developments provide solutions to the circular economy, such as processing solid organic waste into a sustainable economic cycle that is important in reducing greenhouse gas emissions and supporting energy systems and a green environment [22]–[24]. Several studies on solid organic materials have been conducted with the biorefinery approach. Protein extraction has been investigated using a biorefinery approach in the laboratory. The review found that agricultural food residues can produce various proteins with dry or wet extraction [25]. In addition, research related to biorefinery uses coffee grounds as fuel. A review of research found that coffee grounds through biorefinery can produce oil from pyrolysis extraction and be used as biofuel [26]. Research on used coffee grounds is also conducted in a multilevel biorefinery, which produces coffee oil as biodiesel, methane gas, and fermentable sugar [27]. The biorefinery of ginger pulp has also been investigated. Industrial ginger dregs with a biorefinery approach can produce cellulose, starch, microfibrillation with hydrothermal technique, and ginger oil with pyrolysis technique [28].

Research with hydrothermal and enzymatic pretreatment, which then produces bioethanol by Candida tropicalis and *Saccharomyces cerevisiae* on cocoa pods. The best production was produced by *Saccharomyces cerevisiae,* reaching 45.2 g of bioethanol per kg of cocoa pods [6]. Pretreatment with delignification and hydrolysis is required to convert cocoa pods into reducing sugars or glucose [29]. The resulting reduced sugar can be fermented into bioethanol [30]. Research on lignocellulosic biomass through biorefinery from cocoa pods is still limited. Developing lignocellulosic biomass technology through biorefinery is considered feasible for future conversion processes [31]. This study aimed to see the effect of the fermentation time of reduced sugar produced by the hydrolysis of cocoa pods on bioethanol production using the biorefinery approach.

II. Material and Method

A. Material

The materials used include cocoa pod husks of the Ferestero variety, anhydrous citric acid 6%, 95% alcohol, NaOH 0.1N, NaOH 0.05 N, HCl 5%, HCl 0.25 N, NaOH, 0.5 ml solution of phenol 5%, 2.5 ml concentrated H_2SO_4, H_2SO_4 1.5 N, 10.6 g of 3,5-dinitro salicylic acid, 19.8 g NaOH, 306 g Na-K tartrate, 7.6 g, 8.3 g Na-Metabisulfite, HCl 0.1 N, 2 grams of NaOH, phenolphthalein indicator, *Saccharomyces cerevisiae* yeast. The growth medium for the *Saccharomyces cerevisiae* inoculum consists of 20 grams/L of sucrose, 5 g/L yeast extract, K_2HPO_4, 1.5 kg m-3 NH_4Cl, 1.15 kg m-3 KCl, and 0.65 kg m-3 $MgSO_4.7H_2O$. A total of 50 mL.

B. Apparatus

The equipment used in this study includes blender, water bath, analytical balance, plastic container, 80-mesh sieve, beaker, oven, muffle furnace, porcelain crucible, aluminum crucible, cone and plate viscometer, rotary vacuum evaporator, desiccator, pH meter, hot plate, mortar and pestle, Erlenmeyer flask 250 ml, electric furnace, magnetic stirrer, screen cloth 60 T, autoclave, Erlenmeyer flask 500 ml, vacuum filter, paper filter, sieve, fermentation apparatus set (bioreactor), distillation apparatus, colorimeter, Erlenmeyer flask 500-1000 ml, vacuum separation apparatus, analytical balance, and Gas Chromatography-mass spectrometry Shimadzu QP2010s.

C. Research Design

This study aims to convert cocoa husks into bioethanol through a series of processes, including delignification, hydrolysis, and fermentation. This research's main focus is to evaluate fermentation time's influence on cocoa pod husks. Fermentation time was varied within the optimal temperature range of 30 °C, maintaining pH at 5, and conducted over 1 to 7 days under anaerobic conditions. *Saccharomyces cerevisiae* was employed at a concentration of 10%.

D. Delignification Process

Cocoa husks were initially cleaned and cut into small pieces approximately ± 5 cm x 5 cm, then finely ground using a blender. Next, water was added at a ratio of 1:4. The result obtained was referred to as cocoa husk slurry, which is left to stand for 30 minutes. The chocolate slurry from cocoa husk waste is mixed with a citric acid solution with a pH of 2.5. The acid slurry is heated to a temperature of 95°C while stirring for 3 hours. After heating, the acid slurry was filtered using a vacuum suction filter to separate the filtrate and the residue. Subsequently, the residue, with a substrate/solvent ratio of 58 grams/ml, was placed in a 500 ml Erlenmeyer flask, and NaOH 2.7% pre-treatment reagent was added. The mixture is stirred using an orbital shaker at a speed of 2.5 rpm for 118 minutes. This process is known as delignification, to remove lignin.

E. Hydrolysis Process

The raffinate from the delignification process was then subjected to the cocoa husk hydrolysis process to obtain cellulose. Hydrolysis was conducted using a solvent of H_2SO_4 1.5 N, a reaction time of 120 minutes, and a substrate/solvent ratio of 1:9 grams/ml [32]. The mixture was then heated using an autoclave. Afterward, the separation between the solid and liquid phases was performed. The liquid phase is subsequently used as a sample in the fermentation process.

F. Fermentation Process

During the hydrolysis process, the liquid phase was fermented using Saccharomyces cerevisiae in a 1000 mL flask at a temperature of 30°C, pH 5, and for a duration of 1 to 7 days (fermentation under anaerobic conditions) on a shaker at 20 rpm. The fermentation was conducted using a fermenter at the laboratory scale. Sampling was conducted every 24 hours during fermentation with two repetitions for ethanol content analysis. Distillation was also performed to obtain pure ethanol. The distillation process utilized a temperature of 78°C, the boiling point of alcohol. The procedure involved introducing the fermented ethanol into the distillation flask. The ethanol vapor from the distillation was directed to the condenser and condensed back into ethanol. Subsequently, the distilled product was collected in an Erlenmeyer flask. The ethanol content was then determined using Gas Chromatography-mass spectrometry Shimadzu QP2010s. The ethanol and residual sugar content were used to calculate the yield.

III. Results and Discussion

A. Objective and Experimental Setup

This study aimed to utilize cocoa rind as an ingredient to produce bioethanol, considering it a potential alternative to fossil fuels. The fermentation process, using 10% Saccharomyces cerevisiae, was conducted in an Erlenmeyer connected to a U-pipe with a plastic hose and Vaseline-sealed connections to prevent air ingress during fermentation (Figure 1).

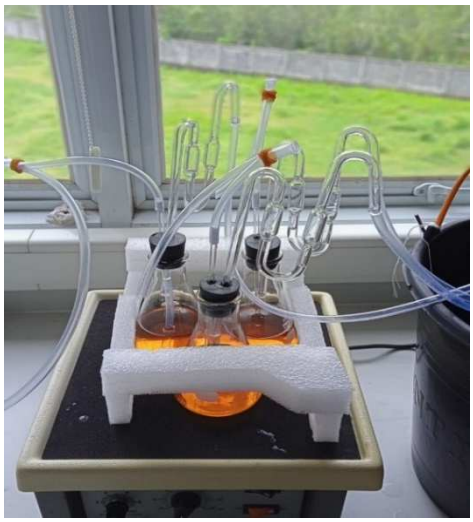

Fig. 1 Cocoa Pod Husk Bioethanol Fermentation Bioreactor

Hydrolyzed with 1.5 N sulfuric acid, the substrate contains (18.45 ± 1.34) % reducing sugar. The fermentation process was carried out in 1 to 7 days with conditions at pH 5 at 30 °C room temperature. Bioethanol produced from fermentation is distilled at a temperature of 78 °C. Bioethanol results obtained from the fermentation process after distillation were analyzed using Gas Chromatography (GC). The results of the bioethanol research obtained are as follows:

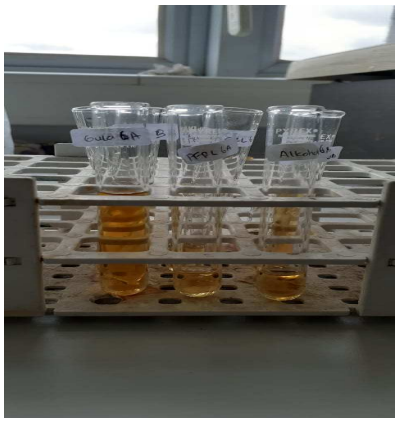

Fig. 2 Cocoa Pod Husk Substrate Fermentation Results

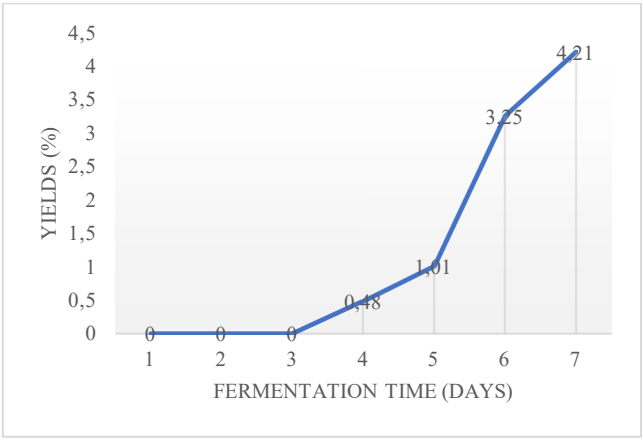

Fig. 3 Graph of Bioethanol Analysis Results Using Gas Chromatography

Determining the color of cocoa husks, as depicted in Figure 2, was significant as it provided a visual indication of the fermentation process. The color of the liquid from the fermentation results presented a clear yellow hue on the cocoa husks, serving as a visual parameter to comprehend changes in raw materials during the fermentation process. The trend of increasing yields, as observed in Figure 3, had a crucial foundation. The observed increase in ethanol production over the fermentation period indicated the presence of specific factors influencing bioethanol production. The rise in bioethanol yields over the fermentation period demonstrated a significant relationship between the fermentation duration and bioethanol production [33].

The biochemical processes occurring during fermentation, such as the conversion of sugar into ethanol by Saccharomyces cerevisiae, along with factors like sustained enzyme activity and nutrient availability during fermentation time, were noteworthy. The increase on the fourth day, by 0.48%, might be associated with the early adaptation of Saccharomyces cerevisiae to the environment. On the fifth day, the 1.01% increase reflected faster growth as microorganisms actively metabolized the substrate [34]. The significant increases on the sixth and seventh days, 3.25% and 4.21%, respectively, indicated the peak of bioethanol production attributable to optimal fermentation conditions.

B. Reducing Sugar Content Analysis

The analysis of reducing sugar content, as depicted in Table 1, reveals a notable trend of continuous reduction,

reaching a significant decline on the fourth day of fermentation. This observation suggests dynamic changes in the substrate composition, specifically the breakdown of sugars during the fermentation process. Understanding these variations is crucial for comprehending the efficacy of bioethanol production from cocoa pod husks. In the subsequent sections, we delve into the temporal dynamics of sugar content, correlating it with the stages of fermentation and the resultant bioethanol yields.

TABLE I.
RESULTS OF ANALYSIS OF REDUCING SUGAR LEVELS PER FERMENTATION DAY

Fermentation Time (days)	Reducing Sugar Content (%)
1	18,45 ± 1,34
2	18,45 ± 1,34
3	17,15 ± 0,48
4	10,97 ± 1,12
5	9,64 ± 1,21
6	7,74 ± 0,81
7	5,53 ± 0,91

Table 1 shows the reduced sugar content progressively decreasing until the seventh day. On the first, second, and third days, the change in reducing sugar scores was not visible. However, on the fourth day, the reducing sugar content was significantly reduced. Likewise, the yield formed was visible on the fourth day (Figure 3). On the seventh day, the remaining reduced sugar content was 5.53%, whereas the original was 18.45%.

C. GC Analysis of Bioethanol

Standard charts and analysis of cocoa pod husk bioethanol fermentation using GC are also presented to see the formation of bioethanol. Standard graphs and analysis of cocoa hull bioethanol fermentation using GC are presented on day 3 to day 7, which can be seen in Figures 4–9.

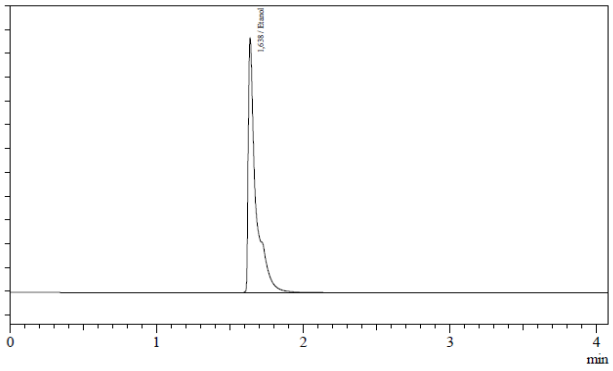

Fig. 4 Graph of Ethanol Standard GC Analysis.

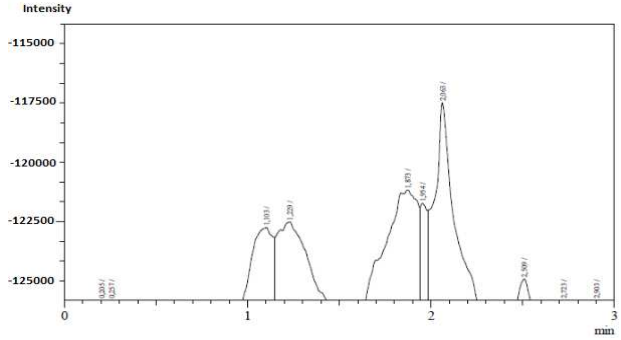

Fig. 5 Analysis of GC Bioethanol on Day 3 Fermentation.

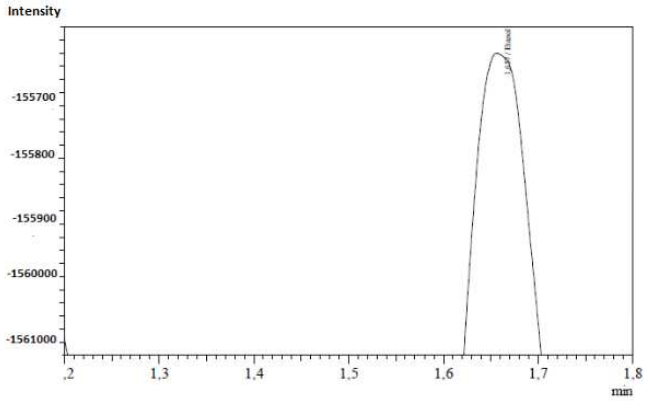

Fig. 6 Analysis of GC Bioethanol on Day 4 Fermentation.

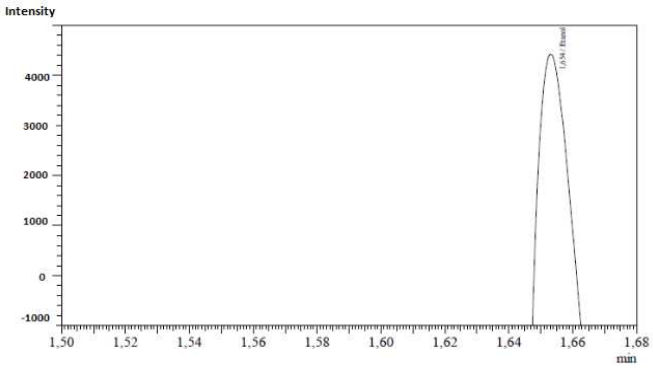

Fig. 7 Analysis of GC Bioethanol on Day 5 Fermentation.

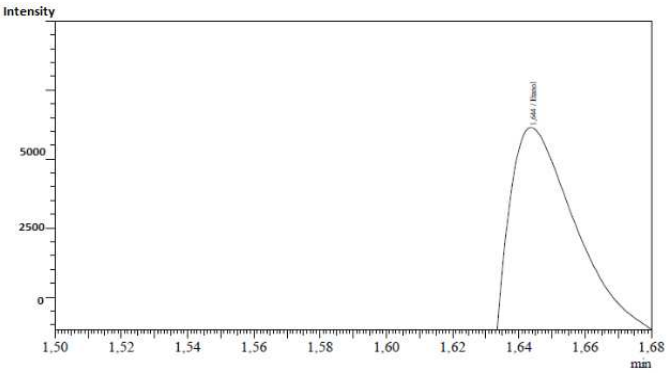

Fig. 8 Analysis of GC Bioethanol on Day 6 Fermentation.

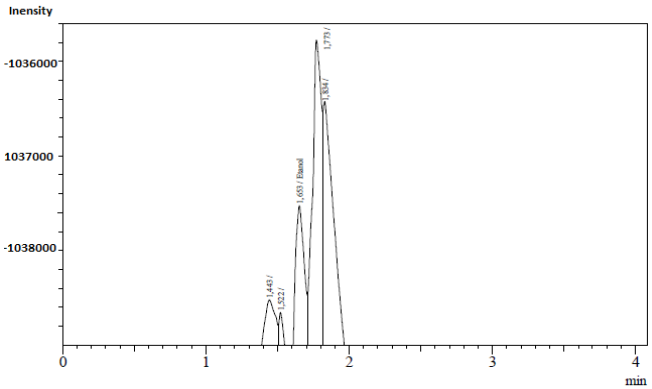

Fig. 9 Analysis of GC Bioethanol on Day 7 Fermentation.

Graph of GC analysis of bioethanol from day 3 to day seven fermentation (Figure 5-9). Each peak of the GC analysis results obtained a retention time value close to the standard value. The standard GC retention time value was 1.6537 (Figure 4), and the retention time value on the fourth day was

1.657 (Figure 6), the fifth day was 1.654 (Figure 7), the sixth day was 1.644 (Figure 8), and on the seventh day was 1.653 (Figure 9). From the retention time value obtained from the GC analysis, it can be stated that bioethanol was not formed on the third day. Bioethanol was only formed on the fourth day, and the highest was on the seventh day.

D. Discussion of Fermentation Phases

The general fermentation process was the aerobic breakdown of carbohydrates into alcohol using microbes [35]. The basic principle of fermentation is to activate certain microbial activities to change the material's properties (substrate) to obtain the desired results. The microbe used to produce alcohol was the yeast *Saccharomyces cerevisiae* [36]. Yeast *Saccharomyces cerevisiae* had a high tolerance for alcohol so that it could produce more bioethanol [37].

Several growth phases affect the fermentation process: substrate, temperature, pH, and fermentation time. The length of fermentation time is closely related to the growth of *Saccharomyces cerevisiae.* In general, microbial growth can also be described by a growth curve that shows the growth phase. There are 4 phases of microbial growth: the adaptation phase, the fast-growing phase, the stationary phase, and the death phase [38].

The adaptation phase is the initial phase where microbes are grown. This study observed the adaptation phase from the first to the third day (Figure 3). The absence of the graph clearly shows ethanol formation (yield of 0%). The adaptation phase was drawn from the zero-curve line. Then there was a slight increase. In this phase, *Saccharomyces cerevisiae* undergoes a period of adaptation to its environment [39].

The fast-growing phase was depicted by a curve line that shows a sharp increase. In this study, the growth phase occurred on the fourth day, where the graph shows the formation of ethanol (yield 0.48%). In this phase, *Saccharomyces cerevisiae* experienced rapid growth. In this phase, there were many anaerobic sugar breakdowns for the growth of *Saccharomyces cerevisiae* to produce alcohol (ethanol). High ethanol was produced in this phase. The increase in ethanol yield and the reduction in sugar content were caused by an enzymatic process carried out by *Saccharomyces cerevisiae* It converts glucose in the hydrolysate of lignocellulosic biomass into ethanol and carbon dioxide [40]. In addition, the immobilized *Saccharomyces cerevisiae* will convert glucose into ethanol more quickly. This is evidenced by the faster depletion of reducing sugar concentrations [41]. The high decrease in sugar content occurred on day 7, indicating that the highest activity of *Saccharomyces cerevisiae* to form ethanol was on day 7 [42].

Next is the stationary phase, which is a phase that describes a horizontal curve line, meaning that the number of live *Saccharomyces cerevisiae* was proportional to the dead. A descending curve depicted the death phase. This phase shows that the number of dead *Saccharomyces cerevisiae* was more significant than the living ones until all existing *Saccharomyces cerevisiae* died [43]. Based on the graph in Figure 3, it can be said that the stationary and death phases of *Saccharomyces cerevisiae* did not occur in this study. The study's findings hold significant implications for bioethanol production and sustainable waste management. By successfully converting cocoa pod husks into bioethanol, the research demonstrates the potential for expanding feedstock sources in pursuing renewable energy alternatives. This addresses the global need for cleaner energy and contributes to environmental and economic benefits through waste reduction and creating a renewable energy source. The study underscores the importance of optimizing agricultural practices by repurposing waste, promoting sustainability in the cocoa industry, and encouraging the exploration of innovative waste-to-resource solutions. However, it is crucial to acknowledge limitations, such as challenges in the fermentation process, to guide future research and refine bioethanol production methods. The study provides valuable insights into sustainable practices and renewable energy possibilities within the agricultural sector.

IV. CONCLUSION

The study successfully demonstrated that hydrolyzing cocoa pod husks with 1.5N sulfuric acid and fermenting them with Saccharomyces cerevisiae leads to bioethanol production. Notably, the findings underscore the significant impact of fermentation time on ethanol formation. The fermentation duration plays a crucial role in converting reducing sugars from cocoa pod hydrolysis to bioethanol, with a peak yield of 4.21% observed over seven days. In emphasizing the main findings, it becomes evident that optimizing fermentation time is key to enhancing bioethanol production from cocoa pod husks. Moving forward, to enhance the completeness of this research, future studies could explore additional factors influencing fermentation efficiency, consider variations in hydrolysis conditions, and assess the scalability of the biorefinery approach for larger-scale applications.

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