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# Bioethanol Synthesis from Durian Seeds Using *Saccharomyces cerevisiae* in Aerobic Fermenter and Bioethanol Enrichment by Batch Vacuum Distillation

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## Abstract

Bioethanol is an alternative energy of environmentally friendly as a substitute for petroleum. Sucrose, starch, and fibrous cellulose (lignocellulose) are the main ingredients for bioethanol production. The material is very easy and abundant to get from the waste of agricultural crops. One of these agricultural wastes in Indonesia that have not been used optimally is durian seeds. Durian seeds only become waste and are not commercially useful, even though they contain high carbohydrates, which is possible as a potential new source for bioethanol production. In this work, an experimental study was conducted on bioethanol synthesis from durian seeds through fermentation by *Saccharomyces cerevisiae* yeast in aerobic fermenter. The process for the production of starch-based bioethanol includes milling, hydrolysis, detoxification, fermentation, and distillation. At the stage of fermentation, variations in the duration of fermentation were applied for 1-11 days. Carbohydrates contained in durian seed flour are 11.541%, which is the largest content. The highest result of ethanol content is 14.72 % (v/v) in 9 day fermentation periods by using *Saccharomyces cerevisiae* in aerobic conditions. Distillation to enrich bioethanol was carried out by batch vacuum distillation at 68°C for ± 180 minutes and produced bioethanol with a purity of 95%.

## INTRODUCTION

Nowadays, fossil fuel is decreasing at an alarming rate, and its combustion is causing environmental pollution (Lainez et al., 2019). Therefore, renewable and sustainable energy sources are needed because they do not affect the environment and ecosystem (Moraiz et al., 2019). One of the innovative solutions is producing bioethanol from fruit waste (Datta et al., 2008). Bioethanol ($C_2H_5OH$) is an environmentally alternative that can be used as a source of oil. Sucrose, starch, and lignocelluloses are the main substances used for bioethanol production (Maryam & Santosa, 2016).

Bioethanol production can be used to replace fossil fuel for transportation sources as it has cheaper production cost and lower carbon emulsion instead of high bioethanol content per liter (Carvalho et al., 2016; Marzo et al., 2019; Dutta et al., 2014). In Indonesia, bioethanol is very potential to be produced and developed since its material is from sugar. It is a plant that widely grows in this country, and it is well known for the

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public. Durian is the name of a fruit from the tropical plant growing well in the Asian region, especially in Southeast Asia, such as Malaysia, Indonesia, Thailand, and The Philippines (Amid & Mirhosseini, 2012). Durian fruit seeds have a high enough carbohydrate content in starch so that carbohydrates can be processed and appropriately utilized. One of which is processed into second-generation bioethanol (ethanol produced from lignocellulosic biomass) (Sims et al., 2009; Robak & Balcerek, 2018; Arlofa et al., 2019).

The process steps for bioethanol production are milling, hydrolysis, fermentation, and distillation (Szambelan et al., 2018). However, due to the complex nature of lignocellulosic biomass present in durian seeds, pretreatment steps such as hydrolysis are essential for the release of sugars which can then be fermented with bioethanol-producing microorganisms (*Saccharomyces cerevisiae*) (Han et al., 2013; Khattab & Kodaki, 2014; Oh et al., 2019).

Although there have been many studies on bioethanol from agricultural waste, new sources of durian seeds are still limited. Especially in the fermentation stage, using aerobic fermenter and bioethanol enrichment using batch vacuum distillation has never been studied. In this study, we also carried out a detoxification process, usually a major inhibitor in the fermentation process using natural weak bases. The study begins by converting starch from durian seeds into glucose through acid hydrolysis with the heating process. Then, the inhibitors in lignocellulosic hydrolysates can be reduced by detoxification using a base solution. Variations of the research are carried out in the fermentation process with different fermentation times. Ethanol content was analyzed by GC using the *n*-propanol method. Also, characterization was also carried out by using FTIR to determine changes in functional groups and SEM to determine the morphology of material changes during the synthesis process.

## MATERIALS AND METHODS

### Materials

The durian (*Durio zibethinus*) seeds waste was collected from around the campus area of Universitas Negeri Semarang, Gunungpati, Semarang, Indonesia. *Saccharomyces cerevisiae* yeast was obtained from LIPI (Indonesian Institute of Sciences), Indonesia. All the chemicals used in these studies were pure analytical reagents from Merck (Germany).

### Pretreatment of Durian Seed

Durian seeds were turned into durian seed flour, which is smaller in size so that it can be hydrolyzed into glucose or sucrose. Durian seeds that have been collected from around the campus area of Universitas Negeri Semarang, Gunungpati, Semarang, Indonesia were washed, pounded, and dried for 2 to 4 days under the sun light, after the durian seeds were dried, were then mashed using a blender. The mixed Durian seeds were then sieved with 100 mesh sieve. The produced Durian seed flour was then used for the next process.

### Hydrolysis of Cellulose, Hemicellulose, and Lignin from Durian Seed

200 g mass fraction of durian seed flour were mixed with 1000 mL HCl (1-3%, v/v) and put into 1500 mL beaker glass. Then the solution was stirred for 30 minutes. After the solution had mixed completely, then the solutions were put in a water bath at 70°C for 3 hours (Salsabila et al., 2013; Alighiri et al., 2015). The solution that had been hydrolyzed by heating then allowed standing for 15 minutes until the filtrate and residue are visible. The filtrate and residue were separated using a filter cloth. The filtrate obtained from the hydrolysis process was used for the next process.

### Detoxification of Hydrolysis Solution

The inhibitors in lignocellulosic hydrolysates can be reduced by detoxification of hydrolyzed filtrate to remove toxins from the acidic solution in the hydrolysis solution. The sample will be detoxified using a weak natural base. 3 M base solution of $Na_2CO_3$ was added to the hydrolyzed filtrate. The amount of base addition is adjusted so that the filtrate has a pH of 4.5 to 5.5. This pH is a condition for the *Saccharomyces cerevisiae* yeast to live optimally. Then, the solution was stirred at room temperature (28-30°C) for 45 minutes. After the mixing process, this solution containing glucose or monosaccharides are left for 24 hours and are ready for use at the fermentation stage.

### Fermentation of Detoxification Solution Using *Saccharomyces cerevisiae*

The fermentation was performed using *Saccharomyces cerevisiae* yeast (Kang & Lee, 2015;

Masturi et al., 2017; Azzar et al., 2017) in an aerobic fermenter. The aerobic fermenters consisted of cylindrical tanks with air introduced at the base via networks of perforated pipes. The fermenter temperature is maintained at around 30–37°C. The experimental setup is shown in Figure 1.

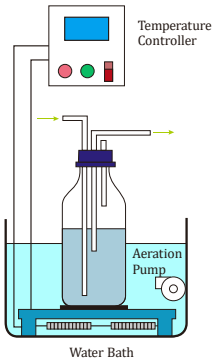



Figure 1. Experimental setup of aerobic fermenter for bioethanol production using *Saccharomyces cerevisiae*.

Before conducting the fermentation process, the inoculum was prepared by 100 mL inoculum solution of distilled water with 5 g of glucose, 0.5 g of yeast extract, 0.5 g of $KH_2PO_4$, 0.15 g of $NH_4Cl$, 0.07 g of $MgSO_4$, and 0.17 g of KCl which mixed until homogeneous and tightly closed with sterile cotton. The inoculum medium was sterilized into an autoclave at 121°C for 15 minutes after the inoculation medium was cooled to room temperature. After the temperature of the inoculation medium reached room temperature, *Saccharomyces cerevisiae* was inserted as much as 2-3 oases, then inoculated for 24 hours (Carrasco et al., 2010; Siburian et al., 2015; Parapouli et al., 2020). In this stage, the variation of fermentation time was 1, 3, 5, 7, 9, and 11 days to determine the levels of bioethanol obtained from the fermentation process.

The fermentation process began by mixing 600 mL of the liquid phase of the hydrolysis containing sugar with pH 4 with 2-3 oases of *Saccharomyces cerevisiae* and adding 12 mL of inoculum. The homogeneous mixed solution was ready for fermentation for 1, 3, 5, 7, 9, and 11 days and each time variation. The sample was taken then distilled and stored in a freeze (Alegre et al. 2003; Karimi et al. 2006; Rouhollah et al. 2007).

**Bioethanol Enrichment from The Fermentation Process by Vacuum Distillation**

Fermentation results with time variations of 1, 3, 5, 7, 9 and 11 days were then distilled to separate the ethanol portion from other compounds. This process was commonly referred to as the purification process by utilizing the different boiling points of compounds. Distillation to separate bioethanol in this study was carried out by setting the temperature at 68°C for ± 180 minutes by batch vacuum distillation (Alighiri et al., 2018). The results obtained had a liquid phase with the characteristic pungent odor of ethanol.

**Sample Characterization**

There were several stages of testing carried out in each process. Tests carried out on the material are FTIR (Fourier-Transform Infrared, Perkin Elmer Spectrum Version 10.03.06 FT-IR spectrophotometer), SEM (Scanning Electron Microscope, Phenom Pro X, manufactured by the Netherlands), UV-Vis Spectrophotometer (sugar content), and moisture content. Furthermore, in the process of distillation,

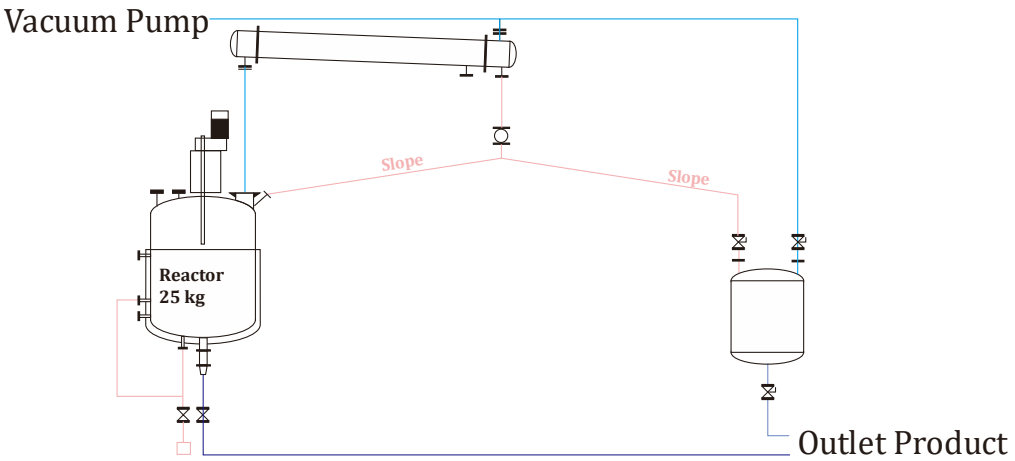



Figure 2. Batch vacuum distillation reactor for enrichment bioethanol (Alighiri et al., 2018)

characterization was conducted by GC (Gas Chromatography) using the *n*-propanol method. The results of the GC analysis were expressed int the ethanol percentage (%, v/v) (Park et al., 2017). In this method, ethanol was made in concentrations variation of 10, 20, 30, 40, 50, 60, 70% (v/v) by adding an internal standard of 5% (v/v) *n*-propanol to each standard solution. The next step is calculating the peak area of ethanol and *n*-propanol from the chromatogram, then looking for the ratio of the peak area of ethanol and *n*-propanol by using Eq. (1) (Toikka, 2011).

$$\text{Total area} = \frac{\text{Total area of etanol}}{\text{Total area of } n-\text{propanol}} \qquad (1)$$

Finally, GC analysis were carried out on an Agilent Technologies GC instrument equipped with a GC 7890A gas chromatograph and provided with an HP-5MS capillary column. The data acquisition and data processing were performed using the MSD Chemstation E.01.01.335 (Agilent) software.

## RESULTS AND DISCUSSION

Carbohydrates in durian seed flour amount are 11.54%. Carbohydrates in starch compounds are the highest content in durian seeds. However, the starch structure in durian seeds cannot be directly fermented, so hydrolysis needs to be done to get glucose (Devarapalli & Atiyeh, 2015). The composition of materials in the hydrolysis process affects the final output of ethanol production (Masturi et al., 2017). Sugar, starch, and cellulose are included in the carbohydrate component containing amylose and amylopectin with the chemical formula $C_x(H_2O)$, which is found in many parts of plants such as stems, fruits, seeds, and tubers as food reserves (Max & Chapados, 2007; Alighiri et al., 2015). HCl was evaluated at concentrations of 1-3% (v/v) for durian seeds flour of pretreatment hydrolyzation.

Table 1 shows that durian seed flour pretreated with 3% HCl was yielded the highest amount of fermentable sugar (9.70 %, v/v). After hydrolysis, glucose content rises to, as shown in Table 1. Curve measurement of absorbance of durian seeds hydrolysis was done by UV-Vis spectrophotometer. Figure 3, which is the result of UV-Vis, calibration of curve concentration of the solution hydrolysis in durian seeds results was obtained from eight absorbance data of the hydrolysis solution of durian seeds flour with a minimum concentration of 200 ppm and a maximum concentration of 450 ppm. The absorbance value is converted to the glucose standard curve equation on the line equation (y = 0.0004x, with $R^2$ = 0.9904). These equations can show the amount of glucose from the hydrolysis of durian seeds. The highest glucose level in the hydrolysis of durian seeds is 367 ppm, with the absorbance of 0.147. So it is known that the glucose content in the hydrolysis of durian seeds is 36%. Furthermore, the glucose in the hydrolysis of durian seeds can be used as a source of bioethanol because it has high glucose levels and is converted into ethanol by microbes under suitable conditions.

Table 1. Composition of durian seed flour and hydrolysis process using hydrochloric acid (HCl) in various concentration.

| Concentration (%) | Durian seed flour | Hydrolysis process using HCl in various concentration (%, v/v) | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Total carbohydrates | 71.54 | 15.68 | 15.52 | 15.48 |
| Glucose in filtrate | - | 9.28 | 9.42 | 9.70 |

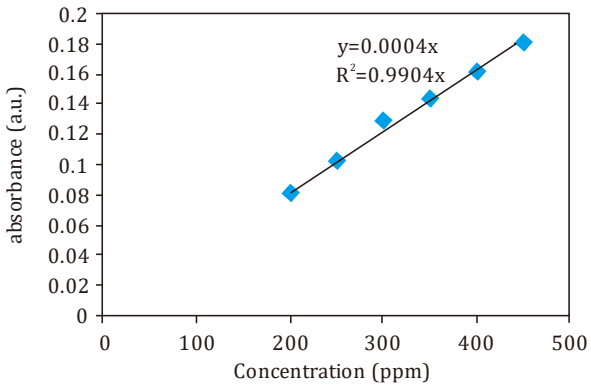



Figure 3. Calibration curve of UV-Vis spectrophotometer using glucose as a standard solution at a concentration of 200–400 ppm.

Glucose content due to the hydrolysis process of durian seed flour was identified through its functional group content by using FTIR

(Wang et al., 2015; Alighiri et al., 2015). Figure 4 shows the FTIR spectra used to analyze vibrations from the functional group of durian seed flour, hydrolysis filtrate, and hydrolysis residues. The main components of durian seeds are amylose and amylopectin, known as starch. This component, if hydrolyzed, will produce glucose compounds, which are monosaccharides. Glucose uptake bands in the hydrolysis FTIR spectra showed the success of the hydrolysis process.

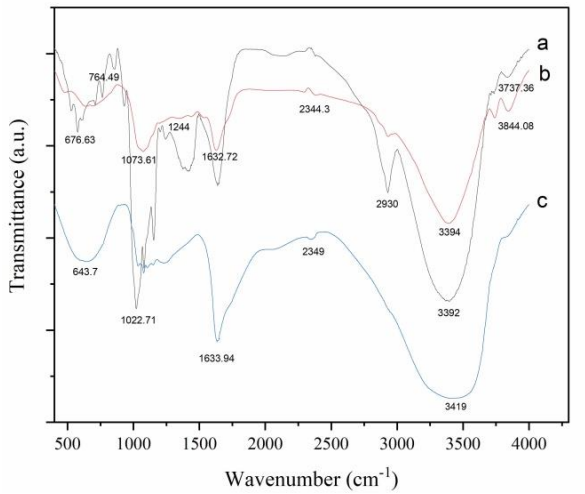



Figure 4. FTIR spectra of (a) durian seed flour, (b) hydrolysis filtrate, and (c) hydrolysis residue

In general, glucose has a typical infrared absorption region around wavenumbers below 800 and 800-1500 $cm^{-1}$ (the fingerprint region), 2800-3000 $cm^{-1}$ (C─H stretch region), and regions between 3000-3600 $cm^{-1}$ (O─H stretch region) (Kizil et al., 2002). The hydrolysis reaction that occurs as shown in Eq. (2) that is starch is hydrolyzed by water using an acid catalyst in this study using hydrolyzed HCl, which is monosaccharide is glucose (Fatimah et al., 2013).

$$\underset{\text{Starch}}{(C_6H_{10}O_5)n} + \underset{\text{water}}{nH_2O} \rightarrow \underset{\text{glucose}}{n(C_6H_{12}O_6)} \quad (2)$$

Complex vibration modes below 800 $cm^{-1}$ are skeletal mode vibrations from the glucose pyranose ring (Kizil et al., 2012). This durian seed flour area is shown at 527.76, 576.63, and 764.49 $cm^{-1}$, while the residue and filtrate of the hydrolysis results respectively show the wavenumbers at 648.7 and 643.7 $cm^{-1}$.

The deformation of molecular complexes simultaneously produces peaks in the fingerprint region (Gilbert et al., 2017). This area of durian seed flour has shown a peak of 1022.71 and 1080.11 $cm^{-1}$, which indicates C─H and C─O─H bonds (Pavlovic & Brandao, 2003). Meanwhile, the *fingerprint* area of the hydrolysis filtrate and residue shows a shift in the peak of the wavenumber of 1073.61 and 1078.2 $cm^{-1}$.

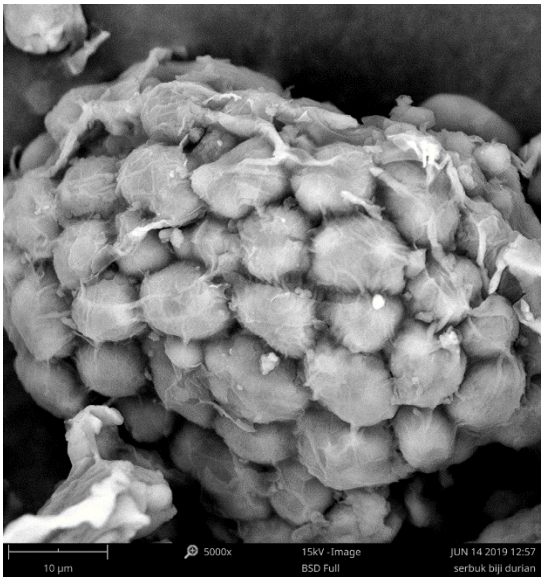
The area between 2800- 3000 $cm^{-1}$ shows the stretching vibration of C─H to observe the variation of amylose and amylopectin in the starch to change the transmittance (Movasaghi et al., 2008). The CH bond (where the hydrogen is attached to carbon having a single bond with other elements) absorbs light at a range of about 2853-2962 $cm^{-1}$ to be exact at 2929.5 $cm^{-1}$. The carbon-oxygen double bond, C=O, is a very useful absorption, which can be found at 1632.72 and 1633.97 $cm^{-1}$ for hydrolyzed filtrate and residues (Zambare et al., 2011).

The stretching vibration of starch O─H in durian seed flour is shown at 3392 $cm^{-1}$, which indicates a characteristic of infrared absorption due to stretching of CO bond in the peak of 1050-1200 $cm^{-1}$. The precisely CO was at 1244 $cm^{-1}$. The emergence of a peak in the wavenumber area 1154.94 $cm^{-1}$ in the spectra of durian seed flour is caused by a bond between C─O and C─C (Gilbert et al., 2017). While the OH bonds in acid groups arise in the area are about 2300-3300 $cm^{-1}$, and those bonds to the chain in the hydrolysis filtrate are in the region of 2349 $cm^{-1}$, and the hydrolysis residue is at 2344.3 $cm^{-1}$.

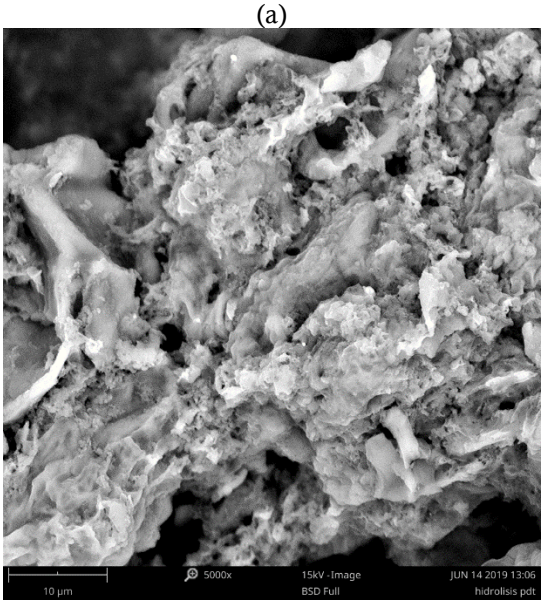
Ether compounds provide strong absorption in the fingerprint area. However, since the absorption is governed by the framework vibrations, which include CO bonds, the absorption frequency varies, i.e. 1000-1250 $cm^{-1}$. Glucose characterization in the filtrate is associated with 643.7 (glucose pyranose ring), 1078.2 (C─O─H bending), and 1633.97 ($CH_2$ bending). Whereas the residues are associated with 648.7 (glucose pyranose ring), 1073.61 (C─O─H bending), and 1632.72 $cm^{-1}$ ($CH_2$ bending) (Pavlovic & Brandao, 2003). These spectra are dominated by the water component, there is a stretching vibration of O─H, and the water is absorbed that can be seen from the results of the residue at 3394.04, 3737.36, and 3844.08 $cm^{-1}$, while the filtrate results at 3418.9 $cm^{-1}$. The absorptions show that there are still water molecules due to the vibration of the OH group from water (Czarneck, 2015), that, of course, still makes sense because the solution being tested is a sugar solution that the biggest

component is water. The interaction between water and starch due to simple heating is affecting several peaks having a similar peak with the spectrum of durian seed flour and the hydrolysis product. It shows that glucose is bonded to a water molecule.

Interpretation of Figure 5 which displays micrographs, can show a correlation between particle size and the content of starch granules (Cui et al., 2014).




(a)




(b)

Figure 5. Scanning electron micrographs for (a) durian seed flour and (b) hydrolysis product

Figure 5(a) shows that the average size of durian seed flour granules is 7.03723 µm with maximum value is 10.908 µm, and a minimum is 1.01905 µm. Whereas Figure 5(b) is a micrograph from durian seed flour that has been hydrolyzed with 3% (v/v) of HCl acid at 70°C for 3 hours. Acid catalysts will damage the cellulose and hemicellulose polymer chains to form monosaccharides such as glucose (Alighiri et al., 2015). This is clearly seen in Figure 5 (b) that the polymer chain is damaged by the hydrolysis process.

The effect of acid hydrolysis on some starch properties on durian seeds gives inconsistent results, including changes in amylose content and digestibility. The amount of amylose or linear fraction increases in the early stages of the acid hydrolysis process. Therefore, starch digestibility is reported to be increased due to acid hydrolysis (Faridah et al., 2010). Other researchers report that acid hydrolysis reduces amylose levels, as reported by Jyoti et al. (2006).

At the surface of the hydrolysis, granules begin to appear shrinkage, indentation, or cracks, some starch granules split into smaller pieces due to severe erosion on the surface of the granules, as reported by Hoover (2000) and Miao et al. (2011). The surface of the granules contains pores and cavities, which are the result of increased diffusion in the interior of the granules (Hoover, 2000). As reported by Miao et al. (2011) that from SEM, broken granules can be concluded that starch granules form holes or tunnels that develop in the interior of the granules before they are split.

Further, glucose fermentation aims to convert sugars into organic acids or alcohols using the help of microorganisms (Paulová et al., 2014). The glucose obtained was carried out by fermentation or fermentation process by adding yeast to obtain bioethanol (Tri & Nuri, 2011). The yeast used for the production of bioethanol was *Saccharomyces cerevisiae*, which has a high tolerance to ethanol and sugar (Azhar et al., 2017). Fermentation was carried out at pH 4.5 because, according to Yingling et al. (2011), who studied the effects of pH on bioethanol concentration. It showed that a low initial pH of 4.5 prevented bacteria from being contaminated. In fermentation media with pH levels higher than 4.5, the cell response and growth in *Saccharomyces cerevisiae* will occur with a long pause phase (Liu et al., 2018; De Klerk et al., 2018). Therefore, pH is one of the most important factors for producing bioethanol.

The fermentation of the results durian seed flour hydrolyzed was fermented by varying

the fermentation time for 1, 3, 5, 7, 9, and 11 days to get optimal ethanol in fermenter aerobic. Analysis of Monir et al. (2019) shows maximum microbial growth at 37°C. Therefore fermentation process was carried out by maintaining the temperature at 37◦C in an aerobic fermenter to achieve maximum bioethanol yield. Then, 12 mL inoculum was added to 600 mL of the ready-to-ferment solution according to the study conducted by El-Mekkawi et al. (2019), who said that 2 mL of inoculum was needed for every 100 mL of the solution to be fermented. The fermentation stage was done in aerobic fermenters. However, fermented ethanol levels still contain water by 95% and others, requiring a separation process carried of such as distillation (Rohman et al., 2013).

The solution containing bioethanol was analyzed by GC (Trivedi et al., 2016). The prepared calibration ethanol curve with *n*-propanol can be seen in Figure 6. The results of the gas chromatogram show that ethanol appeared at a retention time of 2.739 minutes or approached it, and *n*-propanol appeared at a retention time of 10.143 minutes or approached it the same as a study conducted by Shudaker & Jain (2016). It can also be seen that ethanol and *n*-propanol have good peak separation.

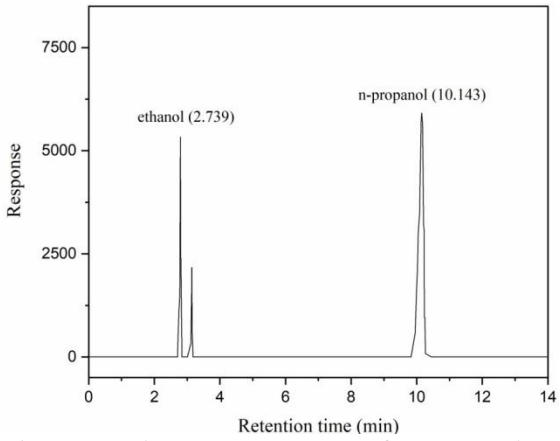



Figure 6. Chromatogram for ethanol determination with an internal standard of *n*-propanol

The next step is to calculate the ratio of the peak area of ethanol and *n*-propanol from the chromatogram using the equation of (1). The value of the correlation coefficient can be used as a parameter to determine linearity. Therefore, the calculation results obtain the regression line equation for the standard compound. Based on the analysis of chromatogram by using *the n*-propanol method, it can be seen in Table 2 that shows the equation of linear regression is y = 0.0133x - 0.1368, with the correlation coefficient value, is 0.9804.

Table 2. Standard curve of ethanol with *n*-propanol as an internal standard at measuring ethanol content using Gas Chromatography.

| Ethanol concentration (%, v/v) | Total Area |
|---|---|
| 10 | 0.0624 |
| 20 | 0.0962 |
| 30 | 0.2056 |
| 40 | 0.3979 |
| 50 | 0.5200 |
| 60 | 0.6518 |
| 70 | 0.8266 |
| $R^2$ = 0.9804<br>y = 0.0133x - 0.1368 | |

By substituting the values of x (concentration) and y (total area of ethanol/*n*-propanol) in the sample, it can show the ethanol concentration in the sample by substituting the value of y. The lowest of ethanol content is 10.93 % (v/v). It is on the first-day fermentation, while the highest ethanol content is 14.72 % (v/v) in 9 days of fermentation time. The increasing levels of ethanol content from fermentation be seen in Figure 7.

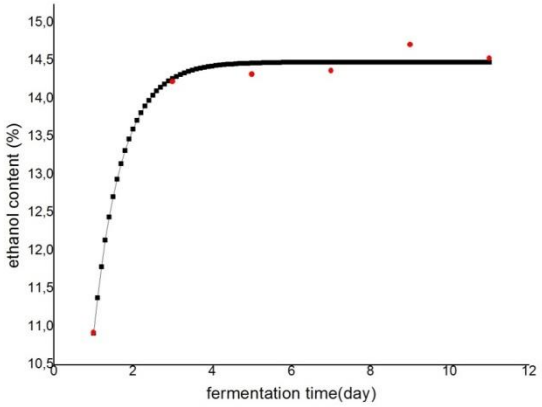



Figure 7. production of ethanol as a function of fermentation time in aerobic fermenter using *Saccharomyces cerevisiae*

The fermentation stage will change the glucose level of the solution into ethanol so that the glucose level will decrease. Then, it can be seen

that the rate of change of glucose with time is proportional to the amount of initial ethanol concentration, or it can be explained using Eq. (3).

$$\frac{dC}{dt} \infty - C_0 \quad (3)$$

From Eq. (3), the initial concentration of ethanol can be found using Eq. (4).

$$C_{etanol}(t) = C_0(1 - e^{-kt}) \quad (4)$$

with $C_0$ is the initial concentration of ethanol, e is the rate of change of glucose, and $k$ is the constant production of ethanol. The equation of (4) was used for the fitting graph shown in Figure 5.

The more glucose in the solution, the faster seed of yeast will grow quickly and can ferment glucose directly into ethanol. The activity of *Saccharomyces cerevisiae* in producing ethanol increases on the first day during fermentation. It will increase steadily until the 9$^{th}$ day. At that time, *Saccharomyces cerevisiae* began to enter the exponential phase, where ethanol as the primary metabolite. Then, *Saccharomyces cerevisiae* began to enter the stationary phase. After the level of ethanol reached its maximum on the 9$^{th}$ day, the ethanol content decreased due to oxidation during the fermentation process, so the glucose which has been converted to ethanol through the glycolysis pathway turned into acetic acid and water. The reduction of levels in ethanol can cause the ethanol converted into other compounds, for example, is esters or carboxylic acid compounds. This condition causes the fermentation media to become more acidic and inhibit the growth phase of *Saccharomyces cerevisiae.* The decreasing ethanol levels are due to the decreased glucose content of fermentation activity by *Saccharomyces cerevisiae*, which uses glucose as a source of nutrition and energy (Sebayang, 2006; Masturi et al., 2017). However, the ethanol content which formed during the fermentation process can affect the length of the fermentation time.

Then, ethanol from fermentation stages was enriched by using batch vacuum distillation with single short of vessel column. Vacuum batch distillation used for bioethanol enrichment resulting from the fermentation stage is made from a tank of 316 L stainless steel (SUS 316 L) consisting of a reactor tank, reflux columns, mixers, condenser, and product tank. All parts are connected to a vacuum pump (Figure. 2) (Alighiri et al., 2018). The sample will be distilled and boiled in a closed reactor tank. The vapors formed will be cooled by the condenser, and distillate is collected in the tank product. The distillation results obtained ethanol purity of 95%.

## CONCLUSION

Ethanol, as renewable alternative energy, can be produced using agricultural waste materials such as durian seeds. The process for the production of starch-based bioethanol includes milling, hydrolysis, detoxification, fermentation, and distillation. The variations time of fermentation using *Saccharomyces cerevisiae* in durian seed flour affects the level of bioethanol product in the aerobic fermenter, so it can produce bioethanol with the highest value is 14.72% (v/v) in 9 days fermentation time. The 100 g durian seed flour can produce 6.18 mL of ethanol with purity 95% after enrichment using batch vacuum distillation.

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