A novel fermentation substrate in bioethanol production by saccharomyces cerevisiae

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ABSTRACT

Pineapple and coconut are part of the largest commodities in Indonesia. In this study, extracts of pineapple skin and coconut water were used as a novel fermentation substrate in bioethanol production by *Saccharomyces cerevisiae*. Those were used as raw materials contain sugar, which makes it a potential medium for bioethanol fermentation. The method used was a milling process to extract sugar into the fermentation medium so that ethanol could be directly produced from the juice. While coconut water is a mixture during the fermentation process. The fermented bioethanol was then purified by the distillation process to obtain pure bioethanol. The sugar content of the ingredients, the operating condition (pH, time of fermentation), and the addition of nitrogen source were studied. *Saccharomyces cerevisiae* was used as a nitrogen source with variations of 14 and 16g/L with fermentation operating conditions for 40 and 44 hours, and pH used were 04 and 05. The sugar content of pineapple skin extracts, coconut water, and the mixture of the two were 4.26; 4.19; and 4.21g/L, respectively. The largest ethanol content produced was 38.42%, with 16g/L of nitrogen sources for 44 hours at pH 5. From these results, the mixture of pineapple skin extract and coconut water is proven to be used as a novel fermentation substrate for bioethanol production.

1. Introduction

The world's energy demand continues to increase over the next 20 years due to the rapid growth of industry, automobiles, and the world's population. This enormous demand for energy is cause for concern given the limited and non-renewable resources. On the other hand, the amount of industrial waste that exists is another problem that has a negative impact on the environment (Suresh, Sivarajasekar, Balasubramani, Ahamad, & Alam, 2020). Agricultural and forestry residues are excellent sources of biomass available for biofuel production. Utilization and waste recycling for energy production lead to zero waste discharge in the circular economy (Salameh, Tawalbeh, Al-shannag, & Saidan, 2020).

In principle, ethanol is divided into three categories based on the source of production. The first generation of ethanol is produced from agricultural products that have high nutritional value, such as corn, sugar cane maize, wheat, rice straw, and others. The second generation of ethanol produced from lignocellulosic non-food plants such as agriculture, forestry and wood residues, residues, and others. Meanwhile, the third generation of ethanol is produced from algae. The

ethanol production from the first generation of raw materials is commercialized, while the second generation of ethanol production of raw materials is not very popular due to the complex conversion process (Ghadge et al., 2020).

Bioethanol is an alternative and non-petroleum energy source produced by the fermentation process of sugar from carbohydrate sources by microorganisms. Bioethanol exists to enhance the supply of oil, in addition to reducing the negative impact of fossil fuels on the environment (Tan et al., 2019). Bioethanol can be converted from renewable natural resources containing lignocellulosic materials. Currently, renewable energy policies seek to reduce greenhouse gas emissions and increase the total renewable fuel volumes (Duarte, Uribe, Sarache, & Calderón, 2020). Bioethanol production is mostly focused on the potential for inedible raw materials enriched with sugar as renewable sugar, where this does not compromise the need for existing resources or food for the next generation. Ethanol that is produced from corn, sugar cane and beet can be a problem in the near future due to the food competition in the use of these materials (Soplah, Abdullah, Shirai, Kamal, & Ali, 2015). So, an alternative way is producing ethanol from renewable sugars, such as pineapple skin waste and coconut water.

Pineapple waste is fundamentally composed of surplus pulp, peel, and cores, that's not considered attractive as an animal feed due to their high fiber content, high soluble carbohydrate, and low protein content. However, the pineapple waste extract contains sugars and organic acids that can be used as the substrates in the production of hydrogen, methane, and ethanol (Chu, Vo, & Chen, 2020). Where pineapple juice contains 06 to 16% (w/v) of fermentable sugar that can be used to reduce the waste of alcohol production from an economic point of view (Alain, Georges, & Aka, 1987). Also, pineapple waste contains relatively high levels of cellulose, hemicellulose, and soluble mono-sugars (Chen et al., 2020). These wastes consist of residual pulp, peels, and skin (Choonut, Saejong, & Sangkharak, 2014). Bioethanol has been successfully produced from industrial pineapple waste (Gil & Maupoey, 2017). Pineapple can be processed in various ways, such as slicing, pulping, and juicing (Banerjee, Ranganathan, Patti, & Arora, 2018). Coconut water is the liquid found in light green coconut, which is not the same as coconut milk (Segura-badilla & Lazcano-hern, 2020). It is known that the sugars of coconut water are derived from coconut fruit (Akpro et al., 2019). While *Saccharomyces cerevisiae* is an ideal biocatalyst for bioethanol production in a sugar-containing nutrient medium (Monir, Aziz, Yousuf, & Alam, 2019). From previous studies, where used banana fond juice as substrate fermentation produce bioethanol (45.75g/L) in a bioreactor using formulated condition of the banana fond juice with 15g/L yeast extract at pH 6.8 (Tan et al., 2019). Bioethanol production by *Saccharomyces cerevisiae* was cultivated on fresh oil palm frond juice, sugarcane juice, and synthetic media as a control, giving a bioethanol yield of 0.38g/g per sugar consumed with sugarcane juice fermentation was completed in 48 hours (Soplah et al., 2015).

Pineapple skin extracts and coconut water were known to contain sugar, which can be used for bioethanol production. This study aims to determine and utilize the sugar concentration contained in pineapple skin waste and coconut water using microorganisms *Saccharomyces cerevisiae*. The density, yield, ethanol concentration of the bioethanol produced was investigated.

2. Methodology

2.1. Materials

2.1.1. Sample sources and preparation

Pineapple skin wastes were collected from unused pineapple skins from pineapple sellers located at Tembalang, Semarang, Central Java, Indonesia. Pineapple skin wastes were

cleaned and washed to remove dirt on the outer surface, then cut for further extraction of the juice. Meanwhile, coconut water was filtered to separate it from impurities.

2.1.2. Chemicals products

Urea $(NH_2)_2CO$, ammonium dihydrogen phosphate ($NH_4H_2PO_4$), hydrochloric acid (HCl), distilled water, glucose anhydrous, anthrone, yeast extract, yeast *saccharomyces cerevisiae* are chemical products used in this work.

2.2. Methods

2.2.1. Pineapple skin extraction

Pineapple skin pieces were weighed, then extracted using a blender which added to distilled water first before. Then filtered and centrifuged to obtain pineapple skin extracts (Tan et al., 2019).

2.2.2. Analyze glucose concentration of samples

Analysis of glucose levels of samples was carried out using the anthrone method. First, make a standard glucose solution. The standard curve was determined by making a standard glucose solution of 0.2mg/ml. Then 10ml of the solution was diluted to 100ml (1ml = 0.2mg glucose). After that, make a pipette into a blank solution of 0ml; 0.2ml; 0.4ml; 0.6ml; 0.8ml; and 1ml of the standard glucose solution, then add water until the total volume of each becomes 1ml. Add 5ml of anthrone reagent and cover tightly. Place the standard glucose solution mixed with the anthrone reagent into a water bath of 100^0C for 12 minutes, cool it and transfer it to the coveted so that the absorbance can be read at a wavelength of 630nm in the UV-vis Spectrophotometer. The sample glucose level test was carried out by taking 1ml each sample of coconut water, pineapple skin extract, and a mixture of coconut water and pineapple peel extract. Then the results are converted into units of g/L (Al-kayyis & Susanti, 2016).

2.2.3. Inoculum culture preparation and fermentation

Inoculum culture preparation aims to adapt yeast cells to the fermentation medium. *Saccharomyces cerevisiae* from packaged yeast was inoculated in 200ml medium (0.2g yeast extract; 0.08g $(NH_2)_2CO$; 0.1g $NH_4H_2PO_4$ and distilled water) in erlenmeyer 500ml. Before that, the medium was steam sterilized at an autoclave temperature of 121^0C for 15 minutes, then cooled down. After that, add 4g of yeast *(Saccharomyces cerevisiae*) to the medium, then stir until homogeneous and incubated for 24 hours. The fermentation process was carried out 08 times running (using a factorial design method) to produce bioethanol, where the mass variation of the nitrogen source was added to the fermentation medium of 14g/L and 16g/L, operating conditions such as pH 4 and 5, also fermentation time for 40 hours and 44 hours (Rahmah, Bahri, & Chairul, 2015).

2.2.4. Distillation process

The distillation process was carried out by inserting the fermented sample into a three-neck flask then installing the flask on the available distillation device. Set the temperature to 78^0C with a distillation time of ±1 hour (Miskah, Saing, & Siburian, 2017).

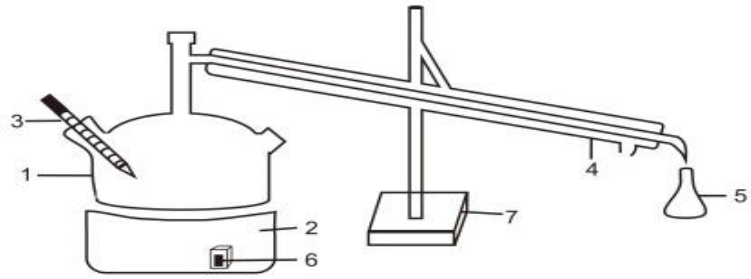

Figure 1. Distillation Process Equipments

Where:

1. Three neck gourds;

2. Heater;

3. Thermometer;

4. Condenser;

5. Erlenmeyer for bioethanol products;

6. Heat scale regulator;

7. Clamp and statif.

2.2.5. Bioethanol analysis

There are several analyzes of bioethanol products carried out, such as density analysis, yield analysis, and ethanol content. The analysis was carried out using the following formula (Hanum, Pohan, Rambe, Primadony, & Ulyana, 2013). The density of bioethanol was determined by weighing a certain sample of bioethanol solution using a pycnometer.

$$\rho = \frac{m}{v} \quad (1)$$

Where ρ is density (g/ml), m is bioethanol mass (g), and v is bioethanol volume (ml). The yield of bioethanol was calculated from the measurement of the volume of bioethanol obtained from the distillation of fermentation, where the volume of the final product was reduced by the volume of the initial product.

$$\text{Rendemen } \% = \frac{volume\ produk\ akhir}{volume\ produk\ awal}\ x\ 100 \quad (2)$$

The ethanol concentration of the bioethanol produced was calculated by interpolating the density and ethanol content data using the conversion table of density - ethanol content (Hanum et al., 2013).

3. Result and discussion

3.1. Result

3.1.1. Pineapple skin extracts

From the extraction, an extract of 0.40L was obtained from 0.50kg of pineapple skin wastes used (Table 1). The advantage of using fruit juice extracted from pineapple skin or other second generation raw materials as a fermentation substrate is that pretreatment of lignocellulosic parts can be eliminated.

Table 1

Material balance of Pineapple skin extraction from pineapple skin wastes

Material balances	Weight
Total wet weight of pineapple skin wastes before pressed (kg)	0.50
Total wet weight of pineapple skin wastes after pressed (kg)	0.25
Total volume of fresh pineapple skin extracts after filtered (L)	0.47
Residues (Losses after extraction) (kg)	0.25
Amount of pineapple skin extracts recovered (L)	0.40

Source: Direct observation in the field

The pineapple skin extract obtained after pressing was brownish yellow (Figure 2). Then centrifuged so that the dirt were sediment. The color of the extracts after centrifugation was bright yellow (Figure 3).

Figure 2. Pineapple skin juice obtained directly after pressing

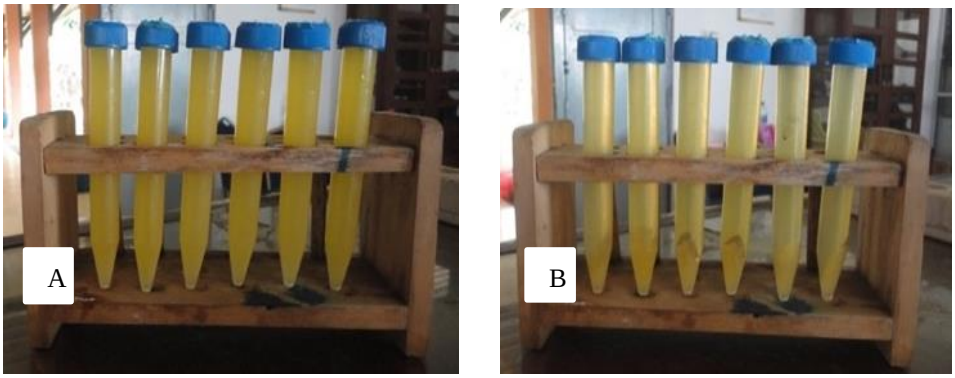

Figure 3. The pineapple skin extracts condition before (A) and after (B) centrifuged

3.1.2. Glucose content of pineapple skin extract and coconut water

Calculation of glucose content was carried out on pineapple skin extracts and coconut water, as well as the fermentation substrate, which is a mixture of pineapple extract and coconut water. The data obtained from the absorbance readings of the glucose standard solution using a UV-vis spectrophotometer are shown in Table 2 as follows.

Table 2

Absorbance reading data in standard glucose solutions

Sugar concentration (g/L)	Absorbance
0.00	0.18
0.20	0.39
0.40	0.40
0.60	0.43
0.80	0.54
1.00	0.69

Source: Direct observation in the field

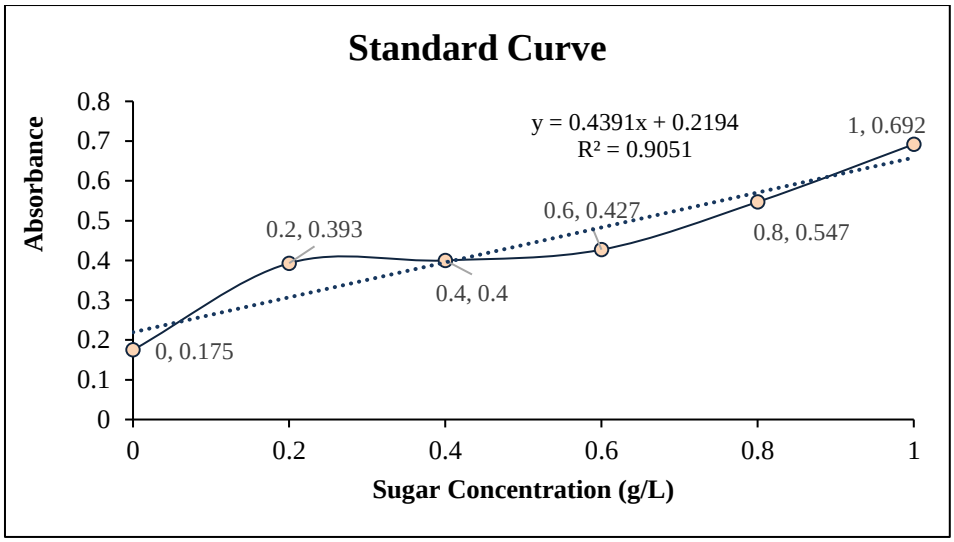

Figure 4. Standard glucose solution curve

From these data, the mathematical equation y = 0.4391x + 0.2194 is obtained where y is the absorbance value of glucose at a wavelength of 630nm, and x is the concentration of glucose. From this equation, the sample glucose content at several substrate concentrations was obtained using the observed data on the absorbance value of each sample. Glucose concentration in each sample can be determined by substituting the absorbance value into the equation obtained from the standard curve.

Table 3

Absorbance reading data in a sample solution

Sample	Absorbance
Pineapple skin extracts	2.09
Coconut water	2.06
The mixture of pineapple skin extracts and coconut water	2.07

Source: Direct observation in the field

The absorbance data of each sample were then substituted into the standard curve equation to determine glucose concentration. Based on the absorbance data of the sample solution, the glucose concentration of each sample is 4.26g/L of pineapple skin extract and 4.16g/L coconut water. Meanwhile, the glucose concentration of the fermentation substrate is 4.21g/L.

3.1.3. Bioethanol analysis

The effect of nitrogen source mass on density (Figure 5), yield analysis (Figure 6), and ethanol concentration (Figure 7) of the bioethanol produced was investigated.

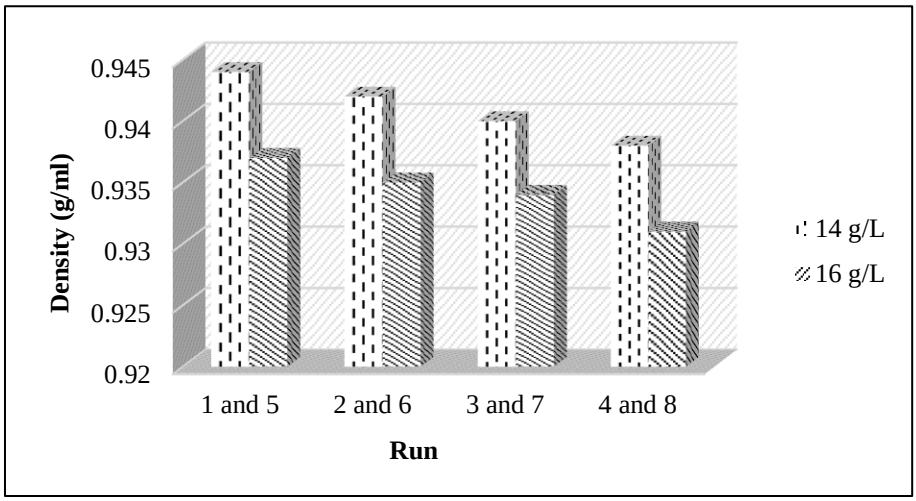

Figure 5. The effect of nitrogen source mass on density bioethanol

Bioethanol density was analyzed; density is the mass of a substance per unit volume (Danil, 2017). The different nitrogen source masses used in this study are 14g/L and 16g/L. The nitrogen source that used in this study was yeast extract with different masses. Based on the diagram in Figure 5, run 1, 2, 3, and 4 with different time and pH and nitrogen source mass of 14g/L yeast extract produced densities were 0.94g/ml; 0.94g/ml; 0.94g/ml; and 0.94g/ml. Whereas at run 5, 6, 7, and 8 with variations in time and pH and the nitrogen source mass of yeast extract is 16g/L were 0.94g/ml; 0.94g/ml; 0.93g/ml; and 0.93g/ml. So, the largest density produced at run 1, 0.94g/ml with a nitrogen source mass of 14g/L of yeast extract.

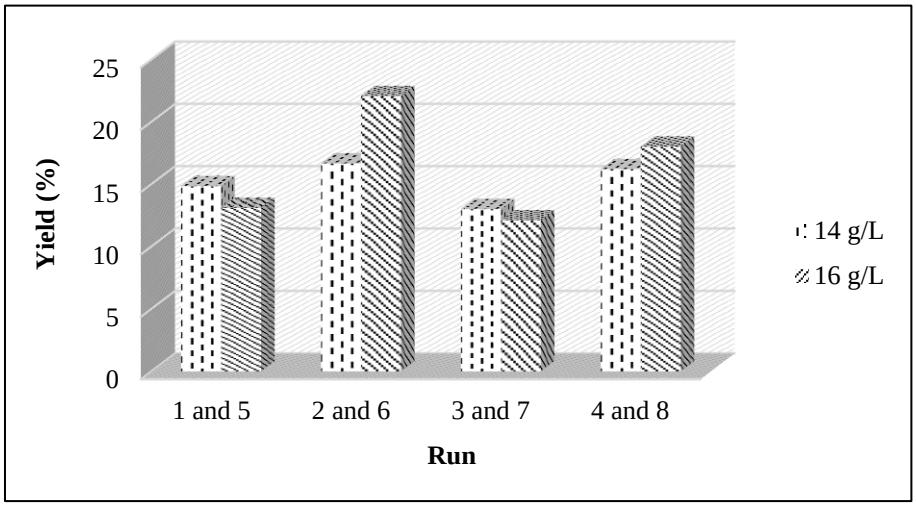

Figure 6. The effect of nitrogen source mass on yield bioethanol

Based on the diagram in Figure 6, runs 1, 2, 3, and 4 with variations in time and pH and 14g/L of yeast extract nutrition produced the yield bioethanol was 14.85%; 16.67%; 13.00%; 16.22%. Meanwhile, at running 5, 6, 7, and 8 with variations in time and pH and yeast extract of 16g/L, the yield bioethanol was 13.10% respectively; 22.07%; 12.11%; 18.02%. So, the highest yield value was produced at run 6, which is 22.07% with 16g/L of yeast extract.

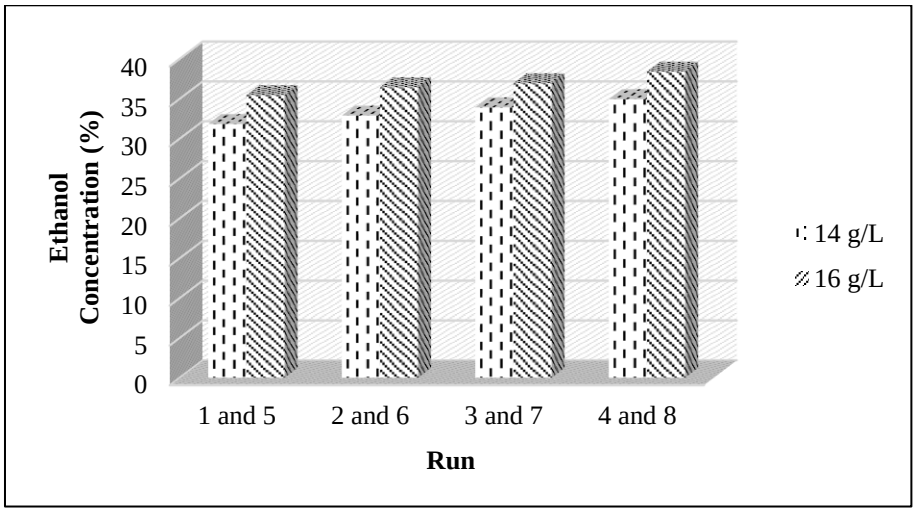

Figure 7. The effect of nitrogen source mass on ethanol concentration

Based on the diagram in Figure 7, runs 1, 2, 3, and 4 with variations in time and pH and 14g/L of yeast extract produced ethanol were 31.84%; 32.89%; 33.93%; and 34.95%. Meanwhile, at run 5, 6, 7, and 8 with variations in time and pH and 16g/L yeast extract produced ethanol was 35.45%; 36.45%; 36.95; and 38.42%. So, the highest ethanol produced at run 8, which is 38.415% with 16g/L yeast extract. The ethanol concentration increases with the increase in nutrient mass given to the bioethanol fermentation substrate.

The effect of operating conditions in the form of pH and fermentation time on density (Figure 8), yield analysis (Figure 9), and ethanol content (Figure 10) of the bioethanol produced were investigated.

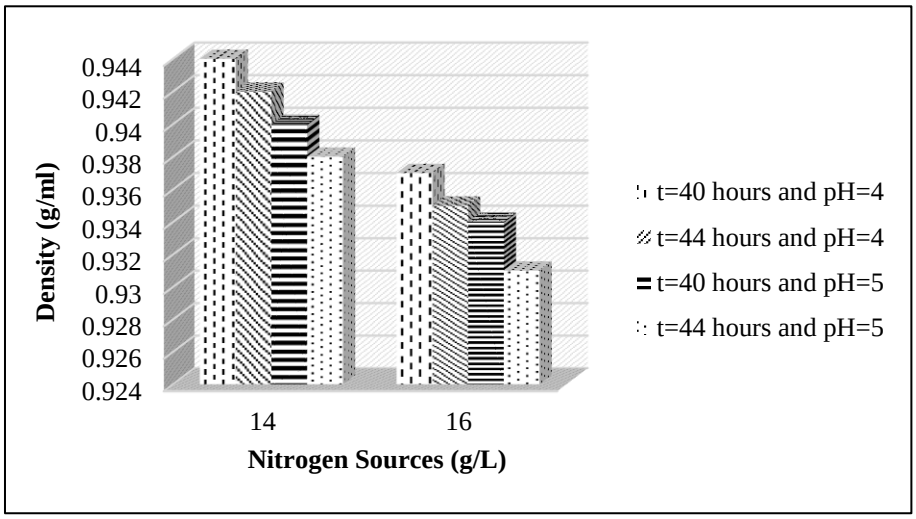

Figure 8. The effect of operating conditions on density bioethanol

Based on the diagram in Figure 8, the operating conditions where pH 4 and fermentation time for 40 hours produced bioethanol with density were 0.94g/ml and 0.94g/ml at run 1 and run 5. Then for operating conditions with a pH of 4 and fermentation time, 44 hours resulted in bioethanol density values of 0.94g/ml and 0.94g/ml, namely at run 2 and run 6. As for the operating conditions with a pH of 5 and a fermentation time of 40 hours, the density bioetanol was 0.94g/ml and 0.93g/ml. Meanwhile, in operating conditions with a pH of 5 and a bioethanol fermentation time of 44 hours, the density was 0.94g/ml and 0.93g/ml.

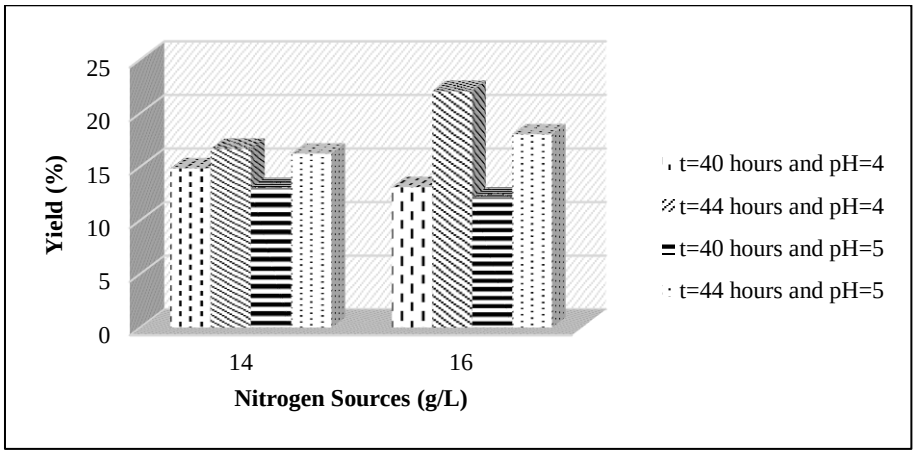

Figure 9. The effect of operating conditions on yield bioethanol

Based on the diagram in Figure 9, the operating conditions where pH 4 and fermentation time for 40 hours the yield value were 14.85% and 13.10% at run 1 and run 5. Then for, operating conditions with a pH of 4 and 44 hours of fermentation time resulted in bioethanol yield values were 16.67% and 22.07% at run 2 and run 6. While in operating conditions with a pH of 5 and a fermentation time of 40 hours produced a yield of bioethanol were 13.00% and 12.11%. Meanwhile, in operating conditions with a pH of 5 and a bioethanol fermentation time of 44 hours, the yield bioethanol was 16.22% and 18.02%. In this study, the highest yield bioethanol produced in operating conditions where the pH used was 4 with a fermentation time of 44 hours, 22.07%.

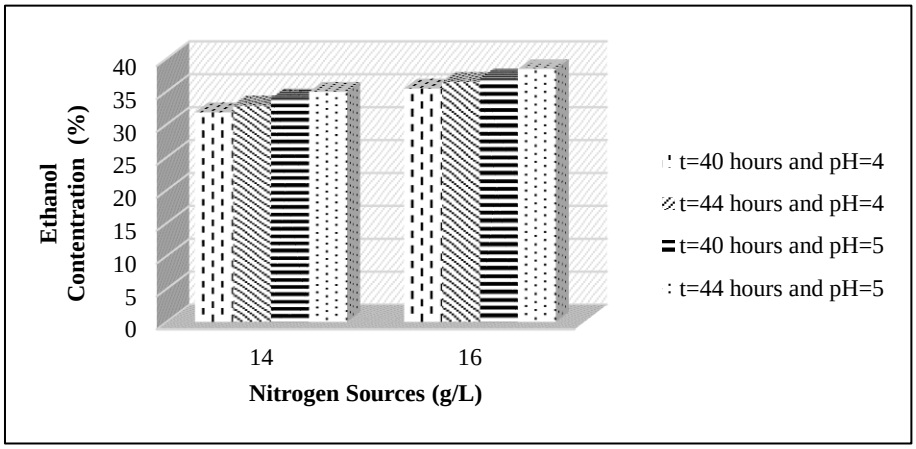

Figure 10. The effect of operating conditions on ethanol concentration

Based on the diagram in Figure 10, the operating conditions where pH 4 and fermentation time for 40 hours produced bioethanol ethanol content were 31.84% and 35.45% at running 1 and run 5. Then, operating conditions with pH 4 and fermentation time for 44 hours produced ethanol 32.89% and 36.45% at run 2 and run 6. As for the operating conditions with pH 5 and fermentation time for 40 hours, produced ethanol was 33.93% and 36.95% at runs 3 and 7. Whereas in operating conditions with a pH of 5 and fermentation time for 44 hours produced ethanol content of bioethanol were 34.95% and 38.42% at runs 4 and 8. In this study, the highest ethanol content was produced in operating conditions where the pH 5 with fermentation time for 44 hours which is 38.42%.

From the analysis that was investigated, density has a close relationship with ethanol concentration. The relationship between the density value and the ethanol concentration was presented in the curve below.

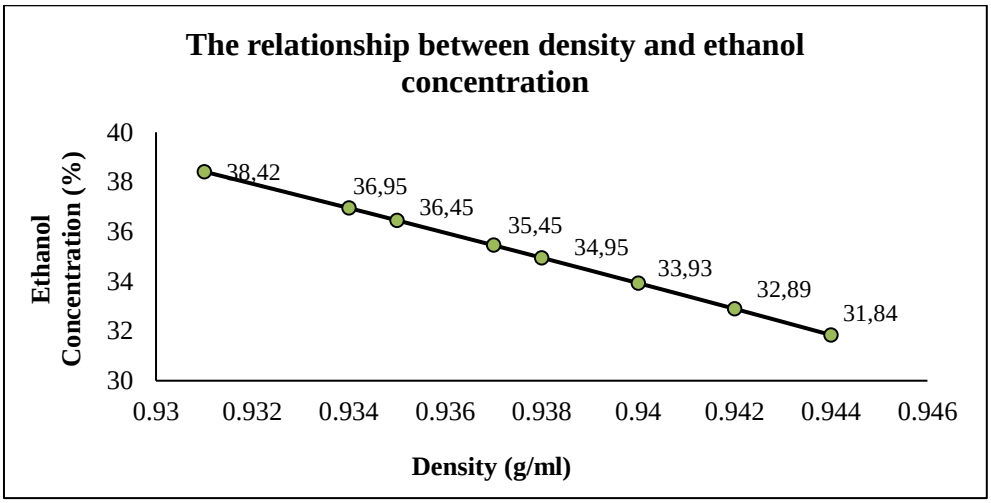

Figure 11. The relationship between density and ethanol concentration curve

The density and ethanol concentration have an inverse relationship. The higher density, the lower the ethanol concentration produced, and vice versa. If seen from the previous analysis that has been carried out, the greatest density was produced at the fermentation time of 40 hours, while the largest concentration of ethanol was produced at the fermentation time of 44 hours. The decrease in density was due to the longer the fermentation; the microbial activity has grown by multiplying more and more. So that the increasing number of *Saccharomyces cerevisiae* bacteria, the more carbohydrates break down into alcohol. As the number of alcohol increases, the density value of the alcohol and water mixture in the resulting Bioethanol will be lower (Miskah et al., 2017). Meanwhile, the concentration of ethanol was higher because the longer the fermentation time, the more starch was converted into sugar; as a result, there will be more sugar that was converted into ethanol so that the ethanol content increases with the length of fermentation time (Danil, 2017).

3.2. Discussion

The effect of nitrogen source mass on density bioethanol was investigated, where the highest density was produced at the less nitrogen source mass, which is 14g/L. This is consistent with research from Tan et al. (2019), which states that the *Saccharomyces cerevisiae* bacteria grow effectively with a minimum concentration of yeast extract. Where the addition of yeast extract in fermentation media can increase the ethanol production in the manufacture of bioethanol by *Saccharomyces cerevisiae's* bacteria at low concentrations of yeast extract. Then, the highest yield value was produced with 16g/L of yeast extract. The yield bioethanol increases as the mass of the nitrogen source is given by the bioethanol fermentation substrate. This is due to the increasing nitrogen source mass; the more *Saccharomyces cerevisiae's* bacteria will grow, so that the alcohol produced from glucose overhaul increases and affects the yield value of the bioethanol produced (Danil, 2017). The ethanol concentration increases with the increase in nutrient mass given to the bioethanol fermentation substrate. The higher the mass of nutrients, the microorganisms present in the material will be higher. This means that the greater number of microbes that break down glucose into alcohol the alcohol or ethanol concentration produced will be higher (Danil, 2017).

While the effect of operating conditions on density bioethanol also was investigated. In this study, the highest density value was produced under operating conditions where the pH used was 4 with a fermentation time of 40 hours. *Saccharomyces cerevisiae's* bacteria work optimally at a pH of 4 - 5.5 in fermentation for ethanol production (Tan et al., 2019). The pH will decrease with the length of the fermentation time; the decrease of pH occurs due to CO_2 formation and other

organic acids (Dompeipen & Dewa, 2015). The highest yield bioethanol was produced under operating conditions with low pH and long fermentation time. This is because the length of fermentation time gave the *Saccharomyces cerevisiae's* bacteria to break down more starch and glucose, which results in more alcohol so that the resulting yield is higher (Danil, 2017). Based on the results of this study, ethanol concentration increases with the length of time and the high pH used during the fermentation process. According to Danil (2017), the longer fermentation time, the more starch converted into sugar; as a result, the more sugar converted into ethanol, so ethanol concentration increases with the length of fermentation time.

4. Conclusions

The mixture of pineapple skin extract and coconut water is proven to be used as a novel fermentation substrate for bioethanol production by *Saccharomyces cerevisiae* bacteria. This is indicated by the presence of ethanol content contained in the resulting bioethanol products reaching the highest level of 38.42%. The addition of yeast extract during the fermentation process can affect the bioethanol product that is produced. The different mass of yeast extract that used, 14 and 16g/L, affects the density, yield, and ethanol concentration of bioethanol. Yeast extract as a nitrogen source with a mass of 16g/L produced the highest ethanol concentration with operating conditions at pH 5 and 44 hours of fermentation time. So, with evidence from the results of experiments that have been carried out, a mixture of pineapple skin extracts and coconut water can be used as a novel fermentation substrate in bioethanol production. Based on the research done, the potential of pineapple skin extracts and coconut water to be used as an alternative and non-food fermentation feedstock can be further studied for the improvement of ethanol production as renewable energy on a larger scale and performed other analytical tests.

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