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Optimization of Raw Material Preparation for Bioethanol Production from Banana Peel Waste and Addition of Nutrient Liquid Fertilizer from Fish Processing Waste Using Separate Hydrolysis and Fermentation (SHF) Method

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Abstract

The world has relied on fossil fuels as its primary energy source. While fossil fuels still dominate power generation, renewable energy sources such as biomass are gaining recognition due to the implementation of energy policies and growing awareness of the importance of environmentally friendly energy. Bioethanol as a fuel has emerged as a viable alternative because it can reduce environmental pollution and petroleum consumption. One type of lignocellulosic biomass that can be used as bioethanol is banana peel. This study aims to determine the effect of temperature variations and drying of banana peels on the results of moisture and ash content, hydrolysis temperature variations on reducing sugar content, and variations in pH, nutrients, and fermentation duration on carbon dioxide gas volume, content, and bioethanol yield. Banana peels as the main ingredient in bioethanol production go through three stages that are varied: drying, hydrolysis, and fermentation. The hydrolysis and fermentation processes were optimized using the SHF (Separate Hydrolysis and Fermentation) method to determine the effect of each process parameter. The results of the study showed that the optimal conditions for the banana peel drying process were at a temperature of 80^{o}C for 8 hours with a water content of 6.23 ± 0.03% and an ash content of 9.65 ± 0.10%. Testing of reducing sugar levels using the DNS method resulted in reducing sugar levels of 52.71 ± 0.19 g/L at 80^{o}C and 83.67 ± 0.28 g/L at 100^{o}C. Total carbon dioxide gas volume, concentration and maximum bioethanol yield of 605 mL, 47.60%, and 75.51%, were obtained at a pH of 5.5 nutrients in the form of Liquid Organic Fertilizer (LOF) from fish processing waste, and a fermentation duration of 5 days. These results show that Liquid Organic Fertilizer (LOF) from fish processing wastewater can be used as an alternative nutrient besides urea in the production of bioethanol from banana peel waste.

Keywords: Bioethanol, Banana Peel, Fish Processing Wastewater, Separate Hydrolysis and Fermentation

Introduction

Fossil fuels are non-renewable resources, requiring millions of years to form petroleum reserves, the primary ingredient in fuel production. The dwindling availability of these resources is not commensurate with the increasing use of these resources. As a tropical country, Indonesia is blessed with an abundance of natural resources that can be utilized for bioenergy. Bioethanol, a type of bioenergy, can be used as an alternative fuel to mitigate the negative impacts of fossil fuel use. Bioethanol can be produced from natural materials containing carbohydrates, glucose, and cellulose fibers through the use of microorganisms [1].

Bananas, with the Latin name *Musa paradisiaca* L., are a tropical fruit widely grown in Indonesia. According to data from the Central Statistics Agency (CSA), Indonesia produced 9.34 million tons of bananas in 2024. East Java is the largest banana producer nationally, producing 2.63 million tons per year, which containing banana peel of approximately 30-40% of the fruit's weight [2]. In general, banana peels are not being utilized optimally. Banana peel waste is generally discarded as organic waste or used as animal feed [3].

The selection of banana peel waste as the primary ingredient for bioethanol production is due to high potential value in term of carbohydrate content. Banana peel, as a lignocellulosic biomass, contains cellulose, which can be hydrolyzed into glucose and then fermented into ethanol. Therefore, it can solve the organic waste and energy problems as well.

The process of producing bioethanol from banana peel waste begins with physical and chemical pretreatment. Physical pretreatment involves cutting, drying, and grinding [4]. The physical pretreatment process is also known as the raw material preparation process, where in the drying stage several variations can be carried out to find out the best drying temperature and time which can extend shelf life and also prevent microorganism contamination in the raw materials [5]. The next stage is the chemical pretreatment process which is carried out by delignifying NaOH, then the hydrolysis process is carried out with the help of an acid catalyst

[6]. Glucose is produced through a hydrolysis process, then fermented using the help of the yeast microorganism *Saccharomyces cerevisiae* so that it will produce ethanol which is then purified through a distillation process [7]. The bioethanol fermentation process is carried out using the Separate Hydrolysis and Fermentation (SHF) method.

In the bioethanol fermentation process from banana peel waste, *S. cerevisiae* yeast requires nutrients for the growth and metabolism of yeast cells [8]. The nutritional innovation used is liquid organic fertilizer (LOF) from fish processing wastewater. The nutrient content, such as nitrogen, phosphorus, and potassium, in LOF is a source of nutrients needed for the growth of *S. cerevisiae* [9]. Therefore, LOF from fish processing wastewater can be used as an alternative to urea in the fermentation process for making bioethanol from banana peel waste.

Based on the description that has been presented, the optimization of bioethanol using banana peel waste was carried out by varying the temperature and drying time of banana peels, hydrolysis temperature, nutrients, pH and fermentation duration. This study aims to observe the optimum temperature and time for drying banana peels, the effect of hydrolysis temperature on reducing sugar levels, the potential of LOF from fish processing wastewater as a nutrient in the fermentation process, and determine the effect of pH and fermentation duration on the process of making bioethanol from banana peel waste.

Materials and Methods

Materials

The materials used include sodium hydroxide (NaOH, 98%), hydrochloric acid (HCl, 37%), sulfuric acid (H_2SO_4, 98%), asam 3,5-dinitrosalicylic acid (DNS), KNa-tartrate, molasses, microbial starter EM4, $MgSO_4.7H_2O$, urea, dextrose sugar, KH_2PO_4, yeast extract, $K_2Cr_2O_7$, ammonium chloride, phenol. The inoculum used was a pure isolate of *Saccharomyces cerevisiae ATCC-9763*. The isolate was rejuvenated on Potato Dextrose Agar (PDA) medium

to keep the cells active and able to ferment optimally. The banana peels used as the primary substrate were obtained from a banana-based food industry in Malang City, East Java. The waste was cleaned and stored at 4°C to prevent spoilage and slow microbial growth, thus maintaining the material's composition. Meanwhile, fish processing wastewater used as an alternative nitrogen source was obtained from a fish processing factory in Banyuwangi Regency, East Java. This wastewater is generated from various stages of fish processing, from washing to the final production stage.

Optimization of Banana Peel Drying Temperature and Time

The banana peels were cut into smaller pieces and washed thoroughly. Then, the banana peels were oven-dried at different temperatures, namely 50°C, 60°C, 70°C, 80°C, with varying times of 4 hours, 8 hours, and 16 hours. After that, each variation was tested for water content and ash content. The results of the water and ash content tests were compared with quality standards, namely water content (<10%), ash content (<12.5%) and energy use in the drying process (KWh) to obtain the optimal temperature and drying time for banana peels.

Delignification

Delignification process of banana peel powder using 2% (w/v) NaOH solution. Banana peel powder was weighed using an analytical balance as much as 15 grams in a 250 mL Erlenmeyer flask. Then, 15 grams of banana peel powder was mixed with 150 mL of 2% (w/v) NaOH solution, closed the top with a cotton stopper, and wrapped using plastic wrap. The ratio of banana peel powder and 2% NaOH solution is 1:10 (w/v) [10]. Then, the solution is put into an autoclave for the sterilization process for 15 minutes at a temperature of 121ºC [11]. After 15 minutes, the solution was cooled to room temperature. The cooled delignified banana peel pulp was separated into filtrate and residue using a centrifuge at 3,000 rpm for 20 minutes. The

residue was then washed with distilled water until its pH was neutral. It was then dried in an oven at 100°C until a constant weight was achieved and ground into a powder [12]. This powder is known as delignified banana peel. The banana peel powder before and after delignification was then analyzed using SEM to observe any changes in surface morphology caused by base delignification. Lignin content was also tested to determine the effectiveness of delignification in breaking down lignin bonds in the lignocellulose contained in the banana peel powder using the ISO 21436:2020 method.

Calculation of Acid-Insoluble Lignin (AIL) Content:

$$\%AIL = \frac{m2-m1}{m3} \times 100\%$$

Description: m1 = mass of the filter paper (grams)

m2 = mass of the filter paper + acid-insoluble lignin (grams)

m3 = initial mass of the sample (grams)

Calculation of Acid-Soluble Lignin (ASL) Content:

$$\%ASL = \frac{Abs \times V}{\varepsilon \times w \times L} \times 100\%$$

Description: Abs = absorbance of the sample (A)

V = volume of the sample (L) = 1 L

ε = absorptivity coefficient (L g^{-1} cm^{-1}) = 110 L g^{-1} cm^{-1} at 250 nm

w = mass of the sample (grams)

L = path length of the cuvette (cm) = 1 cm

Total lignin content: %AIL + %ASL

Acid Hydrolysis

Hydrolysis of delignified banana peel is carried out by adding delignified banana peel powdr with 1% HCl solution with a ratio of 1:10 (w/v). Then, the mixed solution in the first Erlenmeyer flask was heated in a water bath shaker at a temperature of 80°C and in the second

Erlenmeyer flask at a temperature of 100ºC for 1 hour. The solution was cooled to room temperature, then the residue and filtrate were separated using a centrifuge at 3000 rpm for 20 minutes [13]. The glucose content of the filtrate obtained was then measured using a visible light spectrophotometer. Determination of reducing sugar content was carried out using the 3,5-dinitrosalicylic acid (DNS) reduction method according to the provided procedure using DNS reagent [14].

Preparation of Liquid Organic Fertilizer (LOF) from Fish Processing Wastewater

The process of making liquid fertilizer from fish processing waste begins with a bioactivator made from a mixture of EM4, molasses, and distilled water (1:1:5 v/v). This bioactivator is allowed to stand for two days. Then, the liquid fish processing waste is mixed with the bioactivator (5:2 v/v) and fermented for two weeks. The bottle cap is opened occasionally to release the fermentation gas [15]. The prepared POC was tested for ammonia, nitrate, and nitrite levels based on the ISO 7150-1:1984, ISO 6777-1984, and ISO 7890-3:1988 methods. These tests were conducted to determine the nitrogen content in the form of ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-).

Bioethanol Fermentation

The bioethanol fermentation process begins with the preparation of culture media and rejuvenation of *S. cerevisiae*. 7.8 grams of PDA was dissolved in 200 mL of distilled water, then the solution was homogenized using a magnetic stirrer, then sterilized using an autoclave at 121ºC for 15 minute. The solution was left at room temperature (25ºC) until cool, then poured into a petri dish. Wait until the media cools and solidifies. After the PDA media was ready, *S. cerevisiae* was transferred from the stock culture using the streak plate method and

incubated for 24 hours at 30ºC [16]. The fermentation inoculum was made using regenerated *S. cerevisiae*.

The preparation of fermentation inoculum is divided into three, namely using nutrients in the form of urea, LOF from fish processing wastewater, and control. The preparation of fermentation inoculum is done by mixing 10 grams of dextrose sugar, 1.0 grams of urea or 1 mL of LOF, 0.2 grams of KH_2PO_4, 1.0 gram of yeast extract, 1.0 grams of $MgSO_4.7H_2O$ into an erlenmeyer flask, then adding 100 mL of distilled water. The mixture is then sterilized by autoclaving at 121ºC for 15 minute. Wait for the mixture to cool, the add 2 oses of *S. cerevisiae* isolate. The mixture is homogenized using a waterbath shaker at 30ºC, 150 rpm for 24 hours [17].

The next process is the fermentation stage. At this stage, the pH of the banana peel hydrolysate is adjusted by adding 15% NaOH and 15% HCl [18]. The pH variations used are pH 3; 5.5; and 10. After adjusting the pH, the banana peel hydrolysate is sterilized by autoclaving at 121ºC for 15 minute. Then, the banana peel hydrolysate is added with fermentation inoculum (10:1 v/v), the top of the bottle is closed with a press and nitrogen is injected for 1-2 minutes to remove carbondioxide so that the fermentation process can be carried out anaerobically. Then it is incubated in a waterbath shaker at 30ºC, 100 rpm.

The fermentation duration was carried out at varying times of 1, 3, and 5 days. The carbon dioxide gas produced during the fermentation process was periodically released and its volume was measured using a gas syringe.

Bioethanol Distillation

In the distillation process, each fermentation solution is poured into a distillation flask and heated to a temperature of 78-80ºC, the boiling point of bioethanol. The distillation process is carried out until 10-15 mL of bioethanol is produced, taking approximately 90-120 minutes.

Qualitative and Quantitative Analysis of Bioethanol

The resulting bioethanol was analyzed using quantitative tests to determine the concentration and yield of bioethanol, as well as qualitative tests using potassium dichromate [19]. Then, the bioethanol with the highest concentration and yield in each nutrient variation was distilled again.

$$\text{Ethanol Yield (\%)} = \frac{total\ mass\ of\ ethanol\ (g)}{total\ mass\ of\ the\ raw\ material\ (g)} \times 100\%$$

Results and Discussion

Optimization of Drying Temperature and Duration

The banana peel drying process using an oven was carried out at temperatures of 50°C, 60°C, 70°C, 80°C, with drying times of 4, 8, and 16 hours. The drying process was carried out to ensure the quality of the banana peel used as raw material for bioethanol production. Suboptimal drying can result in the degradation of important components contained in banana peels. Therefore, tests such as moisture content and ash content were conducted to determine the quality of the raw material after the drying process. The moisture content in banana peel samples affects stability, solid particle flow properties, and microbial growth [19]. The results of moisture content showed in **Figure 1,** from twelve test samples varied between 3.85 ± 0.13% and 84.89 ± 0.58% with a temperature variation of 50°C and a time of 4 hours (84.89 ± 0.58%) as the highest moisture content, while a temperature variation of 80°C and a time variation of 16 hours (3.85 ± 0.13%) as the lowest one. Therefore, from the results obtained, the higher the temperature and the longer the drying time, the lower the moisture content. According to El-Imam et al. (2019), banana peel with a moisture content ranging from 6 to 11% can be used for biomass hydrolysis because hydrolysis efficiency and fuel energy value are negatively affected

by high moisture content or water content [20]. In addition, low moisture content can reduce the risk of fungal growth and samples can also be stored for longer periods of time.

On the other hand, ash content refers to the total amount of inorganic minerals that remain as residue after all organic content is burned at high temperatures. **Figure 2** show that the lowest ash content of banana peels is 2.28 ± 0.15%, was produced by drying at a temperature of 50^{o}C for 4 hours, while the highest ash content, 19.48 ± 0.30%, was produced by drying at a temperature of 80^{o}C for 16 hours. The ash content test results show that the higher the variation in drying temperature and time used, the higher the ash content. A higher ash content percentage indicates a higher inorganic mineral content, while the organic content will be lower. El-Imam et al. (2019) stated that the ash content of banana peel waste with a minimum range is an ideal bioenergy converter [20]. This is because at the minimum range, the organic content, such as cellulose and sugar, which are needed in the bioethanol production process will be higher.

Energy Used in Banana Peel Drying

From the results of moisture content and ash content with variations in initial drying temperature (50^{o}C, 60^{o}C, 70^{o}C, 80^{o}C) and time (4 hours, 8 hours, and 16 hours), the most optimal initial drying temperature and time were selected, namely at a temperature of 80^{o}C for 8 hours. The results of moisture and ash content tests between drying times at 80^{o}C for 8 hours and 60^{o}C for 16 hours were almost the same. However, drying for a longer time resulted in higher costs and energy consumption [21].

Figure 3 demonstrates that energy consumption for 8 hours of drying is lower compared to 16 hours of drying. However, in practice, the oven can increase power consumption at higher temperatures because the heating element works more actively to maintain the heating temperature at 80^{o}C. Nevertheless, the heating duration of 8 hours is still

much shorter than 16 hours, so the total energy consumption is lower at 80°C. Therefore, heating at 80°C for 8 hours (13.6 kWh) is more efficient than heating at 60°C for 16 hours (27.2 kWh) for drying banana peels. This shows that the specific energy required to dry banana peels can be reduced at higher temperatures, because at these temperatures a faster drying rate and shorter drying time are obtained [22]. In addition, the drying temperature of 80°C for 8 hours was chosen because at that temperature and time, the moisture content and ash content were 6.23 ± 0.03% and 9.65 ± 0.10%, respectively. The test results meet the requirements according to the Indonesian Materia Medica (MMI), namely moisture content <10% and ash content <12.5% [23].

Delignification of Banana Peels

Banana peel is the main raw material in the bioethanol production process through chemical pretreatment. The alkali pretreatment process with NaOH delignification can increase the maximum ethanol production from banana peel [24]. NaOH works by breaking the ester bonds between lignin and hemicellulose so that the lignin can be dissolved.

As presented in **Table 1,** the lignin content decreased from 18.41% to 9.16% after the NaOH delignification process. This decrease in lignin content was caused by the fact that NaOH delignification resulted in the breaking of bonds between carbohydrates and lignin, causing the lignin to dissolve in NaOH and thus reducing its content. Strong bases such as NaOH disrupt the ester cross-links between lignin and structural carbohydrates, particularly hemicellulose, resulting in lignin degradation [11]. In addition, the results show that the acid-insoluble lignin content is higher than the acid-soluble lignin content. This is because the larger fraction of lignin in lignocellulosic biomass has a strong structure and is resistant to acid, causing most of it to remain insoluble when reacted with strong acids [2]. This is what causes acid-insoluble lignin is a more dominant component than acid-soluble lignin.

SEM (Scanning Electron Microscope) micrographs of banana peel powder before and after NaOH delignification were obtained at a magnification resolution of 2,500x using a particle size of 40 μm. In **Figure 4a**, it can be seen that the surface structure is dense and rough with larger visible pores. The presence of lignin that has not been completely degraded causes the surface to appear more closed. The dense and compact structure, as indicated by the blue arrow, indicates that lignin still dominates and has not been decomposed, so that not many pores are open. Meanwhile, **Figure 4b**, illustrates that the surface structure is more open, loose, and the pores appear smaller because the fibers have begun to decompose. The NaOH delignification process causes lignin degradation, resulting in a more porous structure. In addition, NaOH also dissolves most of the lignin with a more open and decomposed cellulose structure, as indicated by the green arrow, so that the surface area increases significantly. According to Chen et al. (2020), changes in the microstructure of banana peel after NaOH delignification are caused by a reduction in hemicellulose and lignin, resulting in swelling of the fibers and disruption of the bonds between hemicellulose and lignin, thereby causing the structure to disintegrate [25]. According to Agbede et al. (2020), modifications to the surface of banana peel cause oxidation of lignin, producing hydroxyl, carbonyl, and carboxyl groups [26]. These groups can increase the solubility of lignin in alkaline solutions, namely NaOH.

Effect of Hydrolysis Temperature Variation on Reducing Sugar Content

After undergoing a pretreatment process in the form of NaOH delignification, banana peels undergo hydrolysis. This process is carried out to obtain fermentable sugars from polysaccharides found in lignocellulosic biomass. Polysaccharides such as cellulose and hemicellulose require hydrolysis to become monosaccharides. During the hydrolysis process, important monomers that are included in reducing sugars, such as glucose, can be obtained [27].

Hydrochloric acid is one type of strong acid commonly used for the hydrolysis of various complex biomasses, such as banana peel [28]. This study shows that hydrolysates treated with acid, namely hydrochloric acid (HCl) at hydrolysis temperatures of 80ºC and 100ºC, produce different levels of reducing sugars. A higher reducing sugar content of 83.67 ± 0.28 g/L was recorded in the hydrolysate treated at a hydrolysis temperature of 100ºC compared to other treatments, namely at a hydrolysis temperature of 80ºC of 52,71 ± 0,19 g/L. This is because the reducing sugar content will increase as the hydrolysis temperature increases. A higher temperature during the hydrolysis process will increase the area of interaction between the catalyst and cellulose, resulting in a higher reducing sugar content [29].

Effect of Fermentation Duration and pH on Total Carbon Dioxide (CO_2) Production

Ethanol is produced through the fermentation of lignocellulosic biomass such as banana peels with carbon dioxide as a by-product. The accumulation of gas assumed to be carbon dioxide produced during the bioethanol fermentation process will inhibit the overall efficiency of anaerobic fermentation due to gas buildup [4]. Therefore, continuous gas release is necessary to avoid disrupting the bioethanol production process. The total gas assumed to be carbon dioxide is calculated from the accumulation of gas produced during various fermentation durations, namely 1 day, 3 days, and 5 days.

Figure 5 illustrates that the longer the fermentation duration, the higher the total carbon dioxide gas produced. According to Ullah et al. (2023), the cumulative volume of carbon dioxide gas produced during the fermentation process will be proportional to the amount of glucose consumed and ethanol produced [10]. The results presented in **Figure 6** indicate that the highest total carbon dioxide gas in each nutrient variation was found in the pH 5.5 variation, where pH is the optimum pH for the growth of *S. cerevisiae* [12]. In addition, the highest total carbon dioxide gas was found in the LOF from fish processing wastewater nutrient variation,

where LOF is the best nutrient, resulting in higher yeast metabolic activity, which causes the total carbon dioxide gas produced to increase.

Figure 5a shows the graph of total carbon dioxide gas produced during the fermentation process, while **Figure 7a** demonstrates the bioethanol concentration. These two graphs show significant differences, whereas according to Ullah et al. (2023), the cumulative volume of carbon dioxide gas produced during the fermentation process will be proportional to the amount of glucose consumed and ethanol produced [10]. This can be caused because during the fermentation process other gases can be formed, especially as metabolic by-products or due to non-ideal fermentation conditions. This may be because other gases can form during the fermentation process, mainly as a byproduct of metabolism or due to less-than-ideal fermentation conditions. Gases such as hydrogen sulfide (H_2S) or ammonia (NH_3) can be formed, causing an increase in the total amount of gas produced. Ammonia (NH_3) is formed due to excess nitrogen, which then undergoes decomposition, while hydrogen sulfide (H_2S) is formed when there is insufficient nitrogen, causing disruption in sulfur metabolism [30].

Effect of Nutrient Variations, pH, and Fermentation duration on Bioethanol Concentration and Yield

One variation applied during the bioethanol fermentation process from banana peel waste is varying the nutrients used in the fermentation inoculum. The fermentation inoculum contains several nutrients such as dextrose, yeast extract, KH_2PO_4, $MgSO_4$, urea, and LOF from fish processing wastewater. The addition of nutrients as sources of carbon, nitrogen, phosphate, and sulfate in the fermentation inoculum can increase the growth of *S. cerevisiae* [31]. In Figures 28 and 29, the highest bioethanol concentration and yield were found in the nutrient variation in the form of LOF from fish processing waste. This is because LOF from fish processing waste is rich in inorganic nitrogen (ammonia, nitrate, and nitrite), phosphorus,

potassium, and other micro nutrients that are released during the decomposition of organic matter [32]. In addition, LOF from fish processing waste also contains organic nitrogen in the form of amino acids, proteins, and peptides produced by microbial activity during the LOF production process. Much more complex organic content, such as residual carbohydrates and lipids, can provide additional carbon sources that can be used by yeast for growth during the fermentation process.

The use of LOF from fish processing wastewater as a nutrient source in the fermentation inoculum provides organic and inorganic nitrogen, vitamins, and micro minerals needed by *S. cerevisiae* yeast. In the process, the use of urea which provides inorganic nitrogen as a nutrient source in the fermentation inoculum produces lower bioethanol concentration and yield than LOF from fish processing wastewater. Therefore, the more complex nitrogen content in LOF is more effective in the bioethanol fermentation process from banana peel waste than using urea, which only contains inorganic nitrogen. These results are supported by research Wang et al. (2024), where the bioethanol concentration using *S. cerevisiae* in urea nutrient variations was 31.82 ± 0.47% and 84.91 ± 1.22%, lower than the variation without urea (control) of 33.45 ± 0.78% [33].

The use of LOF from fish processing wastewater as the best nutrient variation was seen from the results of bioethanol concentration and yield, supported by the content of inorganic nitrogen in the form of (NH_4-N), nitrate (NO_3-N), and nitrite (NO_2-N). In this study, the ammonia content increased from 33.58 mg/L to 40.02 mg/L, nitrate decreased from 4.57 mg/L to 1.47 mg/L, and nitrite decreased from 0.26 mg/L to 0.13 mg/L. These results prove that, in addition to containing organic nitrogen, LOF from fish processing wastewater also contains inorganic nitrogen, making its content more complex than urea. The inorganic nitrogen content in the form of ammonia (NH_4-N), nitrate (NO_3-N), and nitrite (NO_2-N) is obtained from fish

processing wastewater through protein degradation and the biological process of nitrification [15].

Of the three nutritional variations applied to the fermentation inoculum, *S. cerevisiae* was still able to grow and ferment even in inoculums that did not use nitrogen sources such as urea or LOF from fish processing waste. This was supported by the concentration and yield of bioethanol produced in **Figures 7** and **8** when urea and LOF were not used as nutrients. Without using these two nutrients, the growth of *S. cerevisiae* will be slightly slower, resulting in a lower ethanol concentration. Apart from nitrogen, the nutrient most absorbed by yeast is carbon [34]. Dextrose serves as a source of carbon and the main energy source for *S. cerevisiae*. The addition of yeast extract also provides an organic nitrogen source for *S. cerevisiae*. Furthermore, KH_2PO_4 and $MgSO_4$ are essential mineral sources that provide phosphorus (P), potassium (K), magnesium (Mg), and sulfur (S). With the addition of these nutrients, the yeast will have sufficient nutrients to grow and carry out the fermentation process.

Figure 7b provides evidence that the highest concentration and yield of bioethanol in the three nutrient variations were at pH 5.5. This is because the optimum pH for bioethanol production using S. cerevisiae is in the range of 5.0–5.5 [35]. At this pH range, the enzymes involved in glycolysis and the conversion of pyruvate to ethanol will work effectively [12]. On the other hand, at pH 3, the concentration and yield of bioethanol are lower because the environment becomes too acidic for *S. cerevisiae*, causing enzyme activity in yeast cells to be inhibited and decreased. At pH 10, the concentration and yield of bioethanol are also lower because the environment becomes too alkaline for *S. cerevisiae*, causing enzymes to denature, cell structure to be damaged, and cell metabolism to be disrupted [35].

Based on the fermentation duration, **Figures 7** and **8** show that the highest bioethanol concentration and yield in all three nutrient variations were at 5 days of fermentation. Bioethanol production increased gradually, starting from 1 day, 3 days, to 5 days. This is

because the fermentable sugar is sufficient for the growth of *S. cerevisiae* microorganisms in digesting sugar into bioethanol [36]. Bioethanol from distillation with the highest concentration in each nutrient variation was redistilled because the moisture content in the bioethanol was still quite high. According to **Figures 7** and **8**, the highest concentration and yield of bioethanol were found in fermentation at the optimum pH of 5.5 for 5 days.

As presented in **Table 3**, the bioethanol distilled from the three nutritional variations contains ethanol, as indicated by a change in the color of the solution from orange to blue-green. Qualitative testing of ethanol using potassium dichromate can detect the presence of ethanol, where ethanol is oxidized so that the orange-colored dichromate ions are reduced to blue-green chromium ions [37].

The bioethanol concentration increased after redistillation as shown in **Figure 9a**. According to Bayu et al. (2022), the bioethanol concentration below 20% is not high enough to be used practically as bioenergy [38]. The low bioethanol concentration is due to the presence of an ethanol-water azeotropic mixture, which increases its density. Therefore, an additional process such as redistillation is needed to increase the bioethanol concentration. The additional method of redistillation has been proven effective in increasing the bioethanol concentration, where the ethanol concentration after 2x distillation is higher when compared to 1x distillation.

It is confirmed in **Figure 9b** that bioethanol yield decreased after 2x distillation. According to Yoon et al. (2019), double distillation results in a reduction in water content as an ethanol mixture, producing a solution with a high concentration but with a smaller volume [39]. The reduction in bioethanol volume after 2x distillation resulted in a decrease in yield. In **Figures 9**, the highest concentrations and yields of bioethanol were found in the LOF from fish processing waste nutrient variation, at 47.59% and 75.51%, respectively. Therefore, it can be

concluded that LOF from fish processing wastewater is the best nutrient that can be used in the production of bioethanol from banana peel waste.

Conclusions

The result of this study showed that the optimum condition of the banana peel drying process was carried out at a temperature of 80°C for 8 hours, resulting in a moisture content of 6.23 ± 0.03% and an ash content of 9.65 ± 0.10%. The acid hydrolysis process with HCl showed that a hydrolysis temperature of 100°C produced a higher reducing sugar content of 83.67 ± 0.28 g/L compared to a hydrolysis temperature of 80°C, which produced 52.71 ± 0.19 g/L. The highest total volume of carbon dioxide gas, concentration and bioethanol yield results were found at a pH variation of 5.5, a fermentation duration of 5 days, and a LOF variation of fish processing wastewater, with results of 605 mL, 47.59%, and 75.51%, respectively.

Abbreviations

***CSA*:** Central Statistics Agency

***LOF*:** Liquid Organic Fertilizer

***SHF*:** Separate Hydrolysis and Fermentation

***SEM*:** Scanning Electron Microscope

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Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics declarations

Ethical approval and consent to participate

Not applicable

Human participation and animals traits

Not applicable

Informed consent

Not applicable

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