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Biothanol from Rice Washing Wastewater with Variations in Addition of NPK and Fermentation Time

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Abstract. Rice-washing wastewater has the potential as a raw material for making bioethanol because it still contains carbohydrates. Adding NPK with the appropriate fermentation time can affect the levels of bioethanol produced. This study aims to determine the effect of the addition of NPK and fermentation time on the levels of bioethanol produced in the manufacture of bioethanol from rice-washing wastewater. Variations in the amount of NPK (5, 10, and 15 g) and variations in fermentation time (3, 5, and 7 days) are seen in their effect on the yield and characteristics of the bioethanol produced. The characteristics of bioethanol include content, specific gravity, API Gravity (American Petroleum Institute), calorific value, water content, density measurement with a densitometer instrument and qualitative analysis of bioethanol with a gas chromatography instrument. The highest bioethanol yield was 34% with the treatment of variations in adding 15 g of NPK and a fermentation time of 7 days. Based on the two-way ANOVA test results, the addition of NPK (5, 10, and 15 g) and the difference in fermentation time (3, 5, and 7 days) significantly affected the levels of bioethanol produced from rice-washing wastewater. The addition of 15 g of NPK and a fermentation time of 7 days provided the best bioethanol characteristics, with a bioethanol content of 20.84%. Using rice-washing wastewater with NPK and optimal fermentation time provides another alternative in developing bioethanol raw materials.

Keywords: Bioethanol, Enzyme, Fermentation, Rice, Waste.

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Introduction

Biofuel is a New Renewable Energy (EBT) derived from biomass processing. Bioethanol is one of the biofuels commonly used today [1]. The raw materials for first-generation bioethanol are widely available in Indonesia, including cassava, corn, sweet potatoes, and sugar cane [2]. These raw materials are biomass rich in carbohydrates and are produced by plants that produce carbohydrates or starch [3]. One of the raw materials that have the potential to produce bioethanol is rice-washing wastewater [4]. In its processing into rice, rice undergoes a washing process before being cooked. This wastewater is usually wasted, even though the content of organic compounds and minerals is diverse.

The content of organic compounds and minerals in rice-washing wastewater includes carbohydrates, nitrogen, phosphorus, potassium, magnesium, sulfur, iron, and vitamin B1 [5]. Rice-washing wastewater with the addition of glucoamylase enzyme as a catalyst and a fermentation time of 4 days can produce bioethanol with a content of 11.17% [6], while at a fermentation time of 7 days, it can produce bioethanol with a content of 13% [3]. Fermentation with a time of 5 days and the provision of optimal glucoamylase produces a bioethanol content of 19.39%. Making bioethanol with different fermentation times can affect the content of bioethanol produced. The longer the fermentation time, the greater the bioethanol content.

Bioethanol is produced from sugar, which is the result of yeast cell fermentation. Good yeast used to produce bioethanol is from the genus Saccharomyces. This yeast can obtain nutrients from NPK fertilizer. This fertilizer is a compound fertilizer containing elements such as Nitrogen (N), Phosphorus (P), and Potassium (K). The addition of NPK to the fermentation of rice-washing wastewater can increase microbial productivity in producing bioethanol. The addition of 20% NPK in the production of bioethanol from rice straw produces the highest bioethanol content [7]. However, the addition of NPK to rice -washing wastewater has not been studied and is a novelty in this study.

Experimental

Sample Preparation. 5 Kg of rice was washed with 5.5 L of water. In this study, washing was carried out once, and the rice was separated from the washing water. All rice-washing wastewater was meticulously collected for the fermentation process, ensuring a comprehensive dataset for our experiment.

Hydrolysis of Rice Washing Wastewater. Before the hydrolysis process, the rice-washing wastewater sample was measured for glucose levels and initial pH with utmost precision. The pH was meticulously set between 4-5 by adding HCl 0.1 N. Once the desired pH was reached, the rice-washing wastewater was added with 50 grams of glucoamylase enzyme, and heated for 3 hours at a temperature of 60. After the hydrolysis process, the sample was measured with a refractometer, ensuring the accuracy of our results.

Determination of Reducing Sugars in Rice-Washing Wastewater (RWW). Measurement of sugar content in rice-washing wastewater using a refractometer. Rice-washing wastewater was measured by dropping a sample of rice-washing water on the appliance. The °Brix scale of the refractometer is equal to the weight of the grams of glucose in the measured solution.

Bioethanol Production. The bioethanol production process begins with 4.5 L of RWW, adding 25 grams of yeast and stirring until homogeneous. The rice washing water sample was divided into three parts of 1.5 L each. NPK was added in varying amounts of 5, 10, and 15 grams to each sample. The samples are then poured into a fermentation bottle of 250 mL of treatment dully (2 repetitions) with each variation. The samples are left to ferment for 3, 5, and 7 days at room temperature (26-28°C).

The fermentation results are separated using a rotary evaporator with a heating temperature of 78 °C and a time of 1 hour. The bioethanol produced was tested for bioethanol characteristics. In this study, the two-way ANOVA RAL test was carried out to analyze the influence of the amount of NPK mass and fermentation time. The test was conducted using the Statistical Product and Service Solution (SPSS) Statistics 25 application, and the bioethanol with the best levels was qualitatively analyzed with gas chromatography instruments. Bioethanol yield was calculated from the results of the measurement of bioethanol volume obtained from the distillation of fermented rice washing water waste divided by the volume of the basic material/initial product.

Specific Gravity Analysis of Bioethanol

(Specific Gravity). Ethanol content can be determined based on the specific Gravity of the distillate using the table of specific Gravity and ethanol content in the IV Edition of the Indonesian Pharmacopoeia in Appendix 10. This value is the unit weight of the volume of a material. The chronometer was cleaned by rinsing it with aqueducts and rinsing it again with ethanol, and then the pedometer was dried. An empty picometer was weighed by weight (W0), filled with aqua dest at a temperature of 25, and then a pycnometer containing aqua dest was wiped on the outside and weighed (W1). A pycnometer contains distillates by weight (W2), and the specific Gravity is calculated according to the formula.

$$\text{Specific gravity } (\rho) = \frac{W_2 - W_0}{W_1 - W_0} \quad (1)$$

Where, ρ = Specific Gravity (kg/m^3); W_0 = Empty Piknometer Weight; W_1 = Weight of the Pyknometer containing aquadest; W_2 = Weight of the Pyknometer containing the distillate.

Gravity API Analysis (G). The API (American Petroleum Institute) gravity value is an inverse measure used to determine the weight of petroleum liquids compared to water. A liquid with a gravity API of more than 10 is considered a light oil that floats on water. If the liquid's API gravity is less than 10, it will sink into the heavy oil category. Meanwhile, API gravity measures the relative density of petroleum liquids and water. Mathematically, the Gravity API has no dimensions. However, the measurements are assessed in degrees using a custom-made hydrometer instrument. Thanks to the strategic API scale design, most petroleum liquids will be categorized between 10 and 70 degrees of API gravity. The magnitude of API gravity ranges from 0-100, while specific Gravity is the relative price of a material's density to water. The specific gravity value and API gravity are then used to calculate the calorific value. In the following formulation in calculating API Gravity, constants 141.5 and 131.5 are Baume scales to measure the specific Gravity of liquids less dense than water [14].

$$G = \frac{141{,}5}{sg} - 131{,}5 \quad (2)$$

Analysis of Calorific Value (NK). This analysis aims to determine the heat energy produced in each bioethanol composition to be tested. The calorific value can be calculated using the following equation:

$$NK \times = \frac{2{,}2046226}{3{,}9673727} \times (18{,}650 + 40)(G - 10)kkal/kg \quad (3)$$

Where, NK = Calorific value (kcal/kg); G = API Gravity.

Moisture Content Analysis. The bioethanol moisture content test was carried out by calculating the initial weight of the fermentation results minus the final weight after distillation divided by the initial weight. The formulation in calculating the moisture content is:

$$\text{Moisture Content} = \frac{A - B}{A} x\ 100\%$$

Where, A = Initial Sample Weight (mL); B = Final Sample Weight (mL).

Density of Bioethanol. The bioethanol level test of rice washing water waste was carried out in two ways: reading the ethanol specific gravity alcohol metric table based on the Indonesian Pharmacopoeia IV Edition in Appendix 10 and using the Anton Paar brand densitometer instrument. In the Anton Paar densitometer, only the optimum level of bioethanol from rice washing water waste is measured, namely by the pinched sample, and the results will be read on the device screen.

Bioethanol Analysis with Gas Chromatography Instruments. The best fermentation results obtained at the optimum time were analyzed qualitatively using gas chromatography instruments using internal standard comparators and HP Agilent DB-624 as the column.

Result and Discussion

Reducing Sugar of Rice Washing Wastewater.

The initial pH of the rice-washing wastewater sample was pH 6.68, which is a neutral pH. Based on the initial pH, the acidity level was increased before hydrolysis by lowering the pH to 4-4.5. The pH of the rice-washing wastewater sample was 4.8. The pH adjustment aims to achieve the optimum performance of enzymes and yeast. The optimum temperature of the glucoamylase enzyme ranges from 60

C, and the optimum pH is 4–5, with the main result of its breakdown being glucose [8,9]. The sugar content contained in rice-washing wastewater is 1.5%. This content is very small, so if the fermentation process is carried out directly, it will produce low levels of ethanol. The starch contained in rice washing wastewater can be hydrolyzed to produce glucose. The hydrolysis carried out in this study was enzymatic and heating. The enzyme used in the hydrolysis process is the glucoamylase enzyme, which plays a role in starch saccharification. The saccharification stage using the glucoamylase enzyme shows an increase in reduced sugar levels [10]. This increase in reduced sugar levels is due to the hydrolysis of maltose and dextrin into glucose by the glucoamylase enzyme during the saccharification stage. After hydrolysis, the sugar content in the rice washing water increased to 5.2%.

The principle of starch hydrolysis is the breaking of the starch polymer chain into monosaccharide units, namely glucose (C6H12O6). The reaction mechanism of the glucoamylase enzyme is to cut the alpha-1,4 glycosidic bond in the starch molecule. This enzyme can also break the alpha-1,6 bond but at a lower frequency. The main result of the breakdown is glucose, a simple form of carbohydrate molecule [9]. The glucoamylase enzyme can lower the activation energy to accelerate the breakdown of the polysaccharide polymer chain into its constituent sugar monomers [6]. Sufficient starch or carbohydrate content in rice-washing wastewater can be broken down into glucose through an enzymatic hydrolysis process using the glucoamylase enzyme at a constant temperature of 60°C.

Bioethanol Yield. Yield is one of the parameters to determine the ratio of the results (volume of distillate) to the raw material in rice-washing wastewater. In each treatment, different yield results were obtained. This happens because bacteria need time to convert glucose into bioethanol. In addition to fermentation time, adding NPK mass in the fermentation process of rice washing water into bioethanol also affects the resulting yield.

Based on Figure 1, the average yield of the highest bioethanol was on the seventh day of fermentation (34%), and the average yield of the lowest bioethanol yield was on the third day of fermentation (13.4%). This is because the longer the fermentation time treatment carried out, the greater the bioethanol yield obtained. The amount of yeast and fermentation time during fermentation greatly affect the bioethanol yield obtained [11]. The high yield is due to the long fermentation time of rice-washing wastewater, allowing yeast to break down more starch and produce more alcohol [12].

Bioethanol Specific Gravity. Specific gravity is a quantity that states the ratio between mass (g) and volume (mL). The lowest specific gravity of bioethanol was obtained at 0.9748 g/mL at a fermentation time of 7 days, and the amount of NPK was 15 grams (Figure 2). This specific gravity exceeds the absolute specific gravity of bioethanol, which is 0.789 g/mL (National Standards Agency, 2009). A lower specific gravity or approaching the best characteristics of bioethanol indicates the high ethanol compounds contained in the product [13]. So, the low specific gravity indicates that its properties can easily evaporate. In the research that has been conducted, the specific gravity obtained does not match absolute bioethanol. This result shows that the bioethanol is not pure because it is still mixed with water. Different water content will cause differences in specific gravity and boiling points.

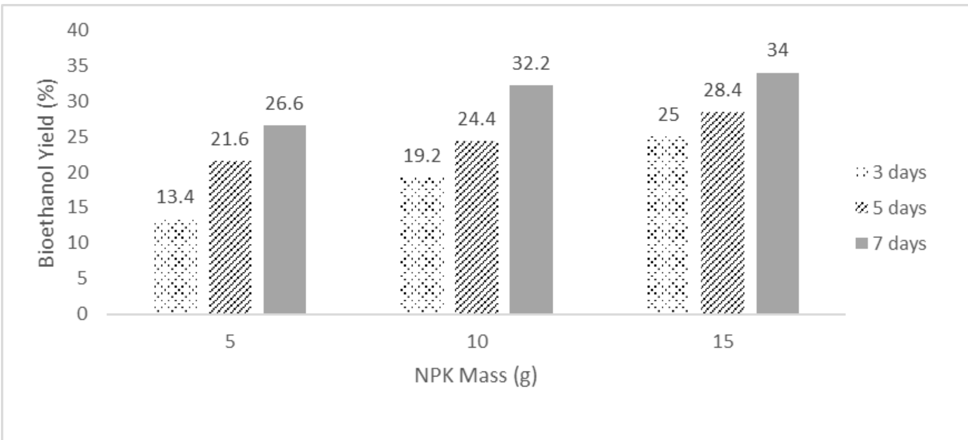

Figure 1. The Yield of Bioethanol from Rice-Washing Wastewater

API Gravity (G) Value. The API gravity value, a measure used to determine the weight of petroleum fluids compared to water, is of significant importance in the context of bioethanol from rice-washing wastewater. A fluid with an API gravity of more than 10 is considered a light oil that floats on water. The results of this study (Figure 3) reveal that bioethanol from rice-washing wastewater has the lowest API Gravity value at a variation of 5 grams of NPK (10.8). Conversely, the highest value of the API Gravity analysis results was obtained from a variation of 15 grams of NPK (13.7). It is important to note that the API Gravity value has a very close relationship with the calorific value of bioethanol. This relationship is direct and proportional- the greater the API Gravity value, the higher the calorific value. Conversely, if the API Gravity value is low, the calorific value is low.

Bioethanol Calorific Value. The calorific value obtained from the research results shows that rice-washing wastewater has not approached the bioethanol quality requirement value. The National Standards Agency (BSN) sets the bioethanol quality standard at a maximum calorific value of 5000 kcal/kg. The lowest average is seen at an NPK content of 5 g of raw materials, showing a calorific value of 28.13 kcal/kg. The highest average calorific value is at an NPK content of 15 g of 91.98 kcal/kg.

The API Gravity value and specific gravity influence the calorific value in the results of this study. This is because the specific gravity of the fuel will affect the fuel consumption rate. The greater the specific gravity, the greater the fuel consumption. This means that a considerable specific gravity value produces a small API gravity value and calorific value, producing low-quality bioethanol.

A considerable calorific value will make the combustion process easier, so the quality of bioethanol can be said to be good [14]. The research results show that the calorific value of bioethanol from rice-washing wastewater is still very low. The greater the addition of the amount of NPK and the variation in the length of fermentation time given in the fermentation process, the calorific value of the bioethanol produced will increase but is not yet optimal (Figure 4).

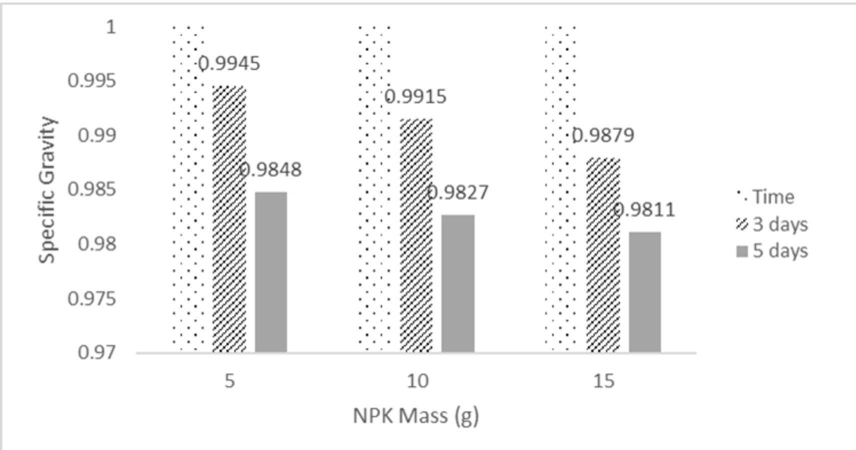

Figure 2. The Specific Gravity of Bioethanol from Rice-Washing Wastewater

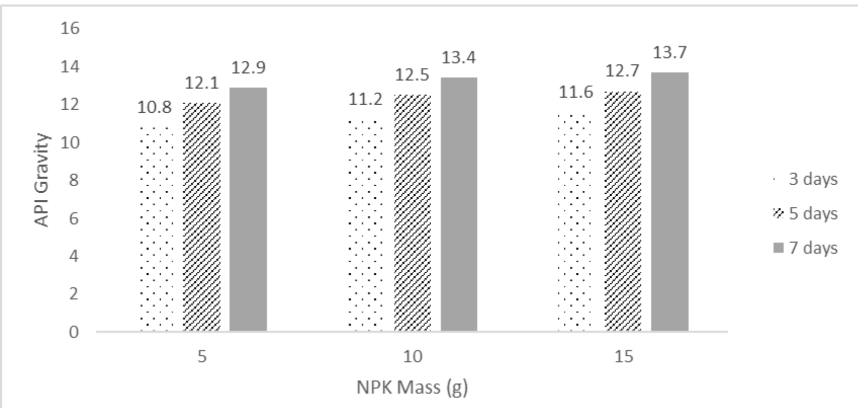

Figure 3. API Gravity Values

Water Content. The bioethanol water content test was carried out by calculating the initial weight of the fermentation results minus the final weight after distillation divided by the initial weight. The water content produced from several variations of NPK and fermentation time showed that the highest water content was obtained from the NPK content of 5 grams, namely 86.8%. In comparison, the lowest water content was obtained from the yeast content of 15 grams, namely 67.2%.

The greater the amount of NPK given, the lower the water content value obtained (Figure 5). The data generated from this study show that bioethanol's water content value does not meet bioethanol's quality requirements, with a maximum value of 2% [14]. This is because the ethanol produced is still not pure. After all, it is mixed with water. The distillation process is conventional, so bioethanol from rice washing water waste still contains much water. The higher the water content, the lower the heat of combustion.

Bioethanol Levels. Table 1 shows the highest average bioethanol level of 20.84% with the addition of 15 grams of NPK and a fermentation time of 7 days. The research results show that variations in the amount of NPK and the longer fermentation time can affect the levels of bioethanol produced. The longer the fermentation time is, the higher the volume of bioethanol produced [15]. This is because the longer the fermentation time, the more microbes reproduce, so with the increasing number of microbes, the more carbohydrate polymers break down into alcohol. Microbes need essential nutrients as food and energy sources [16], such as nitrogen and phosphate [17]. The nutrients for the growth of Saccharomyces cerevisiae are increasingly sufficient, so the bioethanol produced is also more optimal. The more NPK or nutrients added to the fermentation medium, the greater the bioethanol produced.

Based on the results of the Completely Randomized Series (CRD) ANOVA test, two ways of NPK Mass factor (M) and fermentation time factor (H)

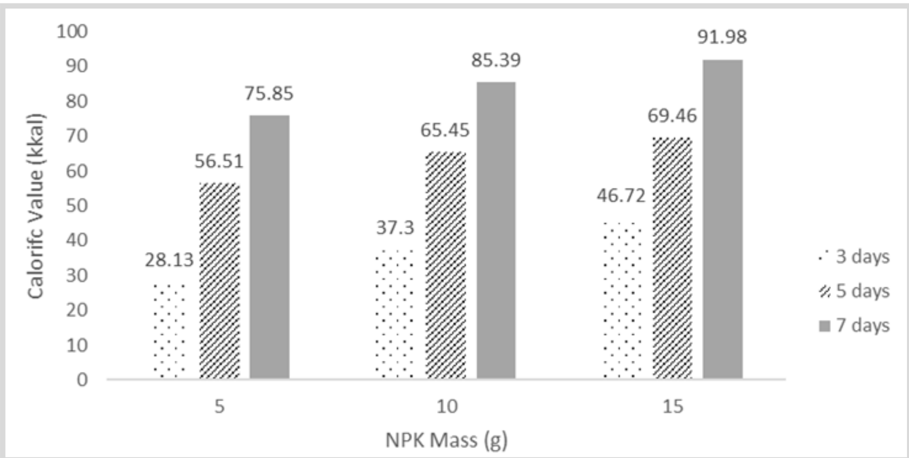

Figure 4. Calorific Value of Bioethanol in Rice-Washing Wastewater

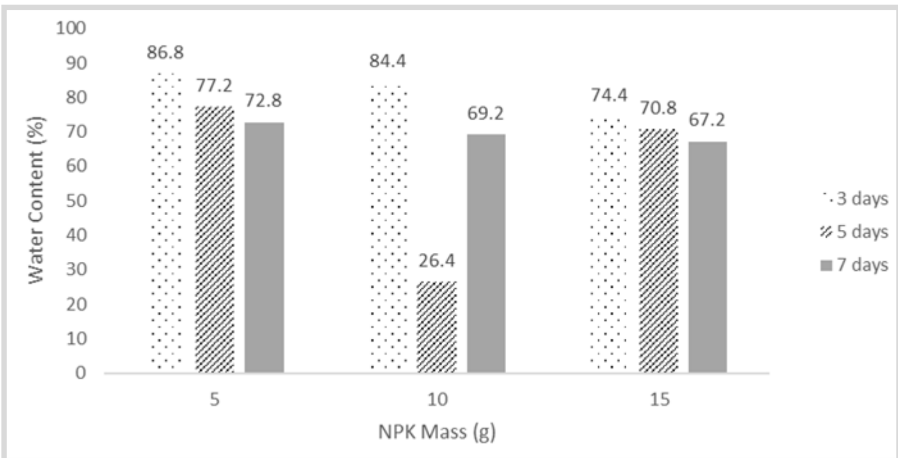

Figure 5. Water Content Analysis

Table 1. Bioethanol Levels of Rice Washing Water After Distillation

NPK Mass (g)	Bioethanol Levels (%) Time of Fermentation (Days)		
	3	5	7
5	3.76 ± 0.01[a]	9.92 ± 0.03[d]	14.85 ± 0.01[g]
10	6.25 ± 0.01[b]	12.39 ± 0.01[e]	17.26 ± 0.01[h]
15	7.47 ± 0.02[c]	13.61 ± 0.01[f]	20.84 ± 0.06[i]

have a significance of 0.000 less than 0.01 ($p < 0.05$), so the treatment is very significantly different. It means that the difference in the amount of NPK mass and fermentation time significantly affects bioethanol levels. The interaction or relationship between NPK Mass (M) and fermentation time (H) has a significance of 0.000 less than 0.01 ($p < 0.05$), so the treatment is very significantly different. This means that the interaction of the concentration of the amount of NPK with fermentation time affects bioethanol levels. Duncan's further test was carried out on factors (treatments) that were significantly different so that they were carried out on factors M, H factors and MH interactions. he further ANOVA test, namely the Duncan Test, the interaction between the treatment of adding the amount of NPK mass (M) and variations in fermentation time (H) was significantly different. Because the ANOVA interaction states that the bioethanol levels are significantly different or that the interaction produces different bioethanol levels statistically, the best treatment for bioethanol levels is the variation in the mass amount of 15 grams and fermentation time for 7 days with the highest average.

Conclusion

Rice washing wastewater has the potential to be used as a raw material for bioethanol with further research. The amount of NPK and fermentation time affect the yield, content and character of the bioethanol produced. The best treatment for bioethanol levels is the variation in the mass amount of 15 grams and fermentation time for 7 days.

Author Contributions

Nur Rahmah Maulina designed the research, collected data and composed the manuscript. Devy Susanty and Nurlela contributed to design the research and composed the manuscript. Nina Ariesta aided in the review of the results and discussion

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