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Effects of concentration of NPK fertilizer as a Nitrogen Source in fermentation of Bioethanol

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ABSTRACT

Bioethanol is a versatile raw material widely used in the production of ethanol derivatives, pharmaceuticals, fuel additives, alcoholic beverages, solvents, and medicines. Its production involves a fermentation process that can be optimized by adjusting nutrient concentrations. This study investigates the effects of NPK fertilizer concentration as a nitrogen source on the yield and density of bioethanol produced from sugarcane fermentation. The concentrations of NPK fertilizer tested were 0.00%, 0.10%, 1.00%, and 2.00%. The results indicate that low concentrations of NPK, particularly 0.10%, significantly enhanced ethanol production, achieving the highest bioethanol yield of 3.71%. This increase in yield is attributed to the essential nutrients provided by NPK, which support microbial growth and fermentation efficiency. However, as NPK concentration increased to 1.00% and 2.00%, ethanol yield sharply declined to 1.24% and 1.20%, respectively, likely due to osmotic stress and the proliferation of non-ethanol-producing microorganisms, which hindered fermentation efficiency. Regarding bioethanol density, no significant differences were observed across the varying NPK concentrations, with values ranging from 2.31 g/mL to 2.33 g/mL, suggesting that nitrogen supplementation does not influence the physical properties of the bioethanol. The density of the bioethanol produced was far higher than the Indonesian National Standard for fuel-grade ethanol, indicating the need for further purification processes, such as distillation or dehydration, to meet quality standards. These findings highlight the importance of optimizing NPK fertilizer concentrations to maximize ethanol yield while emphasizing the role of post-fermentation treatments for improving bioethanol quality

Keywords: bioethanol, NPK fertilizer, nitrogen source, fermentation, ethanol

1. INTRODUCTION

The world's energy needs have long been dominated by fossil fuels, such as petroleum and coal. However, as global reserves of these fuels decline and environmental concerns rise, there is a growing need for alternative, renewable energy sources. In Indonesia, for instance, the increasing demand for fuel, driven by advancements in transportation and infrastructure, has put immense pressure on the national supply of fossil fuels. The country's national fuel consumption exceeds the supply, with Pertamina, the national oil company, providing around 1.03 million kiloliters per year, while the demand reaches approximately 1.4 million kiloliters per year. This gap underscores the urgency of seeking alternative energy solutions. Bioethanol, a renewable energy source produced through the fermentation of biomass, has emerged as a promising alternative. Bioethanol not only offers a cleaner energy option but also provides a viable solution to reducing dependency on fossil fuels and mitigating environmental impacts[1].

The production of bioethanol through fermentation involves the conversion of organic materials, such as tubers, legumes, and agricultural residues, into ethanol[1]. This process utilizes microorganisms, particularly *Saccharomyces cerevisiae*, to convert sugars into ethanol. Fermentation is influenced by several key factors, including the availability of carbon and nitrogen sources. Carbon is typically supplied by sugars or other

carbohydrates in the substrate, while nitrogen is essential for microbial growth and metabolism. Nitrogen is a key component of amino acids, proteins, and other cellular structures, making it crucial for the fermentation process. While various nitrogen sources can be used, one of the most widely utilized and accessible sources is NPK fertilizer, which contains nitrogen, phosphorus, and potassium – three essential nutrients for microbial growth[2], [3].

NPK fertilizer is an attractive option for bioethanol fermentation due to its affordability and easy availability. Nitrogen, phosphorus, and potassium play vital roles in promoting the growth of *Saccharomyces cerevisiae*, the primary yeast used in bioethanol production. Nitrogen supports protein synthesis and cellular function, while phosphorus is involved in energy transfer within cells, and potassium helps regulate cellular processes such as osmoregulation and enzyme activation. The balanced combination of these three nutrients in NPK fertilizer creates an optimal environment for the fermentation process, enhancing ethanol yield. However, while NPK fertilizer is commonly used, the concentration of these nutrients is critical, as it can significantly impact the efficiency of the fermentation process[2], [4], [5].

The concentration of NPK fertilizer is a key factor that influences bioethanol production. Excessive amounts of nitrogen, phosphorus, or potassium can lead to nutrient imbalances that hinder microbial growth and fermentation efficiency. On the other hand, insufficient nutrient levels may result in slow fermentation rates and low ethanol yields. Therefore, it is essential to determine the optimal concentration of NPK fertilizer that supports the highest ethanol production without causing detrimental effects on the fermentation process. Previous studies have shown that moderate concentrations of NPK fertilizer, particularly nitrogen, can enhance the growth of *Saccharomyces cerevisiae* and increase ethanol yield. However, the precise concentrations that lead to optimal ethanol production remain an area of active research[1], [6].

This study aims to explore the effects of varying concentrations of NPK fertilizer on bioethanol production. By testing different concentrations of NPK (0%, 0.5%, 5%, and 10%), the research will identify the optimal fertilizer concentration for maximizing ethanol yield during fermentation. The results will provide valuable insights into the role of nitrogen and other nutrients in bioethanol production and contribute to the development of more efficient and cost-effective fermentation processes. Moreover, understanding the effects of NPK fertilizer concentration will help to optimize the fermentation conditions.

2. MATERIALS AND METHODS

2.1 Materials

The materials used for bioethanol production include 70% ethanol, distilled water (aquades), granulated sugar (sucrose), NPK fertilizer, yeast (*Saccharomyces cerevisiae*), and urea.

2.2 Bioethanol Production

The bioethanol production process begins by sterilizing all equipment with 70% ethanol to prevent contamination. Then, 500 mL of distilled water is boiled and mixed with 50 grams of sugar cane as carbon sources[6], [7]. Afterward, varying amounts of NPK fertilizer are added, with concentrations of 0 %, 0.5%, 5%, and 10%. NPK provides essential nutrients for the fermentation process. To supplement the nitrogen source, 5 grams of urea is also added. The mixture is stirred thoroughly to ensure it is uniform. Once the mixture is prepared, yeast is added as the microorganism responsible for fermenting the sugars into ethanol. The solution is allowed to ferment for 7 days. Samples are collected for testing on day 3 and day 7.

2.3 Bioethanol Product Testing

2.3.1 Refractometer Test

The refractometer test is used to measure the ethanol concentration in the bioethanol samples [8] . On days 3 and 7 of fermentation, a few drops of the sample are placed on the refractometer's glass surface. The refractometer is a tool that measures the refractive index of a liquid. The refractive index is a measure of how much light bends as it passes through the sample. The refractometer provides a reading that corresponds to the liquid's refractive index [9]. This value can then be compared to a standard ethanol concentration curve, which relates the refractive index to the ethanol concentration in the liquid. By using the standard curve, the ethanol concentration can be calculated. This test helps determine the progress of fermentation and estimate the ethanol yield. The result of the refractive index range of 1.300 to 1.700, in the concentration of ethanol of 0–95%. The refractive index data gathered from the samples is used to determine the ethanol concentration based

on this standard curve y= 0,1305x + 1,0394; R^2 = 0,9477.

2.3.2 Density Analysis

Density analysis is another method used to assess the bioethanol produced. On day 7, after the fermentation process, the bioethanol solution is filtered to remove any solid particles. The clear liquid, or filtrate, is then transferred into a 10 mL pycnometer, a small glass container used to measure the volume of liquids precisely. The mass of the pycnometer with the sample inside is recorded. The pycnometer is then weighed without the sample to obtain the mass of just the bioethanol solution The density is calculated by dividing the mass of the sample by the volume of the pycnometer.

2.4 Data analysis

One-way ANOVA is used to analyze the effect of different NPK fertilizer concentrations (0%, 0.5%, 5%, and 10%) on ethanol concentration and density in bioethanol production. The null hypothesis assumes no significant difference between the groups, while the alternative hypothesis suggests at least one group differs significantly. The analysis involves calculating the mean ethanol concentration or density for each NPK group, determining the overall grand mean, and calculating the sum of squares between and within groups. The F-statistic is derived by dividing the mean square between groups (MSB) by the mean square within groups (MSW). The F-statistic is then compared to a critical value from the F-distribution table at a 0.05 significance level. If the calculated F-statistic exceeds the critical value, the null hypothesis is rejected, indicating significant differences between the NPK concentrations. If significant differences are found, post-hoc tests like Duncan's HSD can be applied to identify which specific NPK concentrations contribute to the variation in ethanol concentration or density.

3. RESULTS AND DISCUSSION

3.1 Effect of the NPK concentration on the yield bioethanol

The concentration of NPK fertilizer plays a critical role in ethanol production during fermentation, as it directly impacts microbial growth and metabolic activity. The NPK concentrations significantly affect the concentration of bioethanol resulting from the fermentation of sugar cane (**Figure 1**). At low concentrations, such as 0.10%, NPK significantly boosts ethanol yield, achieving the highest recorded output of 3.71%. This can be attributed to the essential nutrients NPK provides, including nitrogen, phosphorus, and potassium, which are vital for microbial enzyme function and fermentation efficiency[3], [10]–[12]. The comparison with 0.00% NPK, which resulted in a lower ethanol yield of 2.47%, emphasizes the importance of these nutrients in supporting optimal microbial activity.

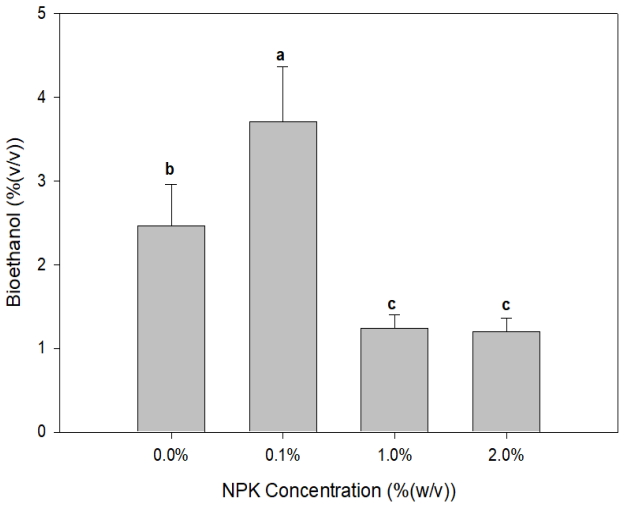

Figure 1. Bioethanol concentrations resulting from fermentation using NPK fertilizer as a nitrogen source at concentrations 0-2%. Differences letters show a statistically significant difference ($p<0.05$) at bioethanol concentration.

As NPK concentrations increase to 1.00% and 2.00%, ethanol production drops sharply to 1.24% and 1.20%, respectively. This decline suggests that excessive nutrients may create adverse conditions for ethanol-producing microorganisms. High concentrations of NPK can lead to osmotic stress, disrupting the balance of microbial cells and reducing their efficiency. Additionally, nutrient overload might encourage the growth of non-ethanol-producing microorganisms, which compete for resources and further inhibit ethanol production[5], [13]. This highlights the delicate balance required to provide sufficient nutrients without exceeding the optimal threshold.

These findings underscore the importance of optimizing NPK fertilizer concentrations in fermentation systems to maximize ethanol production. A concentration of 0.10% appears ideal, providing the necessary nutrients for efficient microbial activity without introducing inhibitory effects. By identifying and maintaining this optimal

level, bioethanol production processes can achieve higher yields while minimizing resource waste and environmental impact. Future research should further explore the interplay between NPK concentration and other factors, such as substrate composition, temperature, and pH, to refine fermentation practices and enhance ethanol production efficiency[4], [5].

Studies on bioethanol production from other sources reinforce the importance of nutrient optimization. For instance, research on cassava peels found that supplementing the fermentation medium with appropriate nutrients increased ethanol yield by up to 4.2% under optimal conditions. Similarly, bioethanol production from sugarcane bagasse hydrolysates achieved a yield of approximately 3.8% when supplemented with nitrogen-rich nutrients. These findings align with the current study, highlighting the significant influence of nutrient availability on microbial performance. Such comparative insights underline the necessity of tailoring nutrient supplementation to the specific feedstock and fermentation parameters to achieve the highest bioethanol yields[1], [14].

3.2 Effect of the NPK concentration on the density of bioethanol from fermentation

The data shows that the concentration of NPK fertilizer does not significantly influence the density of bioethanol produced during fermentation, as the observed differences are statistically insignificant ($p > 0.05$) (**Figure 2**). At 0.00% NPK, the bioethanol density is 2.31 g/mL, and it shows only a slight increase to 2.32 g/mL at 0.10% NPK. Further increases in NPK concentration to 1.00% and 2.00% result in densities of 2.329 g/mL and 2.33 g/mL, respectively. These small variations indicate that the addition of NPK fertilizer as a nitrogen source does not substantially affect the physical property of density in the bioethanol produced.

The similarity in density across the different NPK concentrations suggests that nitrogen supplementation, while critical for microbial activity and ethanol yield, does not significantly alter the composition of the fermentation product regarding density. This implies that other factors, such as substrate type, fermentation duration, or distillation efficiency, may play a more prominent role in determining the ethanol-water mixture ratios and density. Therefore, the effect of NPK fertilizer concentration on bioethanol density may be negligible within the tested range.

It is important to note that the density values observed in this study (ranging from 2.31 g/mL to 2.33 g/mL) are far above the standard for bioethanol as per the Indonesian National Standard (SNI), which specifies a density of 0.789–0.792 g/mL at 20°C for fuel-grade ethanol [15]. This indicates that the bioethanol produced contains significant water content or other impurities. To meet the SNI standard, further purification processes, such as advanced distillation or dehydration techniques, would be necessary. These findings reinforce the importance of post-fermentation processing in achieving ethanol of the required quality and purity.

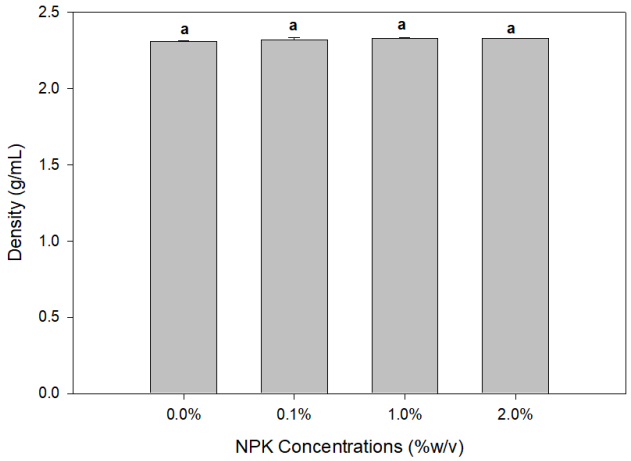

Figure 2. The density of bioethanol resulting from fermentation using NPK fertilizer as a nitrogen source at concentrations 0-2%. Differences letters show a statistically significant difference ($p < 0.05$) at bioethanol concentration.

4. CONCLUSION

The concentration of NPK fertilizer significantly affects the yield of bioethanol from sugarcane fermentation, with the highest concentration (3.71%) observed at 0.10% NPK, while higher concentrations (1.00% and 2.00%) lead to a decrease in yield due to osmotic stress and competition from non-ethanol-producing microorganisms. However, NPK concentration does not notably impact the bioethanol density, which remains relatively constant across different concentrations, suggesting other factors like substrate type and distillation efficiency play a more significant role. The produced bioethanol's density exceeded the

Indonesian National Standard for fuel-grade ethanol, indicating the need for further purification to meet quality standards.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

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Mubshair Naveed: Validation, Writing-review

Alwani Hamad: Conceptualization, Methodology, Supervisor, Writing-review

All authors have read and agreed to the published version of the manuscript.

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