

Production of Bioethanol from Oil Palm Stumps with Varying Levels of Ammonium Sulfate ((NH₄)₂SO₄) Added through a Fermentation Process Using Tape Yeast

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Abstract: Oil palm trunk is a lignocellulosic biomass with significant potential as a raw material for bioethanol production due to its cellulose and hemicellulose content, which can be converted into fermentable sugars. This study aimed to determine the effect of ammonium sulfate ((NH₄)₂SO₄) supplementation on bioethanol production from oil palm trunk through a fermentation process using tape yeast. The research procedure included raw material preparation, hydrolysis to produce simple sugars, fermentation using tape yeast, and analysis of the resulting bioethanol content. Ammonium sulfate was used as a nitrogen source to support the growth and metabolic activity of microorganisms during fermentation. Different concentrations of ammonium sulfate were applied to obtain optimal fermentation conditions. The results showed that the addition of ammonium sulfate affected bioethanol production. Adequate nitrogen availability enhanced microbial growth, resulting in a more efficient conversion of sugars into ethanol. However, excessive nutrient addition could reduce fermentation efficiency by disrupting microbial activity. The findings indicate that oil palm trunk can be used as an alternative feedstock for bioethanol production, and that an appropriate concentration of ammonium sulfate can improve fermentation performance and increase bioethanol yield.

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INTRODUCTION

Global energy demand is growing rapidly in tandem with industrial and population growth. According to a report by the International Energy Agency (IEA, 2023), energy consumption is projected to rise by 25% by 2040. In Indonesia, approximately 87% of national energy consumption still relies on fossil fuels, which contributes to carbon emissions. Therefore, efforts to develop renewable energy, particularly bioethanol, are becoming increasingly urgent. Indonesia is the world's largest producer of oil palm, yielding more than 47 million metric tons of fresh fruit bunches (FFB) per year (BPS Indonesia, 2023). However, biomass waste such as oil palm trunks (OPT) from replanting activities has not yet been optimally utilized. OPT contains cellulose and hemicellulose that can be processed into bioethanol, making it a potential feedstock for renewable bioenergy (Utari et al., 2024). Bioethanol production from OPT lignocellulose faces significant challenges, particularly due to its high lignin content, which inhibits enzymatic hydrolysis (Rahmawati, 2018). To overcome this obstacle, an effective pretreatment process, such as alkali or enzyme treatment, is required to break down the lignocellulose structure (Kululo et al., 2025; Woźniak et al., 2025).

In addition to oil palm trunks, empty fruit bunches (EFB) are an abundant lignocellulosic waste in Indonesia with potential as a bioethanol feedstock. As one of the world's largest palm oil producers,

Indonesia generates large quantities of EFB, which, if disposed of improperly, can cause air pollution and soil acidification even though EFB contains lignocellulose that can be hydrolyzed into monosaccharides for bioethanol production (Gozal et al., 2024). Domestic demand for bioethanol in Indonesia is projected to rise sharply from 0.22 billion liters in 2019 to 10.38 billion liters in 2025 (Suhartini et al., 2022); thus, utilizing EFB waste aligns with this demand while simultaneously reducing the environmental burden caused by palm oil agro-industrial waste.

The fermentation process is a crucial stage in the production of bioethanol from lignocellulosic materials such as oil palm stalks. Fermentation efficiency is greatly influenced by the type of microorganisms used and the availability of supplemental nutrients, particularly nitrogen (Akpogheli et al., 2024). Previous research has shown that adding nutrients, such as ammonium sulfate ((NH₄)₂SO₄), during fermentation can accelerate microbial metabolism and increase ethanol concentration. In that study, oil palm trunks pretreated with sulfuric acid were successfully converted into bioethanol using *Saccharomyces cerevisiae*, yielding promising results on a laboratory scale. This underscores the importance of optimizing fermentation conditions, including nutrient composition, to efficiently convert lignocellulose into bioethanol (Saragih, 2023). Furthermore, local tape yeast containing *Saccharomyces cerevisiae* holds great potential for small- to medium-scale bioethanol production (Soetarto & Putri, 2016).

In addition to ammonium sulfate, several studies have examined the use of other nutritional supplements to enhance *S. cerevisiae*'s fermentation capacity on lignocellulosic substrates. Propagation conditions have been shown to significantly influence the fermentation capacity of *S. cerevisiae*, where the yeast's limited tolerance to inhibitory compounds in lignocellulosic hydrolysates poses a major challenge for second-generation bioethanol production (van Dijk et al., 2020). The addition of a mixture of pyridoxine, thiamine, and biotin to the propagation culture was shown to enhance cell growth and ethanol yield in the fermentation of wheat straw hydrolysate from 0.04 g/g to 0.19 g/g, and in corn stover hydrolysate from 0.02 g/g to 0.08 g/g (van Dijk et al., 2020). These findings indicate that nutrient supplementation, including sources of nitrogen and vitamins, plays a crucial role in enhancing yeast resilience and productivity under challenging lignocellulosic substrate conditions.

A study published in the journal AgriTECH states that variations in the *S. cerevisiae* strains used in the fermentation of oil palm trunk sap result in significant differences in ethanol content. This indicates that selecting yeast strains and adapting them to local substrate characteristics, such as OPT (oil palm trunks), must be considered to ensure optimal fermentation. The use of locally available tape yeast can be an economical and practical approach to biofuel development in oil palm-producing regions (Ulum et al., 2021). Theoretically, this study contributes to understanding the synergy between nitrogen sources and *S. cerevisiae* fermentation performance in lignocellulosic substrates. In practical terms, the results of this study can be utilized by the bioenergy industry and the palm oil waste processing sector as a reference for improving bioethanol production efficiency while reducing production costs. Thus, the results of this study can serve as a foundation for the implementation of sustainable, environmentally conscious bioenergy technologies in the future.

Although laboratory-scale results are promising, industrial-scale implementation of second-generation bioethanol remains hampered by various multidimensional obstacles. The establishment of integrated biorefineries that produce a variety of fuels, chemicals, and materials from sustainable biomass holds the promise of an industrial transition toward a low-carbon pathway, yet widespread

commercial adoption remains elusive despite two decades of technological progress, due to technical challenges in the biomass conversion process as well as concerns regarding economic viability such as high capital costs and a lack of market competitiveness (Makepa & Chihobo, 2024). In developing countries such as India, for example, the commercialization of second-generation bioethanol remains hampered despite abundant availability of agricultural feedstocks, primarily due to the recalcitrant nature of the feedstocks, byproduct management, and limited subsidies compared to other countries (Pattnaik et al., 2024). Therefore, optimizing cost-efficient pretreatment and fermentation processes is a crucial step toward fostering a commercially viable second-generation bioethanol industry, including in Indonesia.

Based on the literature review, two major research gaps were identified. First, previous studies on nitrogen and vitamin supplementation for lignocellulosic fermentation have predominantly used purified commercial *S. cerevisiae* strains on non-palm substrates (van Dijk et al., 2020) or on oil palm trunk sap (Saragih, 2023; Ulum et al., 2021), whereas the use of locally available tape yeast, despite its documented potential for small- to medium-scale bioethanol production (Soetarto & Putri, 2016), remains unexplored specifically for solid oil palm trunk substrate combined with nitrogen optimization. Second, although nitrogen supplementation has been shown to enhance fermentation performance, the optimal concentration of ammonium sulfate for oil palm trunk fermentation using tape yeast, including the threshold at which excessive nitrogen addition begins to impair microbial activity, has not been systematically determined. These gaps indicate the necessity for research capable of establishing an effective and economical nitrogen supplementation strategy for oil palm trunk bioethanol production at small- to medium-scale.

Based on this gap, the problem addressed in this study can be formulated as follows: how does varying the concentration of ammonium sulfate $((\text{NH}_4)_2\text{SO}_4)$ at 0.5 g, 1 g, and 1.5 g affect the fermentation efficiency and bioethanol yield of oil palm trunk using tape yeast, and which concentration produces the optimal result?

Accordingly, this study aims to determine the effect of ammonium sulfate $((\text{NH}_4)_2\text{SO}_4)$ supplementation at concentrations of 0.5 g, 1 g, and 1.5 g on bioethanol production from oil palm trunk through a fermentation process using tape yeast. The study seeks to evaluate bioethanol yield across three ammonium sulfate concentrations and identify the concentration that yields optimal fermentation performance. The novelty of this research lies in its use of locally available tape yeast rather than purified commercial *S. cerevisiae* strains as an economical fermentation agent for oil palm trunk substrate, and in its systematic evaluation of ammonium sulfate concentrations to determine the optimal nitrogen supplementation threshold. The findings are expected to provide practical contributions to the bioenergy industry and the palm oil waste processing sector, serving as a reference for improving bioethanol production efficiency, while supporting the development of a cost-efficient, commercially viable second-generation bioethanol industry in Indonesia.

RESEARCH METHOD

Time and Location

The research stages were conducted at the Campus 1 Laboratory of the Indonesian Palm Oil Institute of Technology (ITSI), Jl. William Iskandar, Medan Estate, Percut Sei Tuan Subdistrict, Deli Serdang Regency, North Sumatra 20371. Sample testing was performed at the Biology Laboratory of

the Faculty of Mathematics and Natural Sciences (FMIPA), UNIMED, Kenangan Baru, Percut Sei Tuan District, Deli Serdang Regency, North Sumatra 20371.

Equipment and Materials

The materials used in this study were oil palm trunks, ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) at 0.5 g, 1 g, and 1.5 g; 1 M NaOH; 2 M sulfuric acid; and distilled water. The equipment used included a hot plate, glass beakers, Erlenmeyer flasks, a thermometer, an analytical balance, a pH meter, a spatula, dishes, aluminum foil, a magnetic stirrer, a dropper pipette, a 100-mesh sieve, and a gas chromatography-mass spectrometry (GC-MS) system.

Research Procedure

Preparation of Raw Materials

The raw material used in this study was oil palm trunks sourced from Mr. Agus Astaman Butar-Butar's plantation. The topmost section of the trunk was collected. The trunk was then chopped or grated with a coconut grater, and the liquid was squeezed to reduce moisture content. They were then dried in an oven at 120°C for 2 hours. The raw material was subsequently sieved using a 100-mesh sieve (Saragih, 2023).

Pretreatment

The pretreatment process in this study employed a basic method using sodium hydroxide (NaOH). Twenty-five grams of the sieved powder were weighed for each sample. The samples were placed in 500-ml beakers, and 250 ml of 2M NaOH solution was added. The mixture was homogenized on a hot plate stirrer at 90°C for 3 hours at 200 rpm. The pretreated oil palm trunks were neutralized with distilled water until the pH reached 7. The oil palm trunks were dried in an oven at 70°C for 2 hours (Rilek et al., 2017; Saragih, 2023).

Hydrolysis

This study employed a chemical hydrolysis technique, specifically an acid hydrolysis method, using 2 M sulfuric acid (H_2SO_4). Take 100 ml of the H_2SO_4 solution and pour it into a 150-ml beaker, then mix it with the oil palm trunk powder obtained from the pretreatment. Hydrolyze the mixture using a hot plate and stirrer at 90°C for 100 minutes at 100 rpm (Rilek et al., 2017).

Fermentation

The hydrolysis filtrate was transferred to a container, then 1% tape yeast (*Saccharomyces cerevisiae*) and ammonium sulfate in varying amounts of 0.5 grams, 1 gram, and 1.5 grams were added. The fermentation process was carried out for 48 hours at 30°C. Once the fermentation period was complete, the samples were heated in a water bath to halt the reactions within them and inactivate the microorganisms (Miyono & Warstyo, 2021).

Experimental Design

This study was conducted using a Completely Randomized Design (CRD) with a single factor, namely ammonium sulfat concentration, consisting of three treatment levels: 0.5 g, 1 g, and 1.5 g, each replicated three times, resulting in nine experimental units. A 0 g concentration (without ammonium sulfate addition) served as an additional control treatment, as reported in Table 1. Treatments were

arranged randomly across experimental units to minimize the influence of extraneous variables on the fermentation results.

Data Analysis

The fermentation solutions were analyzed using GC-MS. GC-MS (Gas Chromatography-Mass Spectrometry) is an analytical technique that combines two methods: gas chromatography to separate the components in a mixture, and mass spectrometry to identify and quantify each component based on its molecular mass. The resulting ethanol and levoglucosenone content data were statistically analyzed using one-way Analysis of Variance (ANOVA) at a 5% significance level ($\alpha = 0.05$) to determine the effect of ammonium sulfate concentration on each parameter. Because the HMF content data did not meet the assumptions required for ANOVA, the non-parametric Kruskal-Wallis test was used as an alternative to compare HMF content among treatments. A p-value below 0.05 was considered to indicate a statistically significant difference between treatments. Statistical analyses were performed using SPSS software.

RESULTS AND DISCUSSION

Ethanol Concent

Table 1. Ethanol Content from Fermentation

No.	Ammonium= Sulfate Concentration (gram)	EthanolContent (%)
1.	0	0
2.	0.5	0
3.	1	0
4.	1.5	1.25

The ANOVA results showed that the significant difference was due to the 1.5 g ammonium sulfate group, which produced a much higher ethanol content than the 0.5 g and 1.0 g groups, whose results were nearly identical and not significantly different from one another (0). These results indicate that the addition of ammonium sulfate as a new nitrogen source has a significant effect on ethanol production when its concentration is increased to 1.5 g; at concentrations of 0.5 g and 1.0 g, the amount of available nitrogen may not be sufficient to support optimal fermentation activity, resulting in very low ethanol contents.

This finding is consistent with previous reports showing that nitrogen availability can limit yeast fermentation of lignocellulosic hydrolysates. Skiba et al. (2018), for instance, found that a nutrient medium containing 1.82 g/L ammonium sulfate (together with 0.98 g/L potassium monophosphate and 6.47 g/L yeast extract) produced the highest bioethanol yield from oat hull hydrolysate, reaching 89.9% of the theoretical yield using an inhibitor-resistant *Saccharomyces cerevisiae* strain indicating that a nitrogen threshold must be reached before yeast metabolism is fully activated, a pattern broadly consistent with the sharp increase observed only at the 1.5 g treatment in this study. However, the effect of ammonium sulfate on ethanol yield appears to be substrate- and condition-dependent rather than universal. Sanchez et al. (2017), for example, found that ammonium sulfate supplementation (tested at 0, 160, and 1000 mg/L) did not significantly affect ethanol production during the fermentation of cachaza (sugarcane press-mud) using *S. cerevisiae*. However, it markedly reduced the formation of undesirable branched alcohols such as 2-methyl-1-propanol and 3-methyl-1-butanol.

This contrast suggests that nitrogen supplementation does not always translate into higher ethanol yield, and that its effectiveness likely depends on the baseline nitrogen content of the substrate, the severity of inhibitory compounds present, and the specific yeast strain used, factors that may explain why a significant effect was only observed at the highest ammonium sulfate dose (1.5 g) in the present study.

Compared to other lignocellulosic fermentation studies, the ethanol content obtained in this study (1.25% at the optimal treatment) remains considerably lower than, for example, onion waste fermentation using a monoculture of *S. cerevisiae*, which achieved an ethanol titer of 30.56 g/L under optimized pH, temperature, and time conditions (Shahid et al., 2023). This gap suggests that nitrogen supplementation alone was insufficient to overcome other limiting factors in the present system, most notably the residual fermentation inhibitors (HMF and levoglucosenone) detected in this study, discussed further below. This interpretation is reinforced by the observation that the 1.5 g treatment, despite yielding the highest ethanol content, also exhibited the highest HMF content (30.19%) among all treatments (Table 3), suggesting that ethanol production in this study was likely governed by a balance between nitrogen sufficiency and inhibitor tolerance rather than nitrogen availability alone.

Levoglucosenone Content

Table 2. Levoglucosenone Content

No.	Ammonium Sulfate Concentration (grams)	Levoglucosenone Content (%)
1.	0.5	4.66
2.	1	5.01
3.	1.5	5.98

The ANOVA results showed that the variations in ammonium sulfate concentration used in this study (0.5, 1.0, and 1.5) did not have a significant effect on the Luvoglucosenone content produced. In other words, the differences in the average Luvoglucosenone content between these three concentrations were not statistically significant; therefore, it can be concluded that the addition of ammonium sulfate within the tested concentration range did not result in a significant difference in Luvoglucosenone yield.

Ammonium sulfate generally acts as a catalyst, accelerating the dehydration reaction; however, its effectiveness can be influenced by other, more dominant factors—such as pyrolysis temperature, reaction time, or raw material characteristics—which likely contribute more significantly to the yield than variations in the catalyst dosage itself.

This result can be partly explained by the fact that levoglucosenone is typically reported to form via acid-catalyzed dehydration of cellulose-derived intermediates, a reaction commonly observed under fast-pyrolysis conditions or in anhydrous/polar aprotic solvent systems (Cao et al., 2015). While levoglucosenone formation under the mild aqueous acid hydrolysis conditions applied in this study (2 M H₂SO₄, 90°C) is less commonly reported in the literature than high-temperature pyrolysis, its formation via acid-catalyzed dehydration remains chemically plausible given the acidic environment during hydrolysis. Because this dehydration process occurs primarily during the hydrolysis stage before the introduction of ammonium sulfate in the subsequent fermentation stage, the nitrogen source added later would not be expected to influence levoglucosenone content, consistent with the

non-significant result observed here. This temporal separation between the hydrolysis stage (where levoglucosenone would form, if present) and the fermentation stage (where ammonium sulfate is introduced) offers a plausible explanation for the lack of significant effect. However, confirmation of peak identity via reference standards is recommended to strengthen this interpretation.

HMF (5-Hydroxymethylfurfural) Concent

Table 3. HMF Concent

No.	Ammonium Sulfate Concentration (grams)	HMF Content (%)
1.	0.5	21.78
2.	1	27.61
3.	1.5	30.19

The results of the Kruskal-Wallis test showed a significance value of 0.561 ($p > 0.05$), indicating that variations in the addition of ammonium sulfate at contents of 0.5, 1.0, and 1.5 had no significant effect on HMF content during the bioethanol fermentation process from oil palm trunks. HMF is a fermentation inhibitor that can hinder yeast growth and activity at high concentrations by interfering with glycolytic enzymes and slowing fermentation.

This result is consistent with the well-established mechanism in which HMF forms via acid-catalyzed dehydration of hexose sugars during the hydrolysis stage, before fermentation, rather than during fermentation itself. Since ammonium sulfate is introduced only after hydrolysis is complete, its addition would not be expected to alter HMF content, consistent with the non-significant result observed here. Previous studies have established that HMF is a dose-dependent inhibitor of *S. cerevisiae* fermentation activity; Taherzadeh et al. (2000) reported that the addition of 4.0 g/L HMF inhibited approximately 32% of the CO₂ production rate in *S. cerevisiae*, with the compound being taken up and converted by the yeast at a specific uptake rate of 0.14 ± 0.03 g/g·h under both aerobic and anaerobic conditions. Although a direct quantitative comparison with the present study is limited by differences in measurement units (relative GC-MS peak-area percentage versus g/L), the consistently high HMF levels observed across all treatments (21.78–30.19%) suggest that inhibitory stress from HMF may have been present throughout the fermentation process regardless of ammonium sulfate dosage. This may help explain why ethanol content remained at 0% in the 0.5 g and 1.0 g treatments despite the presence of fermentable sugars: at lower nitrogen availability, yeast cells may have been less able to simultaneously cope with HMF-induced stress and sustain active fermentation, whereas the additional nitrogen at 1.5 g may have enhanced cellular resilience sufficiently to allow limited ethanol production despite the inhibitor load. This interpretation remains a plausible hypothesis based on the observed data pattern rather than a directly tested mechanism. It would benefit from confirmation through yeast viability or stress-response assays in future work.

CONCLUSION

Oil palm trunks (OPT) that were pretreated with NaOH and hydrolyzed using H₂SO₄ were found to have potential as a substrate for bioethanol production using tape yeast (*Saccharomyces cerevisiae*), and the addition of ammonium sulfate ((NH₄)₂SO₄) affected fermentation performance; a

concentration of 1.5 g significantly increased ethanol content while a concentration of 1.0 g minimized the formation of the HMF inhibitor; meanwhile, a concentration of 1.5 g also tended to increase the levoglucosenone (LGO) precursor. The findings indicate a trade-off between maximizing ethanol production and controlling inhibitor formation; therefore, nutrient dose optimization and further studies (pilot-scale, controlled pretreatment/hydrolysis conditions) are needed to improve bioethanol yield and the feasibility of industrial-scale application.

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