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Pineapple Peel Waste Juice as Low-Cost Fermentation Substrate for Bioethanol Production using Commercial Baker's Yeast

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Abstract:- The energy currently being utilized comes from non-renewable sources, primarily fossil energy, which is anticipated to run out shortly. As a result, investigating alternate and sustainable energy sources is imperative, such as biofuels. Bioethanol, a biofuel derived from raw materials like lignocellulosic biomass from agricultural waste, is a promising option. It is produced through the fermentation reaction of sugars or polysaccharides facilitated by microorganisms. On an industrial scale, bioethanol is commonly produced from sugars obtained from basic starch products like cassava, corn or sugarcane. But the fact that these feedstocks—which are high in sugar and starch—are primarily utilized for food and feed has an impact on their steady supply. In Indonesia, pineapple production has been steadily increasing. Pineapple skin constitutes 41% of the total pineapple fruit, often discarded or used as animal feed. In 2022, pineapple production reached 3.2 million tons, with the potential for 1.3 million tons of pineapple peel waste. If not managed properly, this waste has the potential to pollute the environment. Pineapple peel waste (PPW) allows for the direct production of ethanol from the juice by requiring only a milling process to extract sugar into the fermentation medium. The characteristics and mineral composition of Pineapple peel waste (PPW) juice, along with the effects of adding nitrogen sources ($(NH_4)_2SO_4$, $(NH_4)_3PO_4$, $(CO(NH_2)_2)$) and micronutrient ($ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 7H_2O$) were studied. It was discovered that the PPW juice had a 15.23% total sugar content. Utilizing Pineapple peel waste (PPW) juice as a raw material, bioethanol production was carried out using Baker's yeast (Fermipan from Sangra Ratu Boga Company) with fermentation at 30°C for 72 hours at pH 5.5. The results revealed that the sample with urea supplementation (2.3595 g/l) produced the highest alcohol content at 13.052%, while the sample with Copper Sulfate supplementation (0.05 g/l) yielded 11.723% alcohol. These findings underscore the potential for sugar production from Pineapple peel waste (PPW) for subsequent bioethanol production. Further optimization of bioreactor parameters is required to enhance bioethanol production.

Keywords:- *Bioethanol; Fermentation; Pineapple Peel; Waste; Baker's Yeast; Nutrient; Renewable Sugars.*

I. INTRODUCTION

Energy conservation and environmental protection have become two pivotal concerns in today's world, escalating the demand for alternative and environmentally friendly energy sources. This was also a significant focal point at the G20 summit held in Bali in November 2022. Two specific points related to the energy sector stood out, as G20 leaders reached an agreement to expedite efforts in ensuring a sustainable, fair, affordable, and inclusive investment in energy transition. The emphasis on G20 renewable energy and new and renewable energy aligns with Indonesia's strategic vision for future development [1].

In addition, the world's fossil fuel reserves are anticipated to deplete within the next 40 to 50 years, primarily as a result of the quickly increasing use of non-renewable fuels. Burning fossil fuels releases a lot of greenhouse gases into the atmosphere, which causes climate change and global warming. [2].

The current energy consumption relies heavily on non-renewable sources, particularly fossil energy, which is predicted to quickly run out. Consequently, the need to investigate alternate, renewable sources of energy, such as biofuels. Among these alternatives, bioethanol stands out as one of the most promising options, as it can be produced from various renewable sources [3].

Bioethanol (C_2H_5OH) is a biofuel produced through the fermentation of sugars or polysaccharides facilitated by microorganisms. As an environmentally friendly product, bioethanol generates lower carbon monoxide levels (approximately 12-15%) and is primarily derived from sugar, starch, or cellulose. Renowned as one of the most popular renewable fuels, bioethanol boasts attributes such as a low boiling point, low environmental toxicity, a high octane number, and a substantial energy content [4]. Bioethanol, presently a widely produced fuel globally, represents the first generation of bioethanol derived from the fermentation process of food sources rich in sugar or starch, including corn, sugar cane, sweet potatoes, potatoes, and cassava. However, this production method has raised concerns about the potential competition between food and energy, potentially leading to a food crisis [5]. The raw materials for the production of 2G bioethanol, or second-generation bioethanol, are

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lignocellulosic biomass derived from agricultural waste. including sweet sorghum bagasse [6] sugar cane bagasse [7], cassava starch [8], wheat straw [9], oil palm frond [10], banana frond [11], and melon peel [12]. The advantages of using these materials have been documented in various publications. Microalgae, macroalgae, and food waste are used to produce third generation (3G) of bioethanol [13], [14]. The second generation of bioethanol from lignocellulosic biomass has great potential due to its abundant, inexpensive, and easily obtainable raw materials [15]. Pineapple peel is one of the waste materials that can be used as a feedstock for production of bioethanol [16]–[18].

Pineapples are among the largest agricultural commodities in Indonesia, with production steadily increasing each year. In 2022, pineapple production in Indonesia reached 3.2 million tons [19]. Pineapples are not seasonal fruits, allowing for year-round cultivation. They are consumed either directly when fresh or in the form of canned pineapple. The continuous cultivation of pineapples leads to an increase in waste production. The weight composition of a pineapple is as follows: 31% pulp, 6% pineapple core, 41% pineapple skin, and 20% pineapple crown [20]. Pineapple skin constitutes 41% of the total pineapple fruit and is typically discarded or used as animal feed. In 2022, pineapple production reached 3.2 million tons, resulting in a substantial amount of pineapple waste estimated at around 1.3 million tons. The pineapple peel, with its high global production and significant content of sugar, protein, and minerals, holds potential as a cost-effective raw material in order to produce bioethanol.

Sugar is converted to bioethanol by yeast cells' fermentation process. Specifically, yeast from the genus Saccharomyces is known for its effectiveness in producing bioethanol. Pereira et al. [21], state that *Saccharomyces cerevisiae*, a yeast, is the most widely used microorganism among the different microorganism used to produce bioethanol. This preference is attributed to its superior fermentation ability, strong resistance to ethanol and other inhibitors, which can be created during fermentation or during raw material pre-treatment, and its quick growth in anaerobic environments, which is typical in large-scale production. Saccharomyces cerevisiae produces the enzymes zymase and invertase. Invertase breaks down sucrose to monosaccharides (glucose/fructose), whereas zymase turns glucose into bioethanol.

How much bioethanol is produced through fermentation is influenced by factors such as the choice of microorganisms and the fermentation medium. The fermentation medium, serving as a growth environment for microorganisms, must supply all the compound components necessary for the metabolic processes of microorganism cells. Trace elements like Fe, Zn, Cu, Mn, and Co, as well as a carbon and nitrogen source, are necessary components of fermentation media [22]. Nitrogen is essential for the growth of yeast and contributes a crucial role in influencing both the ability to produce and tolerate ethanol [23]. Micronutrients, also known as trace elements, are essential for cell growth and the fermentation of ethanol. [24]. According to reports, the micronutrients zinc (Zn^{2+}) and manganese (Mn^{2+}) are essential for yeast growth and fermentation of bioethanol. [25]. Reference [26] conducted research showing that Zinc indicates that Zinc supplementation affects ethanol concentration and enhances ethanol tolerance, correlating with an increase in yeast tolerance to ethanol [27]. The addition of Mn^{2+} has been found to promote cell growth and increase ethanol concentration [28].

Even though Pineapple peel waste (PPW) juice contains all the nutrients required for yeast growth and ethanol fermentation, their concentration may not be optimal. Therefore, supplementation of Pineapple peel waste (PPW) juice with specific nutrients may be necessary.

The objective of this study is to understand the characteristics of juice from pineapple peel waste to identify the best macronutrient and micronutrient sources for the fermentation of juice from pineapple peel using commercial yeast to produce bioethanol.

II. MATERIALS AND METHOD

This research took place out in Laboratory of Microbiology and Bioprocess, Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Indonesia.

A. Materials

Fresh pineapple peel waste (PPW) for this study was collected from a local seller in Semarang City, Central Java, Indonesia. Before extraction into juice, the peel of the pineapple is crushed into small pieces.

Baker's yeast (Fermipan from Sangra Ratu Boga Company), distilled water, NaOH, HCL, $(NH_4)_2SO_4$ (ammonium sulphate), $(NH_4)_3PO_4$ (ammonium phosphate), Urea $(CO(NH_2)_2$ are purchased from Indrasari Store for Chemical Supplies in Semarang, Indonesia. Zinc sulfate $ZnSO_4{\cdot}7H_2O$, Copper sulfate $CuSO_4{\cdot}7H_2O$, Mangan sulfate $MnSO_4{\cdot}7H_2O$, Yeast Extract (Merck) sourced from the Nitrakimia Chemistry Store in Yogyakarta, Indonesia.

B. Method

- *Sample Sources and Preparation*

To prepare pineapple peel waste juice, pineapple peels were acquired from a local seller in Semarang City, Central Java, Indonesia. To get rid of dirt and soil from the outside, the peels were carefully cleaned and then rinsed with tap water. Subsequently, the pineapple peels were sliced into tiny pieces. of equivalent size before being blended using a blender. The pineapple skin pieces were weighed, then blended with distilled water. After filtering the mixture, pineapple peel extracts were obtained, which were further squeezed through muslin cloth and filtered again using filter paper. The filtered pineapple peel juice was then stored at -4°C for subsequent experiments [11].

- *Fermentation*

The fermentations processes took place in 250 ml containers with a fermentation medium volume of 200 ml. The medium's pH was adjusted to 5.5 using normal HCl and NaOH. Both flasks were covered with aluminum foil, after that, the substrate was sterilized for 15 minutes at 121°C. using autoclave. Commercial baker's yeast S. cerevisiae (Fermipan from Sangra Ratu Boga Company) was added at a rate of 15 g per 1000 ml of medium [29], [30]. Fermentation was conducted under anaerobic conditions in an incubator shaker for 72 hours at a temperature of 30°C and an agitation rate of 100 rpm [31].

- *Bioethanol Production by Baker's Yeast using PPW Juice*

The feasibility of employing Pineapple Peel Waste (PPW) juice as a substrate for bioethanol production using Baker's yeast, different medium formulations containing different kinds and amounts of micronutrient sources (g/L) and nitrogen sources (g/L) were investigated. Specifically, five types of nitrogen sources— yeast extract, urea, ammonium phosphate, ammonium sulfate, and a mix of ammonium phosphate and ammonium sulfate (1:1)—were investigated. Three types of micronutrient source ($ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 7H_2O$) were investigated too.

- *Analysis of Bioethanol Content Using High Performance Liquid Chromatography (HPLC)*

All samples were analyzed using High-Pressure Liquid Chromatography (HPLC) with detectors for UV-Vis and Refractive Index (Shimadzu) to determine the bioethanol content (%v/v).

III. RESULT AND DISCUSSION

- *The Characteristics of Pineapple Peel Waste*

Pineapples (Figure 1) represent one of the largest agricultural commodities in Indonesia. In 2022, pineapple production reached 3.2 million tons, and with this substantial production, it is estimated that a significant amount of pineapple waste is generated, approximately 1.3 million tons, as depicted in Figure 2. If not utilized, pineapple peel waste has the potential to pollute the environment. As outlined in Table 1, 3 kg of fresh pineapple peel can yield 2.5 L of juice extract.

Fig 1 Pineapple

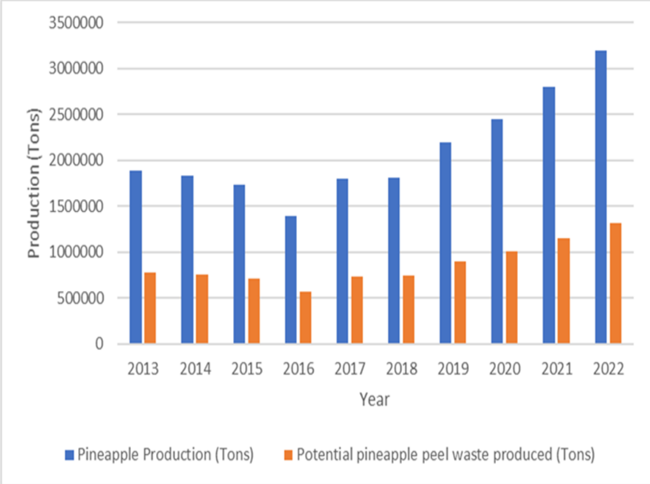

Fig 2 Pineapple Production and its Potential Waste in Indonesia [19]

Table 1 Mass Balance of PPW Juice Extraction

Mass balance	Mass
The total wet weight of pineapple peel before pressed, (kg)	3
The total Amount of distilled water added, (L)	0.5
The total wet weight of pineapple peel after pressed, (kg)	1
Total volume of fresh pineapple peel juice, (L)	2.5

Source: Direct Observation in the Field

Pineapple peel discarded from a market were analyzed by sorting the waste and then extracting juice to determine its main attributes, as illustrated in Table 2. The benefit of using pineapple peel juice as a fermentation substrate is that the pretreatment —such as milling, heating, acid/alkaline/enzymatic hydrolysis— able to be removed. The potential for pineapple peel as a raw material for making bioethanol is quite substantial.

The mineral content in Pineapple peel juice was analyzed using ICP-OES (Inductively Coupled Plasma equipped with Optical Emission Spectrometer). The minerals analyzed included copper (Cu), manganese (Mn), and zinc (Zn), and their concentrations were measured in mg/L. Before injection into ICP-OES/AAS, the system required determining and setting the wavelength for each mineral element [11].

Table 2 Characteristic of Pineapple Peel Waste (PPW) Juice

No	Parameters	[32]	This work
1	Total Sugar (%)	20.41	15,23
2	Ash (%)	4.05	0,32
3	Moisture content (%)	87.5	92,68
4	Carbohydrate (%)	-	6,75
5	Protein (%)	5.18	0,26
6	Nitrogen (%)	0.83	0,04
7	Phosphor (%)	0.14	0,35
8	Sulfur (%)	-	6,70
9	*Trace element Mn (mg/L)*	0.01	1,12
10	*Trace element Zn (mg/L)*	0.02	1.9
11	*Trace element Cu (mg/L)*	0.03	Not detected
12	PH	4.3	4.52

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Previous studies showed that parameter values for each pineapple produced different results. The composition of pineapple residues varies based on factors such as the type of analysis chosen, type of pineapple, area where the pineapple grows, plant morphology, fruit maturity level, and other factors. Proximate analysis of food ingredients for each of these parameters varies depending on the method used and the tools used. In this research, analysis of proximate used the Indonesian standard method. While trace element analysis used ICP-OES/AAS. Other factors that influence the nutritional content of plants include plant genetics, soil conditions where they are grown, climate, physiological conditions of the fruit, and the harvesting. The approximate composition of various pineapple residues highlights their potential for value addition. The specific type of pineapple used in this research is the queen pineapple (Figure 1), characterized by its deep yellow color, lighter weight, and sweeter taste compared to other varieties. The area of origin of this pineapple is Pemalang City, Central Java, Indonesia, while Abdullah & Mat [32] used pineapple solid waste obtained from Pineapple Canning Factory, Johor, Malaysia.

The results of the analysis of the characteristics of pineapple peel juice show that pineapple peel juice can be used as a carbon source in the fermentation process of *Saccharomyces cerevisiae* to make bioethanol because it has a high sugar content. As shown in Table 2, while the PPW juice contains all the necessary nutrients for yeast growth and ethanol fermentation, their concentrations may not be optimal. Therefore, supplementation of Pineapple peel waste (PPW) juice with specific nutrients may be necessary to improve bioethanol production.

Fig 3 Pineapple Peel Waste before Extraction

Fig 4 Pineapple Peel Waste Juice After Extraction

- *The Effect of Nitrogen Source Addition on Pineapple Peel Waste (PPW) Juice on Ethanol Content*

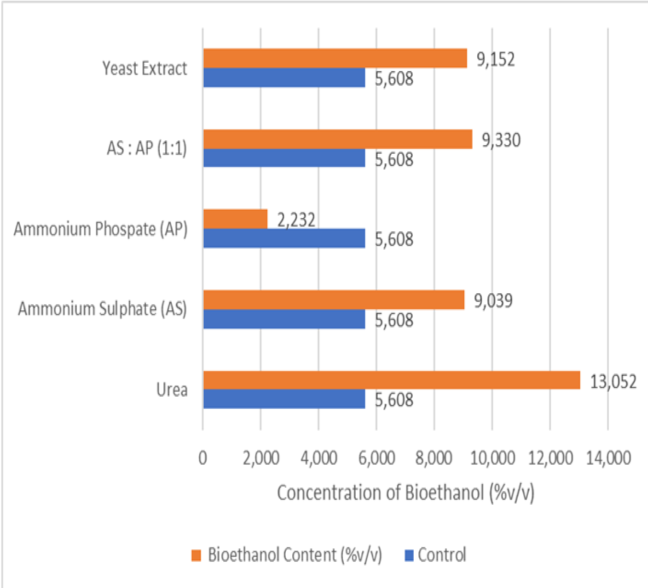

Fig 5 Effect of Nitrogen Addition on Bioethanol Content using Commercial Yeast Fermipan

The findings from this research indicate a positive impact of nitrogen source addition on the increased production of bioethanol, with the exception of ammonium phosphate. Urea emerged as the most effective nitrogen source, yielding the highest levels of bioethanol. The sequence of effectiveness, in terms of bioethanol production during the 72-hour fermentation, was as follows: urea (13.052%), Ammonium Sulfate and Ammonium Phosphate (1:1) (9.330%), yeast extract (9.152%), Ammonium Sulfate (9.039%), and Ammonium Phosphate (2.232%).

In Figure 5, the impact of adding urea ($CO(NH_2)_2$ as a nitrogen source in bioethanol fermentation is evident, resulting in the highest bioethanol content at 13.052%, marking a remarkable 133% increase compared to the absence of any nutrient addition. Following this, a mixture of $(NH_4)_2SO_4$ and $(NH_4)_3PO_4$, Yeast Extract, $(NH_4)_2SO_4$ and the lowest is $(NH_4)_3PO_4$. The addition of macronutrients in the form of nitrogen sources demonstrated a positive impact on bioethanol production compared to the control without any addition, except for Ammonium phosphate $(NH_4)_3PO_4$, which exhibited no positive impact on bioethanol levels. The use of ammonium phosphate is deemed unsuitable as a nitrogen source with pineapple peel juice substrate for ethanol production, as it led to a reduction in ethanol production by approximately 60% compared to the pineapple peel juice substrate without additional macronutrients. This decrease in ethanol production with the addition of $(NH_4)_3PO_4$ is likely attributed to the toxicity of ammonium ions against yeast [33]. Similar to another study showed that urea supplementation in bioethanol fermentation from kapok banana peels produced higher bioethanol (22.439%) than ammonium sulfate supplementation (7.799%) with a faster optimum fermentation time using urea than ammonium sulfate [34].

In this study, in comparison to circumstances where no nutrients were added, the addition of yeast extract led to an increase in the production of bioethanol. However, its performance did not match that of urea or the mixture of $(NH_4)_2SO_4$ and $(NH_4)_3PO_4$. This discrepancy may be attributed to the suboptimal concentration of yeast extract.

Various studies consistently highlight the crucial role of nitrogen sources in production of bioethanol using *S. cerevisiae* in anaerobic environments. Among these, urea emerges as particularly promising in enhancing the profitability of alcohol fuel production, especially from sorghum concentrate. Not only does urea increase specific growth rate and ethanol tolerance, but it also increases ethanol yield and reduces the amount of unwanted byproducts that are formed. The prevailing consensus underscores urea as the preferred nitrogen source in the process fermentation of ethanol using sorghum concentrate [35].

Urea, a fertilizer produced by combining NH_4 (ammonia) with CO_2, boasts a total nitrogen (N) content of 46%. This means that every 100 kg of urea contains 46 kg of nitrogen nutrients. The high nitrogen content within urea makes it a valuable food source for the yeast *Saccharomyces cerevisiae*. Urea, characterized by small to fine grains and a white color, was identified as the most effective nitrogen source in this study.

The nitrogen present in urea serves as a crucial supplier of nucleic acids, single amino acids, and vitamins essential for the vitality of *Saccharomyces cerevisiae*. Furthermore, this nitrogen source is utilized by microbes to expedite cell growth during fermentation.

- *The Effect of Micronutrient Source Addition on Pineapple peel waste (PPW) Juice on Ethanol Content*

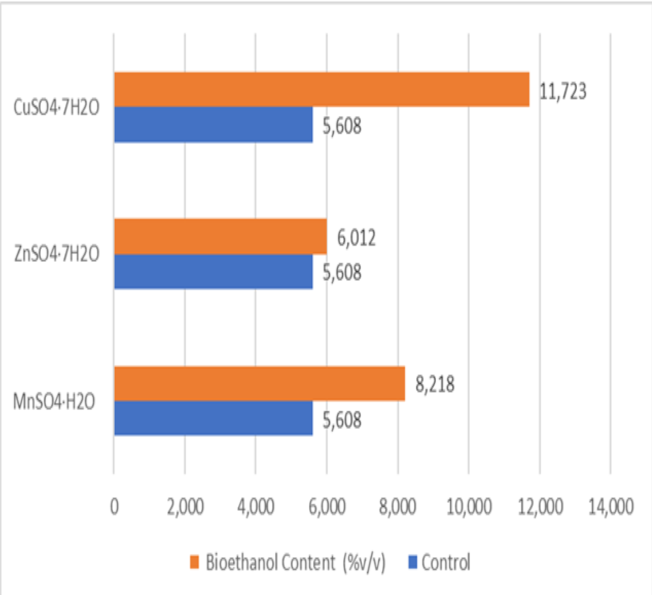

Fig 6 Effect of micronutrient addition on bioethanol content using commercial yeast Fermipan

The addition of micronutrients in the form of metal ions has demonstrated a positive impact on bioethanol production. Specifically, the introduction of Manganese Sulfate ($MnSO_4.7H_2O$), Zinc Sulfate ($ZnSO_4.7H_2O$) and Copper Sulfate ($CuSO_4.7H_2O$) at a concentration of 0.05 g/L each resulted in increased bioethanol production compared to conditions without additional nutrients. Notably, the most substantial increase was observed with the addition of copper metal (Cu), leading to a bioethanol content of 11.723%, marking a significant 109% increase compared to conditions without added copper metal.

Manganese (Mn) plays a crucial role in influencing yeast growth and metabolic processes during fermentation, impacting key parameters such as the rate of sugar to ethanol conversion, yield of ethanol, yeast production, and stress tolerance. Manganese (Mn) serves as an important cofactor required for yeast growth and metabolism, stimulating fermentation. Copper (Cu) acts as a vital nutrient and cofactor for several enzymes at low concentrations, while Zinc (Zn) is a quickly absorbed cofactor for yeast cells.

According to earlier studies, Saccharomyces cerevisiae has been shown to ferment alcohol more quickly when Cu, Zn, and Mn sulfate are added to the molasses medium. This phenomenon occurs in both semi-aerobic and aerobic settings. [36]. Other research shows that the addition of Cu log ions can reduce glycerol production in bioethanol fermentation, but if the addition is too much it will not have a significant effect [37].

IV. CONCLUSION

Pineapple peel juice can be used as a carbon source in the fermentation process of *Saccharomyces cerevisiae* to make bioethanol because it has a high sugar content. The addition of both macronutrient and micronutrient sources has shown positive outcomes in the production of bioethanol. The chemical composition of the resulting mixture appears to be a beneficial nutrient for the cultivation of baker's yeast, making it a potential carbon source for fermentation. Specifically, Pineapple Peel Waste (PPW) juice supplemented with urea $(CO(NH_2)_2$ exhibited a higher ethanol yield than other macronutrients. Similarly, Pineapple Peel Waste (PPW) juice supplemented with copper sulfate $CuSO_4{\cdot}7H_2O$ demonstrated a higher ethanol yield than other micronutrients.

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