Effect of Salinity, Sugar Level, and Fermentation Time for Bioethanol Production from Nipa Palm Sugar

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Abstract. This study aims to determine the effect of salinity, sugar level, and fermentation time on the bioethanol production from nipa (*Nypa fruticans Wurmb.*) palm sugar. The research was conducted in two stages using batch fermentation. The first stage aims to determine the optimal salinity of nipa palm sugar from Nusadadi, Cikembulan, and Pedasong Village, Central Java, for bioethanol production. The results showed that salinity significantly affected bioethanol productivity. Nipa palm sugar from Nusadadi, with the lowest salinity, produced the highest bioethanol content after distillation (39.31% v/v). The second stage was conducted to evaluate the effects of fermentation time (24, 48, and 72 hours) and sugar-to-water ratio (1:4, 1:5, 1:6) on bioethanol production. Nipa palm sugar from Nusadadi was chosen as feedstock due to the highest ethanol yield. The combination of fermentation time and sugar solution ratio had a significant effect on the total sugar and reducing sugar content of the fermentation broth, as well as density and bioethanol content. The treatment with a 48-hour fermentation time and a sugar solution ratio of 1:4 produced the highest ethanol content after distillation (42.97% v/v).

1 Introduction

Indonesia's energy transition emerged to be implemented immediately due to the scarcity of fossil fuels and commitment to achieve Net Zero Emissions (NZE) in 2060. Meanwhile, realisation of the new and renewable energy (EBT) mixes in 2024 reached 14.1%, far from the Indonesian government's target of 23% in 2025. The Ministry of Energy and Mineral Resources (ESDM) of The Republic of Indonesia continues to encourage the development of EBT, including in terms of installed capacity, production, and consumption. In contrast to biodiesel, bioethanol implementation in Indonesia has been facing many obstacles in terms of feedstock availability and price. Therefore, exploration of alternative raw materials is important to meet domestic ethanol needs.

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In Indonesia, Nipa palm (*Nypa fruticans*) is a promising feedstock for bioethanol production due to its abundant availability and high productivity. Presently, the Nipa palm provides of ecological benefits by maintaining soil stability, preventing erosion, and mitigating the impacts of storms and high seas in coastal regions, as an integral component of the mangrove ecosystem. However, its economic potential remains undeveloped [1]. Bioethanol from nipa sap is a promising alternative as a blend with fossil fuels due to its renewable nature and potential to reduce GHG emissions, but has not yet been tested as a pure engine fuel [2], [3].

Nipa (*Nypa fruticans*) is potential alternative feedstock for bioethanol, with high sap productivity, that does not require farmland, high input cultivation, and does not compete with food [3]. The chemical evaluation of nipa sap gave a total sugar composition of 159-214 $g.kg^{-1}$, mainly consisting of sucrose, glucose, and fructose, which are naturally fermented to produce ethanol. The capacity of nipa sap that can be produced varies between 0.4 and 1.2 L d-1 for each nipa palm [4]. Nevertheless, due to its natural sugar content, nipa sap is susceptible to spoilage within just 3 hours of harvesting, as spontaneous fermentation occurs during storage. This fermentation activity is largely caused by the existence of microflora and unhygienic tapping practices [5].

To overcome this problem, nipa sap can be processed into solid sugar by heating before use in the fermentation process, thereby significantly reducing water content and improving stability for longer storage. The first stage of this study aimed to select nipa palm sugar feedstock from three different sites to produce bioethanol. Furthermore, the second-stage fermentation process was varied according to incubation time and sugar solution ratio. These factors are important to determine ethanol productivity and conversion rate.

2 Methodology

2.1 Materials and Tools

Materials used were nipa palm sugar from Nusadadi, Cikembulan, and Pedasong Village, Central Java, dried *S. cerevisiae*, dinitro-salicylic acid (DNS) reagent, NPK, urea, NaCl 0.15 M, bentonite, NaOH 0.5 M, HCl 0.5 M, K-Na Tartrate, sucrose, distilled water, and alcohol 70%. The tools used were autoclave, incubator, hotplate stirrer, sieve (100 mesh), vortex, haemocytometer, microscope, fermenter, distiller, laminar airflow, thermometer, pH meter, salinometer, refractometer, digital density meter Anton Paar DMA 4500.

2.2 Preparation of Nipa Palm Sugar Fermentation Broth

To create a fermentation broth, 75 g of nipa palm sugar was dissolved in 300 mL of distilled water (1:4) at 80-90°C (Brix value of the sugar solution: 18 °brix). As much as 0.4 g/l of urea and 0.5 g/l of NPK were added to the solution. The medium was then homogenised for 1-2 minutes and cooled to 25-30°C. Before use, the pH and salinity of the fermentation broth were measured.

2.3 Yeast Preparation

The starter was prepared using 0.5 g of dried *Saccharomyces cerevisiae*, 5 g of sugar, and 100 mL of distilled water (±40°C). The mixture was then homogenised and left for 30 minutes at room temperature (25-30°C) to activate the microorganism before being ready to use in the fermentation process. The number of yeast cells in the starter medium is calculated using a haemocytometer method with several dilutions.

2.4 First Stage Fermentation

The fermentation process was carried out in a fermenter (Figure 1), using 270 ml of nipa sap broth and 30 ml of *Saccharomyces cerevisiae* starter (9:1 v/v). It was done at 25°C – 30°C for 24 hours. After fermentation was complete, the final pH and sugar content of the fermentation broth were analysed with a pH meter and a refractometer. Furthermore, bioethanol was extracted through vacuum distillation at 78-80°C for 1 – 2 h. Ethanol content and density of distillates were determined with a Digital Densitometer (ASTM D4052). Nipa palm sugar with the highest bioethanol yield was selected for the second-stage fermentation process to determine the best sugar-to-water ratio and fermentation time.

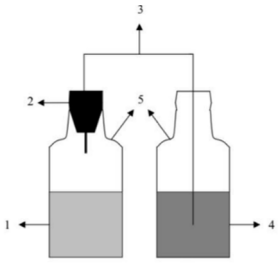

Fig. 1. Fermenter schematic for bioethanol production from nipa palm sugar.

2.5 Second Stage Fermentation

Second-stage fermentation was conducted at a capacity of 300 mL, sugar solution and starter ratio of 9:1, and a temperature of 25-30°C. At this stage, fermentation time was varied to 24, 48, and 72 hours with the sugar solution ratios of 1:4, 1:5, and 1:6. The pH value, total sugar, and reducing sugar content of the broth were analysed before and after fermentation. Reducing sugar content was determined using the dinitro-salicylic acid (DNS) method [6]. Bioethanol was extracted through vacuum distillation at 78-80°C for 1 – 2 h. Ethanol content and density of distillates were determined with a Digital Densitometer (ASTM D4052).

3 Result and Discussion

3.1 The Effect of Nipa Palm Sugar Salinity on The Bioethanol Characteristics

Substrate salinity is an important factor in the bioethanol fermentation process. The presence of soluble ions such as sodium (Na^+), chloride (Cl^-), and sulphate (SO_4^{2-}) in the fermentation medium can disrupt the osmotic balance of yeast cells and inhibit key metabolic activities. Nipa palm sugar, a product of the nipa palm that grows in coastal mangroves, commonly has a higher salt content due to the interaction of groundwater and tides [7]. The salinity of the nipa palm sugar from three sites used in this study is presented in Figure 2.

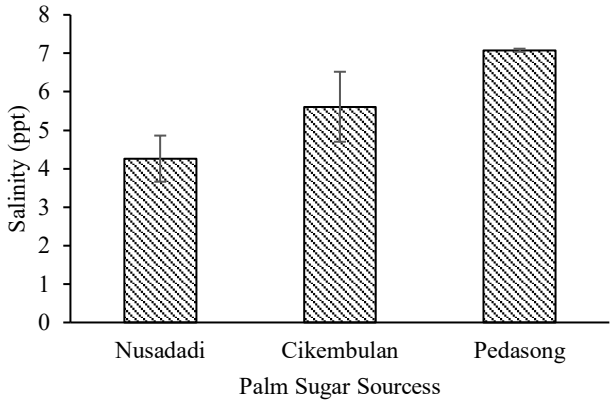

Fig. 2. The salinity of nipa palm sugar.

S. cerevisiae used in the fermentation stage is tolerant to environmental changes within certain limits, but high salinity conditions can trigger osmotic stress, resulting in a compensatory metabolic response. According to Turnip et al. [7], when *S. cerevisiae* cells face hyperosmotic stress due to high salinity, they shift their glucose metabolism to glycerol synthesis. At this point, the carbon source will be used to maintain the cell's internal balance, instead of to produce bioethanol.

Furthermore, salt ions can disrupt membrane protein function, reduce glycolytic enzyme activity, and alter genes expression in the fermentation pathway. Cells in high-salinity media exhibit slower growth rates, longer lag phases, and higher levels of secondary metabolites, such as acetic acid.

In this study, the fermentation of nipa palm sugar by *S. cerevisiae* produced different bioethanol profiles depending on the salinity of each nipa palm sugar. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) results showed significant differences in bioethanol content, density, and pH value of the obtained distillates (Table 2).

Table 2. The effect of nipa palm sugar salinity on the bioethanol content, density, and pH value of distillates.

Nipa Palm Sugar Resources	Bioethanol content (% v/v)	Density (g/cm³)	pH
Nusadadi	39,31[a]	0,94885[c]	3,17[c]
Cikembulan	32,08[b]	0,95958[b]	4,89[b]
Pedasong	21,48[c]	0,97205[a]	5,86[a]

Notes: Mean values followed by different letters indicate significant differences at the 5% level based on Duncan's Multiple Range Test (DMRT)

The highest bioethanol content was obtained from Nusadadi nipa sugar (39.31%). Theoretically, one mole of glucose (180 g) is converted into two moles of ethanol (92 g) with a maximum efficiency of 0.51 g of ethanol per g of glucose. Meanwhile, the bioethanol content of Cikembulan and Pedasong nipa sugars was lower, at 32.08% and 21.48%, respectively, indicating insufficient fermentation process due to higher osmotic pressure.

The density of a bioethanol solution is an indicator of the ethanol concentration in the medium. Solutions with higher bioethanol concentrations have lower densities. Conversely, with a lower ethanol concentration in the distillate, its density will be closer to that of water.

In addition, the pH measurement indicates that the bioethanol solution (distillates) is acidic, with each sample having a pH of 3.17 from Nusadadi, 4.89 from Cikembulan, and 5.86 from Pedasong. These pH values not only originate from bioethanol as the primary fermentation product, but rather from a mixture of other compounds formed and dissolved during the fermentation process, such as organic acids.

3.2 The Effect of Sugar Solution Ratio and Fermentation Time on The Fermentation Process and Bioethanol Productivity

3.2.1 Changes in total sugar content (°Brix)

Total sugar was measured before and after fermentation to determine the amount of sugar consumed by microorganisms during fermentation. The total sugar content of the substrate before fermentation varies with the ratio of sugar solution used. A higher palm sugar ratio results in higher total sugar content in the fermentation medium (Table 3). Total sugar levels decreased by 3.10 to 13.50 °Brix after fermentation. The 72-hour fermentation time with a sugar-to-water ratio of 1:4 resulted in the greatest reduction in total sugar levels.

The decrease in total sugar levels across all treatments indicates that *Saccharomyces cerevisiae* utilises the sugars in the fermentation broth as an energy and carbon source. The total sugar content can influence both yeast cell growth and ethanol production [8]. During the first 24 hours of fermentation, the broth with a higher sugar-to-water ratio (1:4) had the lowest total sugar reduction compared to the other treatments. This may be because *S. cerevisiae* cells may require a longer adaptation period. High initial sugar levels can suppress initial growth of *S. cerevisiae* because cells must divert energy to adapt to osmotic stress [9].

Table 3. Total sugar content of the fermentation broth before and after fermentation at different sugar solution ratios and fermentation time.

Fermentation Time (hours)	Sugar Solution Ratio	Total Sugar Before Fermentation (°Brix)	Total Sugar After Fermentation (°Brix)	Reduction in Total Sugar Content (°Brix)*
24	1:4	18.93 ± 0.12	15.17 ± 0.87	3.77 ± 0.91^{ab}
	1:5	16.10 ± 0.10	13.00 ± 0.66	3.10 ± 0.60^{a}
	1:6	13.97 ± 0.06	9.57 ± 0.25	4.40 ± 0.20^{b}
48	1:4	18.90 ± 0.10	8.57 ± 0.75	10.33 ± 0.80^{d}
	1:5	16.07 ± 0.06	6.63 ± 0.47	9.43 ± 0.42^{cd}
	1:6	13.97 ± 0.06	5.03 ± 0.45	8.93 ± 0.45^{c}
72	1:4	18.97 ± 0.06	5.47 ± 0.50	13.50 ± 0.56^{e}
	1:5	16.03 ± 0.06	5.70 ± 0.62	10.33 ± 0.57^{d}
	1:6	13.90 ± 0.10	4.43 ± 0.51	9.47 ± 0.55^{cd}

*Notes: Mean values followed by different letters indicate significant differences at the 5% level based on Duncan's Multiple Range Test (DMRT)

3.2.2 Changes in reducing sugar content

The fermentation process of nipa palm sugar into bioethanol also affects the reducing sugar content of the fermentation medium. The reducing sugar content in the fermentation medium fluctuated across various treatments (Table 4). In the early stages of fermentation, S. *cerevisiae* produces the enzyme invertase, which hydrolyses sucrose (a non-reducing sugar) into glucose and fructose (reducing sugars) [10]. Consequently, in the treatment with the shortest fermentation time (24 hours), the reducing sugar content actually increased compared to before fermentation. The data showed that in all treatments with a 24-hour

fermentation time, the sugar solution ratio increased in reducing sugar levels (approximately 49.97-55.01 g/L).

Table 4. Reducing sugar content of the fermentation medium before and after fermentation at different treatments.

Treatment		Reducing Sugar Content (g/L)		Change in Reducing Sugar Content (g/L)*
Fermentation Time (h)	Sugar:Water Ratio	Before Fermentation	After Fermentation	
24	1:4	94.29 ± 2.94	144.26 ± 5.03	-49.97 ± 4.08a
	1:5	65.83 ± 0.89	118.85 ± 3.33	-53.02 ± 3.22a
	1:6	29.86 ± 4.38	84.88 ± 5.01	-55.01 ± 4.81a
48	1:4	95.84 ± 4.09	62.08 ± 4.65	33.76 ± 0.89d
	1:5	65.31 ± 2.56	56.05 ± 4.00	9.27 ± 1.44b
	1:6	29.64 ± 2.56	20.30 ± 3.86	9.34 ± 2.25b
72	1:4	93.92 ± 3.11	18.83 ± 2.67	75.09 ± 1.78f
	1:5	61.79 ± 2.43	12.29 ± 2.55	49.50 ± 1.69e
	1:6	28.32 ± 2.55	8.17 ± 2.88	20.15 ± 3.64c

*Notes: Mean values followed by different letters indicate significant differences at the 5% level based on Duncan's Multiple Range Test (DMRT)

The significant decrease in reducing sugar levels after 48 and 72 hours of fermentation, particularly at a more concentrated sugar solution ratio (1:4), reflects the active utilisation of reducing sugars by *S. cerevisiae* to produce bioethanol. This phenomenon is referred to as the "acceleration phase," the stage where reducing sugar consumption is maximised, resulting in a significant increase in the rate of bioethanol production [10].

3.2.3 Changes in pH before and after fermentation

The changes during fermentation process are not only reflected in sugar consumption but also in the medium pH, which indicates metabolic activity of *S. cerevisiae*. Yeast not only produces bioethanol as a primary metabolite but also produces acidic byproducts, which can affect the pH of the fermentation medium.

Table 5. pH value of the fermentation medium before and after fermentation

Fermentation Time (hours)	**Sugar Solution Ratio**	**pH Before Fermentation**	**pH After Fermentation**	**Δ pH**
24	1:4	5.47 ± 0.02	3.28 ± 0.05	2.19 ± 0.04
24	1:5	5.47 ± 0.04	3.26 ± 0.10	2.22 ± 0.07
24	1:6	5.54 ± 0.02	3.15 ± 0.12	2.39 ± 0.03
48	1:4	5.44 ± 0.02	3.23 ± 0.04	2.21 ± 0.05
48	1:5	5.51 ± 0.02	3.18 ± 0.07	2.33 ± 0.06
48	1:6	5.52 ± 0.02	3.13 ± 0.04	2.39 ± 0.06
72	1:4	5.44 ± 0.05	3.08 ± 0.09	2.36 ± 0.06
72	1:5	5.50 ± 0.03	3.09 ± 0.08	2.41 ± 0.09
72	1:6	5.51 ± 0.04	3.09 ± 0.05	2.42 ± 0.09

The pH of the nipa palm sugar substrate is in the range of 5.4-5.5 (Table 5), within the optimal growth range for *S. cerevisiae* of 4-6 [11]. Based on the research results, the medium pH decreased in all treatments after fermentation. Biologically, the decrease in pH after fermentation is due to the accumulation of acidic compounds produced during the fermentation process, which are formed from the secondary metabolic pathway of *S. cerevisiae*. In anaerobic fermentation, pyruvic acid is converted into acetic acid, bioethanol, and carbon dioxide (CO_2), thereby affecting the medium pH at the end of fermentation. Based on the ANOVA test, there was no significant difference among treatments in pH changes, so the DMRT test was not continued.

3.2.4 The effect of sugar solution ratio and fermentation time on the ethanol content, density, and pH of distillates

Based on the research results, the bioethanol content obtained in this study ranged from 14.19% to 42.97% (v/v). The highest ethanol content was achieved with a 48-hour fermentation time and a sugar solution ratio of 1:4 (Table 6). The decrease in ethanol levels with longer fermentation time (72 hours) indicates that fermentation has passed its peak, at which point the metabolic activity of *S. cerevisiae* begins to decline. Furthermore, it can lead to environmental stress, including ethanol accumulation, excessively acidic conditions, and nutrient limitations. These conditions alter *S. cerevisiae* metabolic pathways and increase the production of byproducts, such as acetic acid, which reduces the efficiency of substrate conversion to bioethanol [12], [13].

Table 6. Ethanol content, density, and pH of bioethanol distillates

Fermentation Time (hours)	Sugar Solution Ratio	Bioethanol Content (%)	Density (g/cm³)	pH
24	1:4	32.43 ± 0.76e	0.959 ± 0.0010d	4.96 ± 0.21
24	1:5	29.13 ± 1.05d	0.963 ± 0.0013e	5.51 ± 0.28
24	1:6	14.19 ± 0.83a	0.980 ± 0.0009h	6.43 ± 0.34
48	1:4	42.97 ± 1.37h	0.943 ± 0.0024a	4.51 ± 0.25
48	1:5	38.86 ± 0.71g	0.950 ± 0.0011b	5.03 ± 0.17
48	1:6	17.30 ± 0.37b	0.976 ± 0.0004g	6.08 ± 0.11
72	1:4	35.16 ± 1.01f	0.955 ± 0.0014c	4.41 ± 0.18
72	1:5	33.89 ± 0.55ef	0.957 ± 0.0008cd	4.85 ± 0.23
72	1:6	23.31 ± 0.79c	0.970 ± 0.0009f	5.40 ± 0.27

Notes: Mean values followed by different letters indicate significant differences at the 5% level based on Duncan's Multiple Range Test (DMRT)

Based on the data, a 48-hour fermentation time is optimal, as *S. cerevisiae* is in the exponential phase, with maximum cell growth and metabolism. In this phase, yeast cells intensively consume monosugars (glucose and fructose) to produce bioethanol as a primary metabolite. The sugar content as a carbon source in the fermentation medium remains sufficient, and there has been no accumulation of inhibitory compounds, such as organic acids or bioethanol. The highest ethanol content (42.97%) was obtained at 48-hours fermentation time with a sugar solution ratio of 1:4.

For comparison, Jayanti et al. [8] reported that bioethanol fermentation using molasses produced an ethanol yield of up to 68.67%, an ethanol content of 8.30%, and a total sugar consumption of 57.21 g/L. The ethanol content obtained in this research was higher because the distillates had undergone an evaporation stage. Sugar consumption during fermentation was lower, at 33.76 g/L.

Ethanol content correlates with bioethanol density; the higher the ethanol content of the distillate, the lower its density. However, the density of the distillates obtained in this study was far from the density of pure ethanol (0.789 g/cm^3). This indicates that the distillates still contain some water and non-ethanol compounds. According to Velásquez et al. [14], fermentation by-products, such as organic acids, can also increase the density of the final distillates.

Conclusion

The salinity of nipa palm sugar affects the ethanol yield. The highest ethanol content was obtained from Nusadadi Village nipa palm sugar, which has the lowest salinity. In general, longer fermentation times and concentrated sugar solutions tend to result in higher reduction in total and reducing sugar content. These variables also affect the ethanol content and density of the distillates. The best fermentation conditions for bioethanol production from nipa palm sugar were obtained at a sugar solution ratio of 1:4 with a fermentation time of 48 hours.

References

[1] T. M. Ibrahim, E. Julianti, T. Supriana, and Delvian, "Potential analysis of Nipa Palm (Nypa Fruticans) in Singkil," in *IOP Conference Series: Earth and Environmental Science*, Institute of Physics, 2022. doi: 10.1088/1755-1315/1115/1/012052.

[2] C. P. Prasetyo, A. J. Effendi, and M. Chaerul, "Analysis of the potential and characteristics of Nipa (Nypa fruticans) as a sustainable biofuel alternative source in Indonesia," in *E3S Web of Conferences*, EDP Sciences, Feb. 2024. doi: 10.1051/e3sconf/202448503001.

[3] N. E. R. Mateo, A. D. Calderon, S. C. Agrupis, L. F. T. Manzano, C. C. Baga, and A. S. Fagaragan, "Nipa-based bioethanol as a renewable pure engine fuel: A preliminary performance testing and carbon footprint quantification," *International Journal of Renewable Energy Development*, vol. 14, no. 1, pp. 39–51, Jan. 2025, doi: 10.61435/ijred.2025.60614.

[4] P. Tamunaidu, N. Matsui, Y. Okimori, and S. Saka, "Nipa (Nypa fruticans) sap as a potential feedstock for ethanol production," *Biomass Bioenergy*, vol. 52, pp. 96–102, May 2013, doi: 10.1016/j.biombioe.2013.03.005.

[5] J. Jara'ee, D. S. Awg-Adeni, L. M. Bilung, and P. Azmin, "Effects of selected preservation techniques on the shelf-life of nipa sap," *Food Res*, vol. 9, no. 2, pp. 40–48, Apr. 2025, doi: 10.26656/fr.2017.9(2).403.

[6] G. L. Miller, "Use of DinitrosaIicyIic Acid Reagent for Determination of Reducing Sugar," *Journal of Analytical Chemistry*, vol. 31, pp. 426–428, 1959, doi: http://dx.doi.org/10.1021/ac60147a030.

[7] T. T. Turnip, F. Restuhadi, and E. Rossi, "Coconut water potential in the process of bioethanol fermentation with addition of npk and tween80tm," *Jom Faperta*, vol. 3, no. 2, pp. 1–13, 2016.

[8] A. N. Jayanti, A. Sutrisno, A. K. Wardani, and U. Murdiyatmo, "Bioethanol production from sugarcane molasses by instant dry yeast (effect of pretreatment and fermentation temperature)," in *IOP Conference Series: Earth and Environmental Science*, Institute of Physics Publishing, Feb. 2019. doi: 10.1088/1755-1315/230/1/012102.

[9] M. Gomar-Alba, M. Á. Morcillo-Parra, and M. lí del Olmo, "Response of yeast cells to high glucose involves molecular and physiological differences when compared to

other osmostress conditions," *FEMS Yeast Res*, vol. 15, no. 5, Aug. 2015, doi: 10.1093/femsyr/fov039.

[10] H. Zentou, Z. Zainal Abidin, M. Zouanti, and D. Greetham, "Effect of Operating Conditions on Molasses fermentation for Bioethanol production," *International Journal of Applied Engineering Research*, vol. 12, pp. 5202–5506, 2017, [Online]. Available: http://www.ripublication.com

[11] Y. Wu, B. Li, B. Miao, C. Xie, and Y. Q. Tang, "Saccharomyces cerevisiae employs complex regulation strategies to tolerate low pH stress during ethanol production," *Microb Cell Fact*, vol. 21, no. 1, Dec. 2022, doi: 10.1186/s12934-022-01974-3.

[12] S. Giannattasio, N. Guaragnella, M. Ždralević, and E. Marra, "Molecular mechanisms of Saccharomyces cerevisiae stress adaptation and programmed cell death in response to acetic acid," 2013, *Frontiers Research Foundation*. doi: 10.3389/fmicb.2013.00033.

[13] T. J. Tse, D. J. Wiens, and M. J. T. Reaney, "Production of bioethanol—a review of factors affecting ethanol yield," Dec. 01, 2021, *MDPI*. doi: 10.3390/fermentation7040268.

[14] A. Palacios-Velásquez, V. Quispe-Coquil, E. M. Casimiro-Soriano, K. M. Tapia-Zarate, and A. R. Huamán-De la Cruz, "Acquisition, Characterization, and Optimization of Distilled Bioethanol Generated from Fermented Carrot (Daucus carota) Residues," *Fermentation*, vol. 9, no. 10, Oct. 2023, doi: 10.3390/fermentation9100867.