MULTIDISCIPLINARY APPLIED RESEARCH AND INNOVATION
e-ISSN: 2773-4773

MARI

Vol. 5 No. 1 (2024) 33-38
https://publisher.uthm.edu.my/periodicals/index.php/mari

# Sugar Utilization by *Saccharomyces cerevisiae* in Fermentation to Produce Ethanol

**Auni Jamali, Nur Ainul Mardhiah Min, Siti Fatimah Mohd Noor***

*Department of Science and Mathematics, Centre for Diploma Studies,*
*Universiti Tun Hussein Onn Malaysia, Pagoh Higher Education Hub, 84600, Pagoh, Johor, MALAYSIA*

*Corresponding Author: fatimah@uthm.edu.my
DOI: https://doi.org/10.30880/mari.2024.05.01.006

**Article Info**

Received: 01 September 2023
Accepted: 10 December 2023
Available online: 31 January 2024

**Keywords**

Oil Palm Trunk (OPT), Natural Sugar, Fermentation, Bioethanol, *Saccharomyces Cerevisiae*

**Abstract**

This paper influence of sugar utilization by *Saccharomyces cerevisiae* in fermentation to produce ethanol. Sugar can be characterized by using the Dinitro Salicylic Acid (DNS) method to analyze sugar before further fermentation to see the growth of yeast in the sugar solution. The aims of this research is to identify the production of ethanol by fermenting different type of sugar, fructose, glucose, and sucrose, and ready-to-ferment sugars exist naturally in oil palm trunk sap. Fermenatation process were done for maximum at 144 hours and sample of fermentation product were taken throughout the incubation period for ethanol analysis. Ethanol production from each fermentation set is analyzed by using Gas Chromatography-Flame Ionization Detection (GC-FID). Natural sugar from oil palm trunk sap (OPT) produced the highest ethanol production at the 24th hour with a value of 21.13 mg/mL. This study shows that OPT has a potential to become biomass for ethanol production thus give add value to oil palm industry.

## 1. Introduction

Real life is overly dependent on fossil fuels as a primary source of energy, and as these non-renewable resources are depleting, this condition is what is causing the current energy crisis. Safety and availability of energy have emerged as the world's top concerns. Particularly in Malaysia, the population and economy will rise dramatically [1]. To satisfy the increasing business energy demand, it is necessary to investigate other sources of energy. Malaysia must establish a sustainable energy system because the country has a lot of renewable biomass energy resources. The potential to capture bioenergy as economically advantageous and environmentally good as biofuel was greatly increased by the considerable volume of agricultural and forestry leftovers [2]. Because large number of trees have been cleared for palm oil plantations or destroyed, the production of palm oil on a large scale has drawn criticism and worry from the international community. The transition to using bioethanol as fuel has been made more difficult by the depletion of fossil resources. Even though the ethanol sector generally relies on sugar starch biomass resources, but lignocellulosic biomass could be a significant source of renewable carbohydrates in the future. Due to the enormous biomass production, oil palm trunk (OPT) has attracted the interest of scientists. The large amount of sugar-rich sap found in this soft inner part can be fermented to produce bioethanol. It was discovered that sap from a felled trunk includes significant amounts of carbohydrates like glucose and sucrose. The trunk was discovered to be a substantial resource for the manufacture of fuel ethanol, biochemicals, and bioplastics due to the ease with which these sugars may be processed to ethanol. Yeast, namely Saccharomyces cerevisiae, sometimes referred to as baker's yeast, is the most prevalent fermenting bacterium used to produce

bioethanol. *Saccharomyces cerevisiae* is frequently used to produce ethanol due to its high resistance to ethanol, strong fermentation ability, and potential to grow quickly in anaerobic environments [1].

Oil palm trunk tree can produce fruits for up to 25 years before ageing results in a significant drop in productivity. Aged oil palm tree will be cut down for replantation to maintain oil palm production resulting a tremendous amount of waste will be produced and burden to managed. Early on, a procedure referred to as the "clean clearing technique" is frequently used. This process involves felling, stacking, and burning the OPT, which results in significant smoke and negative environmental effects. The trunk of the oil palm has a high sugar content, which is perfect for the fermentation process. OPT sap contains other compositions besides sugar, which may have positive or negative effects on fermentation. Thus, the capability and efficiency of Saccharomyces cerevisiae in fermenting OPT sap will be studied and compared to chemically produced media with the addition of a certain amount of sugar similar to OPT sap sugar. The aim of this research study is to identify glucose, fructose and sucrose content in the oil palm trunk and the second aim is to compare the production of ethanol in fermentation by using a different type of sugar as the main carbon.

## 2. Materials and Methods

### 2.1 Preparation of Raw Material (Oil Palm Trunk)

An oil palm trunk aged 20 to 25 years was acquired from Kampung Sungai Ranggam, Panchor, Muar, Johor. Oil palm trunk stood at a height of 12m. Three parts of the entire oil palm trunk were removed. To access the softer, sap-rich core, the exterior, harder piece of the trunk core was peeled off. The trunk core was then divided into pieces measuring roughly of the dimension at 20 cm x 20 cm x 1 cm (length x width x thick).

### 2.2 Extraction

The sap was then extracted from the OPT core using a powerful sugar cane juicer. Before being frozen, the sap was homogenized and filtered then stored in clean water bottle to avoid the sap from being contaminated. The estimated time overall for this process will take 12 hours. A powerful sugar cane juicer will be used to extract the oil palm sap sugar in order to extract as much sugar sap from the trunk as possible. After that, all of the sap sugar was mingled and all of the opt trunk pieces were compressed together in a big container. Put 1.5 mL of sap sugar in a jar and freeze it at -20**°C** in the freezer.

### 2.3 Yeast Culture and Inoculation Preparation

11 g of yeast (*Saccharomyces cerevisiae*) was combined with 100 mL of sterile deionized distilled water, and the mixture was allowed for 30 minutes before the bubble appeared. Nutrient agar plate was prepared by mixing 5 g of agar, 4 g of peptone, 2 g of yeast extract and 4 g of glucose with 200 ml of distilled water in a 250 mL bottle reagent and autoclaved at 121°C. Once the temperature fell below 90°C, the agar was put into a sterilized petri plate. The plates were left to allow the agar to solidify. Subsequently, yeast suspension was streaked on all the agar plate and all the plates were closed and sealed, then incubated a day at 30°C. After a day, all the plates were stored in a refrigerator at 4°C until further use.

### 2.4 Fermentation

Yeast Peptone Dextrose media (YPD) was used as fermentation process media. Sample A YPD contains 50 mg/L of glucose, sample B 50 mg/L of fructose, sample C 50 mg/L of sucrose and 55 mg/L mixed sugar that contain 35 mg/L of glucose, 5 mg/L of fructose and 15 mg/L of sucrose into 4 different conical flasks with 100 mL deionized distilled water and autoclaved at 121°C. Then, all the conical flasks were stored in a refrigerator at 4 ˚C until further use. Subsequently, 5 colonies of yeast were inoculated into 50 mL of mixed sugars that contain glucose, fructose and sucrose into 250 mL conical flasks to introduce the yeast for the mixed sugars. In order to prevent any mistakes during the experiment, the yeast must be combined with 50 ml of ideal sugar sap to make sure it is prepared for the fermentation process. The combined yeast and mix sugars were then incubated for a day at 30 ˚C as part of the incubation process. 5 mL of combined yeast and mixed sugars was added into 5 conical flasks that contain 45 mL of glucose, 45 mL of fructose, 45 mL of sucrose, 45 mL of mixed sugar and 45 mL of OPT into each conical flask. After putting all the media and combined yeast into the conical flasks, all the conical flasks were shaken and incubated for 7 days at 30°C.

### 2.5 Sampling

When fermentation process was run, each sample of 3 mL was taken aseptically at various time: 0, 2, 4, 6, 8, 10, 12, 24, 30, 48, 54, 120 and 144 hr. All the collected samples were immediately stored in freezer at – 20 °C before used for analysis. The samples were centrifuged for 5 min at 12,000 rpm.

## 2.6 Sugar Analysis

Using the dinitro salicylic acids (DNS) colorimetric technique, reducing sugar was measured. Using an ultraviolet UV-vis spectrophotometer, samples that had been properly diluted were read at 540 nm to determine the color that had evolved. Readings of absorbance were translated from standard to glucose concentration. Then the sample blank has been prepared for the experiment without containing any sugar for UV-Vis analysis.

## 2.7 Ethanol Analysis

The ethanol produced during fermentation was measured via gas chromatography. Samples taken at specified times were centrifuged at 12000 rpm for 5 minutes and the upper layer was analyzed using GC-FID, (Agilent FID). The instrument was equipped with a 30m x 0.25 mm x 0.25 mm ZB- Wax plus column. The analysis was controlled by an autosampler, and the composition of the samples was detected using a flame ionization detector (FID). During the analysis, the injector and detector temperatures were both set at 230°C and an injection volume of 1 $\mu$L. The total flow rate of GC-FID is 45 mL/min using nitrogen as a carrier gas. The column was programmed with an initial temperature of 40 °C. The temperature was raised to 140°C at a linear gradient of 15 °C /min and to 230 °C at 50°C /min.

## 3. Results and Discussion

In this study, the highest ethanol production from oil palm trunk has been achieved. Due to the difference in sugar used, the production of ethanol produced also varies according to the type of sugar. All sugar were fermented with the same parameters which is volume and temperature at 30 °C. Ethanol production results will be checked through GC-FID. The production of ethanol by OPT will be contrasted with the sugar mixture. This will show that sugar in oil palm trunk can produce a lot of ethanol compared to using existing sugar.

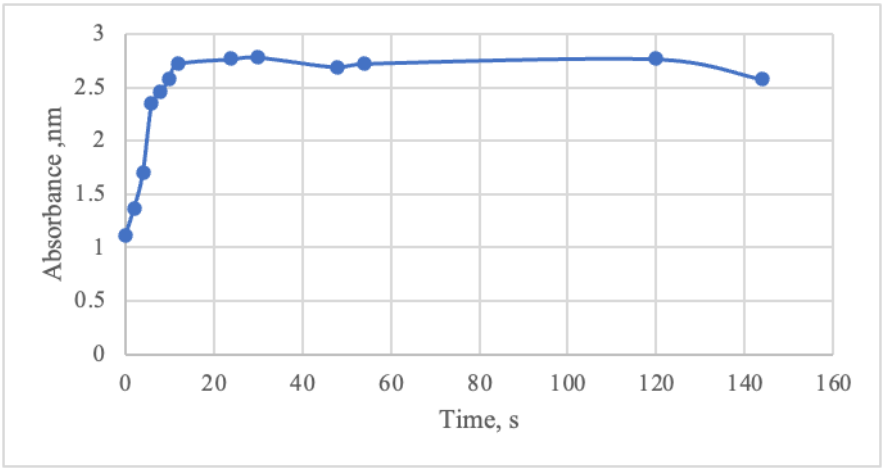
## 3.1 Cell Growth

A typical bacterial growth curve during batch culture displays five distinct development phases, including the lag phase, exponential phase, stationary phase, and death phase. Fig. 1 shows that all three samples A where glucose as sole carbon source, B (fructose), and sample C (sucrose) have a long period of stationary phase from 54 hours to 120 hours. Cell division happens at a steady pace during the exponential phase. It means the beaker's yeast did not produce ethanol at that time. The data analysis above shows that the beaker's yeast is used more by sample C which is sucrose in the ethanol production.

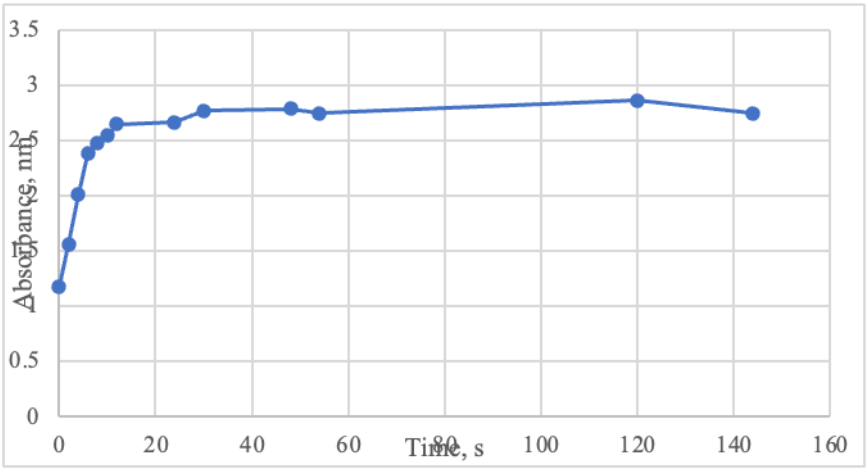
Glucose was consumed more quickly than the other two sugars. Wang et. al., in [3] also agreed with this pattern of sugar consumption where glucose is consumed fastest among other sugars on fermentation. Glucose is more advantageous to the generation of biomass with greater kinetic characteristics. The increased maintenance coefficient (m) on glucose was a direct result of the yeast's quicker metabolism of glucose. Sucrose can be thought of as a mixture of glucose and fructose due to the hydrolysis process that yields equimolar glucose and fructose, and its utilization was in between that of glucose and fructose. Although fructose was utilized slowly, sucrose was utilized more similarly to fructose and had lower values to produce ethanol by using sugar that have been chosen by beaker's yeast.

## 3.2 Ethanol Analysis

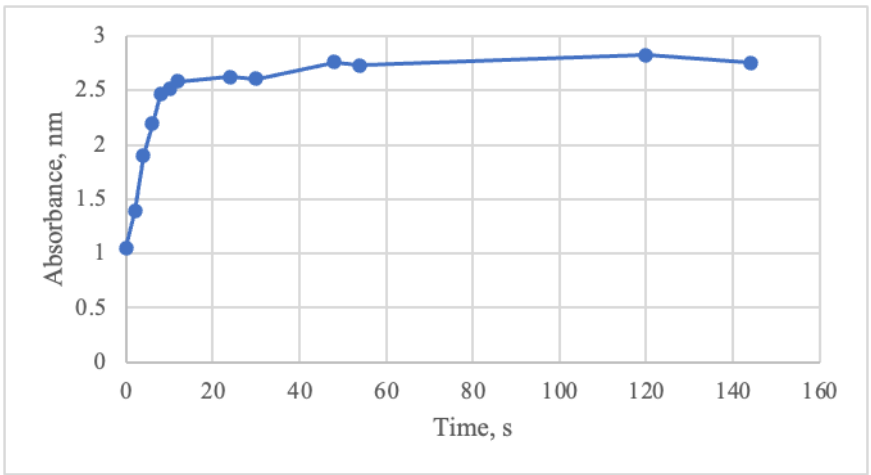
Fig. 2 shows the amount of ethanol produced in fermentation in media with glucose, fructose and sucrose has been checked using GC-FID after 144 hours. Fermentation of one type of sugar as sole carbon sources, media with sucrose produces the highest yield of ethanol, followed by glucose and fructose. During fermentation, sucrose will be break into glucose and fructose by yeast enzyme in order to use as precursor for ethanol production. Therefore, fermentation media contains sucrose compare with fermentation media with the same concentration of glucose or fructose will produce more glucose and fructose molecules [3]. As can be seen, this shows that sucrose produces the highest ethanol and can prove that *Saccharomyces cerevisiae* can produce higher yield ethanol by fermenting sucrose. While fructose, glucose, and sucrose are all forms of sugars, their chemical compositions have differences. While both glucose and fructose are monosaccharides, they are joined to form the disaccharide known as sucrose [4].




**(a)**




**(b)**




**(c)**

**Fig. 1** *Cell growth profile in fermentation of YPD with different type of sugar as sole carbon sources sample (a) glucose; (b) fructose; (c) sucrose*

Multidisciplinary Applied Research and Innovation Vol. 5 No. 1 (2024) p. 33-38

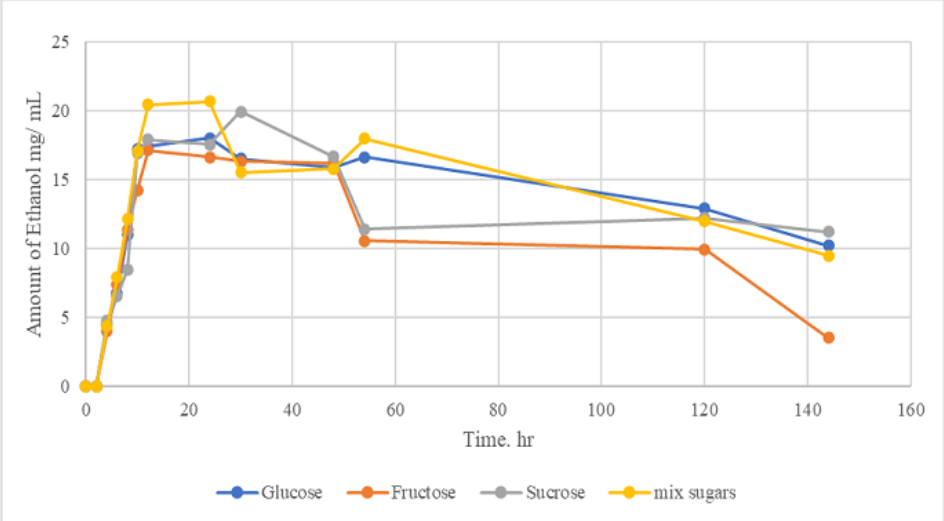



**Fig. 2** *Profile of ethanol production in fermentation of media with different type of sugar*

Fig. 3 shows the amount of ethanol in the mixture of sugar and palm oil trunk (OPT) also has been checked using GC-FID after 144 hours. As we know, OPT is a liquid that does not contain inhibitory substances and has basic nutrients [5] needed to support the fermentation process. This shows that OPT produces the highest ethanol and can prove that *Saccharomyces cerevisiae* can produce higher yield ethanol in OPT compared to chemically prepared media with mixed sugar. Based on Shahirah et. al., in [1], despite the difference type of sugar, the yeast quickly converted sugars to bioethanol. The concentration of all sugars has gradually reduced as bioethanol has been created. At 24 hours into the fermentation, when the total sugars had almost completely been metabolized, the fermentation had reached its peak ethanol concentration. According to the findings, OPT produced ethanol at a rate that was significantly higher than that of glucose, fructose, sucrose and mixture of sugar under comparable fermentation conditions. Based on Murata et. al., [6], it was discovered that a felled trunk carries significant amounts of carbohydrates, including glucose and sucrose, in its sap. The trunk was discovered to be a substantial resource for the production of fuel ethanol, biochemicals, and bioplastics due to the ease with which these sugars may be converted to ethanol and lactic acid.

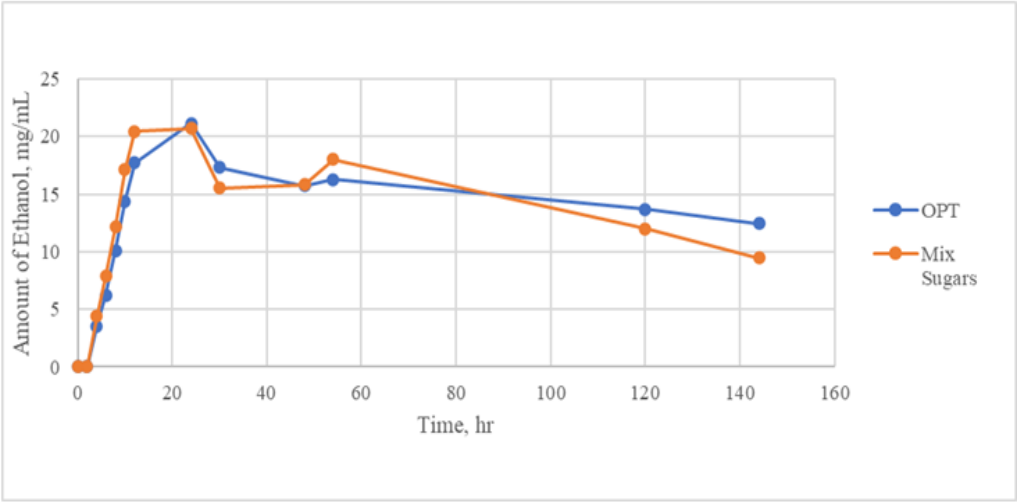



**Fig. 3** *The difference in ethanol production between oil palm trunk and the mixture of sugar*

## 4. Conclusion

The results of this investigation show that baker's yeast uses different sugars efficiently, with glucose being the one that is used up the fastest, followed by fructose and sucrose. In order to improve sugar utilisation and increase ethanol production, the study emphasizes the significance of optimizing fermentation parameters, such as temperature, pH, and oxygen availability. A colored product that may be detected spectrophotometrically is produced because of the reaction between reducing sugars and DNS reagent in the technique. The quantity of

reducing sugars in a sample can be calculated by measuring the absorbance of the colored product at a certain wavelength. These results have significance for the creation of sustainable biofuel production techniques and advance our knowledge of yeast fermentation processes. The highest bioethanol production by *Saccharomyces cerevisiae* was OPT at 24 hours with value 21.13 mg/mL. The addition investigated in this work may improve the production of ethanol from OPT without materially raising the cost of operation.

## Acknowledgement

The research was supported by Universiti Tun Hussein Onn Malaysia (UTHM) through Tier 1 (vot Q512). The authors would also like to thank the Centre for Diploma Studies, Universiti Tun Hussein Onn Malaysia for its support.

## Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

## Author Contribution

*The authors confirm contribution to the paper as follows:* ***study conception and design:*** *Auni Jamali, Nur Ainul Mardhiah Min, Siti Fatimah Mohd Noor;* ***data collection:*** *Auni Jamali, Nur Ainul Mardhiah Min, Siti Fatimah Mohd Noor;* ***analysis and interpretation of results:*** *Auni Jamali, Nur Ainul Mardhiah Min, Siti Fatimah Mohd Noor;* ***draft manuscript preparation:*** *Auni Jamali, Nur Ainul Mardhiah Min, Siti Fatimah Mohd Noor. All authors reviewed the results and approved the final version of the manuscript.*

## References

[1] Shahirah, M. N. N., Gimbun, J., Pang, S. F., Zakria, R. M., Cheng, C. K., Chua, G. K., & Asras, M. F. F. (2015). Influence of nutrient addition on the bioethanol yield from oil palm trunk sap fermented by Saccharomyces cerevisiae. Journal of Industrial and Engineering Chemistry, 23, 213–217. https://doi.org/10.1016/j.jiec.2014.08.018

[2] Hossain, N., Mahlia, T., Miskat, M. I., Chowdhury, T., Barua, P., Chowdhury, H., Nizamuddin, S., Ahmad, N. B., Zaharin, N. a. B., Mazari, S. A., & Soudagar, M. E. M. (2021). Bioethanol production from forest residues and life cycle cost analysis of bioethanol-gasoline blend on transportation sector. Journal of Environmental Chemical Engineering, 9(4), 105542. https://doi.org/10.1016/j.jece.2021.105542

[3] Wang, D., Xu, Y., Hu, J., & Zhao, G. (2004). Fermentation Kinetics of Different Sugars by Apple Wine Yeast *Saccharomyces cerevisiae*. Journal of the Institute of Brewing, 110(4), 340–346. https://doi.org/10.1002/j.2050-0416.2004.tb00630.x

[4] Lee, C. K. & Halim, F. A. A. (2014). Oil Palm Fronds Juice: A Potential Feedstock for Bioethanol Production. International Journal of Scientific and Research Publications, 4(12). www.ijsrp.orghttps://www.ijsrp.org/research-paper-1214/ijsrp-p3683.pdf

[5] Adela, B. N., & SohKheang, L. (2015). Optimisation of fermentation conditions for bioethanol production from oil palm trunk sap by Saccharomyces cerevisiae. Malaysian Journal of Microbiology. https://doi.org/10.21161/mjm.12814

[6] Murata, Y., Tanaka, R., Fujimoto, K., Kosugi, A., Arai, T., Togawa, E., Takano, T., Ibrahim, W. A., Elham, P., Sulaiman, O., Hashim, R., & Mori, Y. (2013). Development of sap compressing systems from oil palm trunk. Biomass and Bioenergy, 51, 8–16. https://doi.org/10.1016/j.biombioe.2012.12.007