# Bioconversion of Oil Palm Trunks Sap to Bioethanol by Different Strains and Co-Cultures at Different Temperatures

A. H. Norhazimah and Faizal C. K. M.
Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Pahang, Malaysia
Email: aimc.azim@gmail.com, ckmfaizal@gmail.com

***Abstract*—Oil palm plantation (OPT) generates a large amount of agricultural waste in a form of oil palm trunk sap. The content of sap (juice) from OPT can be used to produce 'higher value things' including bioethanol. In this research, sap was utilised as the raw material for producing bioethanol using different strains. The relationship between temperature and shaking to the fermentation of OPT sap for bioethanol production was investigated. The experimental results showed that 30 ℃ was the best temperature for most strains except for Pichia stipitis. This study indicated that *Saccharomyces cerevisiae* is the most suitable strains to produce bioethanol from oil palm trunk sap, and thus demonstrated that OPT sap is a promising renewable energy crop.**

***Index Terms*—bioethanol, fermentation, oil palm trunk, sap, *saccharomyces cerevisiae***

## I. Introduction

Changes in operational conditions are quite common in ethanol fermentation plants. These changes are not only due to variation in the quality of raw material, but also because of yeast variations [1]. The yeast commonly used in industrial alcohol production include *Saccharomyces cerevisiae* for fermentation of glucose, fructose, maltose, and maltotriose; *Saccharomyces uvarum* and *Saccharomyces diastaticus* for fermentation of dextrins; and *Kluyveromyces fragilis* and *Kluyveromyces lactis* for fermentation of lactose [2].

The principal wine yeast, *S. cerevisiae* is seemingly the best platform choice for lignocelluloses-derived substrate because , *S. cerevisiae* is relatively tolerant to the growth inhibitors found in the acid hydrolysates of the lignocellulosic biomass [2]. Also, *S. cerevisiae* is particularly suitable for the fermentation of hexoses, therefore suitable to ferment OPT sap that contains a lot of hexoses sugar.

*K. fragilis* or *Candida* sp. can be utilised to produce bioethanol when lactose and pentose are available as raw material. Other pentose- and hexose-fermenting microorganism such as *Clostridium hermosaccharolyticum* and *Thermoanaerobacter ethanolicus* are the thermophilic organisms that grant significant advantages for ethanol fermentation and separation. However, these microorganisms can gain undesirable end product and produce dilute ethanol [3]. Apart from that, a Gram-negative species, *Zymomonas mobilis*, is also considered an alternative organism for the large scale ethanol production. This species has higher sugar uptake, higher ethanol yield, and lower biomass production [4] than *Saccharomyces* species. This species is able to utilise glucose, fructose, and sucrose as the substrates for the ethanol production. Chandel *et al.* [5] workeds on combination of *Pichia stipitis* with *S. cerevisiae* and found that this co-culture was able to achieve higher final ethanol concentration compared to using only single strain of *Pichia stipitis* or *S. cerevisiae*.

OPT is left in a replantation area as a waste. It mainly contains two parts, fibre and liquid sap. For OPT fibre, several researches are currently being carried out to improve the quality of OPT fibre as timber and also for wood-based products. Less research has been done for OPT sap even though it contains high amount of sugar. Researchers have taken different approaches to use this agricultural waste including production of bioethanol from OPT sap [6]-[8]. Some research efforts have shown promising results. However, all of them are directly using *S. cerevisiae* as fermentative microorganism. The usage of other strains is less known for their potential in bioethanol production from OPT sap. It is important to select suitable bacteria or yeast to enhance the production of bioethanol. As different strains ferment sugars at different rates depending on the process conditions especially temperature, there is a need to use different temperatures when making a selection.

## II. Methods

### A. Materials and Methods

*Medium and culture conditions*: The microorganisms used for fermentation were baker's yeast, *S. cerevisiae* (local); *S. cerevisiae* Kyokai no. 7 (ATCC 26622); *Z. mobilis* JCM 10190 (ATCC 29191); *Zymobacter palmae* JCM 21091 (ATCC 51623); *S. cerevisiae* JCM 2220 (ATCC 9804); and *P. stipitis* JCM10742 (ATCC 58376).

*Raw material preparation:* OPT sap was collected from oil palm plantation in Negeri Sembilan, Malaysia. The sap was separated from trunks by mechanical

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pressing. The OPT sap consisted of sucrose, glucose, and fructose as main fermentable sugars.

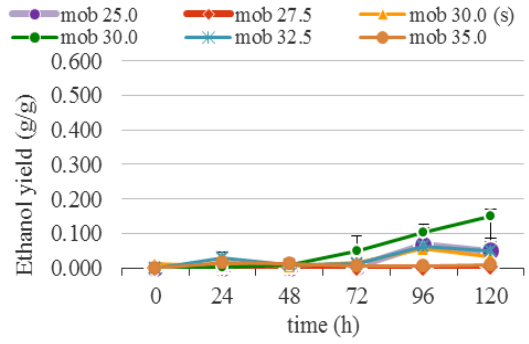
*Fermentation:* Fermentation process was conducted in 250 mL conical flasks with a working volume of 100 mL. About 10% v/v of inoculum was added into fermentation medium.

*Analytical methods:* Total sugar concentration in OPT sap was analysed by HPLC-RID (Agilent Carbohydrate Analysis Column) using acetonitrile and pure water as mobile phase (1.4 mL/min). Ethanol concentration was analysed using gas chromatography with flame ionisation detector (HP-InnoWax column). Carrier gas-helium; oven temperature 85 ℃; detector and injector temperature 250 ℃; internal standard-propanol

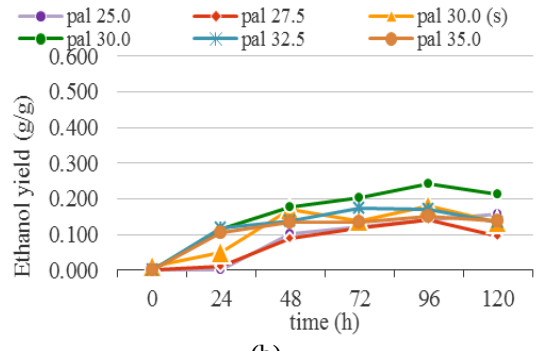
## III. Results and Discussion

### A. *Influences of Different Temperature to the Bioethanol Concentration*

Fig. 1 shows the bioethanol production from OPT sap by different strains at various temperatures.




(a)

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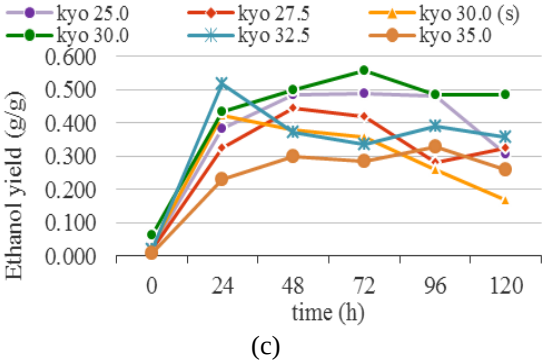


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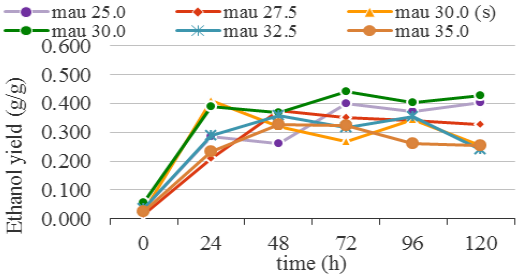


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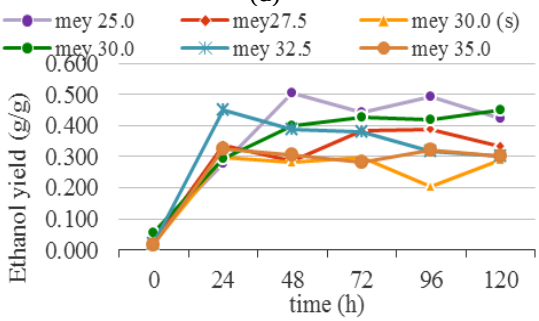


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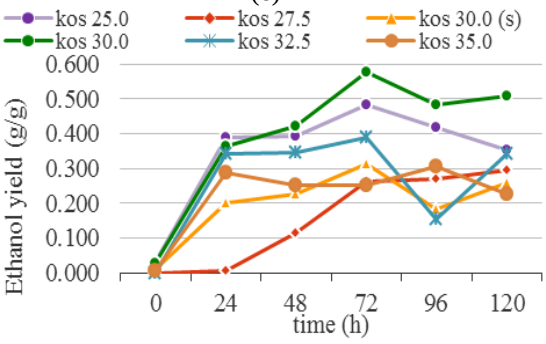
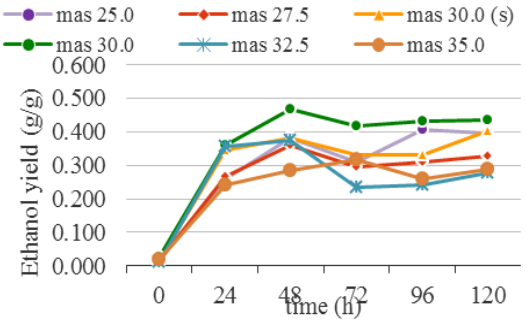
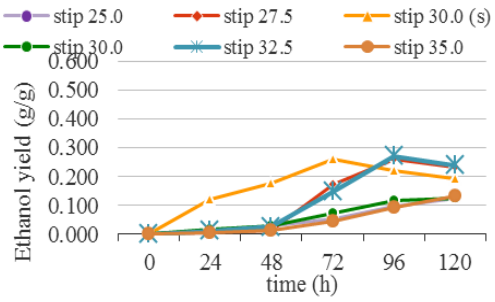



(f)




(g)




(h)

Figure 1. Bioethanol production from OPT sap by different strains at various temperatures using (a) *Z. mobilis*, (b) *Z. palmae*, (c) *P. stipitis*, (d) *S. cerevisiae* (local), (e) *S. cerevisiae* Kyokai no. 7, (f) *S. cerevisiae* JCM 2220, (g) *Co-culture S. cerevisiae* Kyokai no. 7 + *P. stipitis*, (h) *Co-culture S. cerevisiae* (local) + *P. stipitis.*

From the graph, *Z. mobilis* produced better ethanol yield at 30 ℃ without shaking condition compared to other condition. The ethanol yield obtained during fermentation of *Z. mobilis* at 27.5 ℃ and 35 ℃ are the lowest among other conditions. In fermentation using *Z. mobilis* at 30.0 ℃, ethanol was rapidly produced after 48 h, while at 25 ℃, 30 ℃ (shaking), and 32.5 ℃, ethanol concentration only increased after 72 h. Overall, fermentation of OPT sugar to the bioethanol using *Z. mobilis* was slow (increase during 48-72 h). In all conditions of fermentation, *Z. mobilis* was unable to produce more than 0.200 g/g of ethanol yield.

Similar pattern was also observed for *Z. palmae*. The strain produced better ethanol yield at 30 ℃ in static condition compared to other temperature condition. When shaken at 30 ℃, *Z. palmae* gained the lowest ethanol yield. *Z. palmae* produced better ethanol yield in static condition. This observation could be due to the effects of shaking, which can increase surface area contact with air and thus decreasing the anaerobic condition which not favourable to the strain.

TABLE I. FINAL ETHANOL YIELDS FOR DIFFERENT STRAINS AT DIFFERENT TEMPERATURES

| Strains | | | | | | |
|---|---|---|---|---|---|---|
| *Zymomonas mobilis* | | | | | | |
| ℃ | 25.0 | 27.5 | 30.0 (s) | 30.0 | 32.5 | 35.0 |
| g/g | 0.048 | 0.001 | 0.031 | 0.149 | 0.047 | 0.008 |
| *Zymobacter palmae* | | | | | | |
| ℃ | 25.0 | 27.5 | 30.0 (s) | 30.0 | 32.5 | 35.0 |
| g/g | 0.157 | 0.094 | 0.134 | 0.213 | 0.136 | 0.137 |
| *Saccharomyces cerevisiae* Kyokai no. 7 | | | | | | |
| ℃ | 25.0 | 27.5 | 30.0 (s) | 30.0 | 32.5 | 35.0 |
| g/g | 0.308 | 0.325 | 0.170 | 0.483 | 0.356 | 0.260 |
| *Saccharomyces cerevisiae* (local) | | | | | | |
| ℃ | 25.0 | 27.5 | 30.0 (s) | 30.0 | 32.5 | 35.0 |
| g/g | 0.404 | 0.325 | 0.254 | 0.426 | 0.240 | 0.252 |
| *Saccharomyces cerevisiae* JCM2220 | | | | | | |
| ℃ | 25.0 | 27.5 | 30.0 (s) | 30.0 | 32.5 | 35.0 |
| g/g | 0.422 | 0.332 | 0.291 | 0.449 | 0.301 | 0.302 |
| *Saccharomyces cerevisiae* Kyokai no. 7 + *Pichia stipitis* | | | | | | |
| ℃ | 25.0 | 27.5 | 30.0 (s) | 30.0 | 32.5 | 35.0 |
| g/g | 0.352 | 0.297 | 0.257 | 0.510 | 0.343 | 0.227 |
| *Saccharomyces cerevisiae* (local) + *Pichia stipitis* | | | | | | |
| ℃ | 25.0 | 27.5 | 30.0 (s) | 30.0 | 32.5 | 35.0 |
| g/g | 0.394 | 0.328 | 0.404 | 0.435 | 0.277 | 0.288 |
| *Pichia stipitis* | | | | | | |
| ℃ | 25.0 | 27.5 | 30.0 (s) | 30.0 | 32.5 | 35.0 |
| g/g | 0.126 | 0.233 | 0.190 | 0.123 | 0.240 | 0.133 |

All *S. cerevisiae* strains (*S. cerevisiae* Kyokai no. 7, *S. cerevisiae* (local) and *S. cerevisiae*) produced a good amount of final ethanol yield at 30 ℃. For *S. cerevisiae* Kyokai no. 7, the lowest ethanol yield was measured at 35 ℃. This might be due to the limitation of enzyme activation energy at this high temperature. Co-culture *S. cerevisiae* Kyokai no. 7 and *P. stipitis* were better than co-culture of *S. cerevisiae* JCM2220 and *P. stipitis* in terms of final ethanol yield achieved by that combined strain. Co-culture *S. cerevisiae* Kyokai no. 7 and *P. stipitis* rapidly fermented the sugar with a high ethanol yield over 0.500 g/g in static condition at 30 ℃. The final ethanol yields for different strains at different fermentation temperatures are summarised in Table I.

Overall, the ethanol yield from OPT sap using bacteria species was slow compared to using yeast species (except *P. stipitis*). *P. stipitis* was not able to consume a significant amount of total sugar during fermentation and therefore did not generate significant amount of bioethanol.

### *B. Conclusion*

OPT sap can be used to produce bioethanol using different strains of yeast and bacteria. Effects of temperature and shaking were investigated in this study. The suitable strains to produce bioethanol from OPT sap were *S. cerevisiae* (local), *S. cerevisiae* Kyokai no. 7, and *S. cerevisiae* JCM 2220, besides co-cultures containing strains of *S. cerevisiae*.

## ACKNOWLEDGEMENT

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**Dr. Che Ku Mohammad Faiza**l received his Degree in Applied Chemistry & Chemical Engineering from Yamaguchi University, Japan in 2000 and his Ph.D. in Energy & Environmental Engineering from Nagaoka University of Technology, Japan in 2009. He is currently an Associate Professor of Energy and Environmental Engineering in Faculty of Technology, Universiti Malaysia Pahang, Malaysia. His current research interests focus on the areas of biofuel production, CO2 captured and study on synthesis/modification of polymer based advanced materials.