Direct Fermentation of Oil Palm (Elaeis guineensis) Trunk Sap to Bioethanol by Saccharomyces cerevisiae

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Abstract. Oil palm agricultural biomass has a great potential for biofuel production due to its abundance and less pretreatment needed. In this study, bioethanol production from oil palm trunks sap was carried out by using yeast Saccharomyces cerevisiae. The suitability of the oil palm trunk sap for bioethanol production was tested by investigating the relationship between the oil palm trunk sap concentration and ethanol produced. The fermentation was conducted in static condition and without pH adjustment using 60, 80, and 100% oil palm trunk sap concentration. Results showed that the highest bioethanol production rate was achieved for 100% oil palm trunk sap (1.033 g/L.h), followed by 80% (0.744 g/L.h), and 60% (0.645 g/L.h). These results were influenced by the presence of sugars in the initial sap, suggesting that high substrate concentration does not inhibit bioethanol production. Therefore, the oil palm trunks sap showed significant potential as a renewable feedstock for the production of bioethanol without dilution.

Keywords: Bioethanol, Elaeis guineensis, palm oil, sap, trunk

1. Introduction
The recovery of natural energy resources such as petroleum and coal can result in global warming, which affects human lives and cause environmental problems due to the alleviation of earth’s temperature [1, 2]. Rapid expansion of human population and industrial prosperity in developing countries increase the global demands for energy; making the use of bioethanol to replace the non-renewable fuels as attractive. Complete combustion of ethanol produces only carbon dioxide and water which are not harmful to the environment and this has become one of the advantages to accelerate the research momentum.

There is a growing interest among research communities to find alternative bioresource apart from sugar cane and starchy crop for bioethanol production such as biomass [3]. Production of ethanol from sugars in lignocellulosic has been showed to be promising [4] and despite the challenges, it comes with many opportunities for enhancement. The production costs are still the key impediment to the widely use of ethanol as fuel although the fermentative process for ethanol production is well known [4]. Utilization of agricultural wastes helps in reducing the impact of energy demands on food-derived-ethanol without having to compete with the food requirement. Besides that, the massive volume of such waste increase the production capacity while the lower price reduce the production costs [5]. In general, the locally grown agricultural crops are a good choice for reducing the transportation cost and guaranteed continuous supply of biomass. During replantation stage, the oil palm trunk sap is usually
chopped into small pieces, rearrange in rows to avoid road blockage, and left to be rotten naturally in the plantation area (Fig. 1).

![Image of chopped and stacked OPT](https://example.com/image1)

**Fig. 1:** OPT was left to be rotten naturally during replantation

Leaving the trunk just like that is not good for the plantation because the high sugar and starch content in the oil palm trunk sap will attract microflora and microfauna thus increase the possibility of plant diseases [6], especially the younger plants. The presence of starch granules in the parenchyma lumen promotes the growth of the biodeterioration organism [7]. Although oil palm lumber has been successfully utilized as the main material in the production of blackboard, not all parts can be used for plywood manufacturing because only the outer part is relatively strong [2].

The oil palm trunk sap cannot be used as building structure too because it has low specific density, must be dried to remove moisture, and transportation problem. Hence, the inner part will be discarded as a waste due to its weak physical properties but can be considered as an important biomass in Malaysia. Oil palm trunk sap contains liquid (sap) with lower lignin percentage and readily fermentable sugars compared to the other parts of oil palm trees. Therefore, less or no pretreatment (chemical or biological) is needed to delignify or convert the lignocellulose to fermentative sugar, compared with other parts of oil palm tree.

Using oil palm trunk sap have advantages due to simplicity of the procedure, low energy consumption, and lesser time and production cost. Oil palm trunk sap exhibits desirable characteristics as an alternative raw material due to its abundancy and properties. Therefore, the finding of novel innovative method to utilize a cheap material for bioethanol production is important. In order to justify the potential of bioethanol production from oil palm trunks sap to be used as an energy source, it is necessary to analyze the amount of sugar consumption rate and bioethanol. This can provide useful data concerning the relationship between substrate utilization and bioethanol production.

2. Materials and Methods

2.1. Oil Palm Trunks Sap

Oil palm trunk (OPT) sap was used as fermentation medium. Five oil palm trees (26 years old) felled for replantation purposes were freshly obtained from a plantation in Negeri Sembilan, Malaysia. The upper part of the oil palm that contains fruit and frond was removed together with the root. Next, OPT
sap (10 m) was cut into several small pieces to undergo the squeezing process by using sugar cane press machine (Robin brand, 5 HP) to obtain the liquid sap. The OPT sap was collected in a big container, mixed well before divided into 5 L bottles, and stored under −20 °C. The OPT sap media was then filtered with 9.0 μm filter prior to use.

2.2. Microorganism and preculture development
For inoculation purpose, the microorganisms were aerobically incubated in OPT sap medium at 30 °C in static condition for 24 h. The microorganism used are Japan sake yeast *Saccharomyces cerevisiae* Kyokai no.7 (ATCC 26622 obtained from Japan Collection of Microorganisms (JCM)).

2.3. Fermentation
The fermentation was conducted at 30 °C in static condition without pH adjustment. About 10% v/v of inoculum was used as inoculum for fermentation medium. The shake flask was purged with nitrogen to remove oxygen to create anaerobic condition. Samples were withdrawn periodically at predetermined time intervals for analysis. Fermentation to study the effects of substrate inhibition were conducted using 60, 80 and 100% sap concentrations as in Table 1.

![Table 1: Effect of Substrate Inhibition](image)

| Percentage OPT Sap | OPT sap | Distilled Water |
|--------------------|---------|----------------|
| 60%                | 60%     | 40%            |
| 80%                | 80%     | 20%            |
| 100%               | 100%    | 0%             |

2.4. Method of Analysis
Sugar concentration was determined by High Performance Liquid Chromatography (HPLC) with refractive index (RI) detector (Agilent Carbohydrate Analysis Column, ratio acetonitrile to water 4:1, flow rate 1.4 mL/min). Column temperature was set at 60 °C. Injection volume was 10.0 μL. The solvent used for washing and dilution was ultrapure water. All individual sugars in OPT sap can be expressed as equivalent glucose concentration and summed up as total sugar using Equation 1.

\[
\text{Total sugar} = \text{glucose} + \text{fructose} + (1.0526 \times \text{sucrose})
\]  

All calculations are in gram per liter (g/L).

Gas chromatography (Agilent Technologies 6890 Series) equipped with a flame ionization detector (FID) was set up to determine bioethanol concentrations. The column used was HP-INNOWax Polyethylene Glycol (30 m × 250 μm × 0.25 μm nominal). Initial temperature, maximum temperature, and temperature rate in the oven were 50 °C, 170 °C and 20 °C/min, respectively. Temperature of injector and detector were set at 250 °C. Helium carrier gas at a flow rate of 45 mL/min was used. The measured value is “peak area” which was then converted into ethanol concentration (g/L) using a standard curve. Serial dilution was performed using pure ethanol. The solvent used for washing and dilution was n-propanol. One part of sample was mixed with 9 part of n-propanol and filtered through 0.2-micron nylon membrane (Fisher Scientific™). Ethanol productivity was calculated as the amount of ethanol concentration per time (h).
3. Results

Comparison for the effect of substrate concentration on the bioethanol production curve is presented in Table 2.

Table 2: Comparison between ethanol productions using different substrate concentrations at 30 °C.

| Time | 60% OPT sap |  | 80% OPT sap |  | 100% OPT sap |  |
|------|-------------|---|-------------|---|-------------|---|
|      | Ethanol (g/L) | Productivity (g/L.h) | Ethanol (g/L) | Productivity (g/L.h) | Ethanol (g/L) | Productivity (g/L.h) |
| 0    | 2.20 | - | 1.94 | - | 2.10 | - |
| 2    | 2.52 | 1.259 | 2.92 | 1.460 | 2.68 | 1.341 |
| 4    | 3.60 | 0.900 | 3.73 | 0.932 | 4.10 | 1.026 |
| 6    | 5.16 | 0.861 | 5.40 | 0.901 | 5.88 | 0.980 |
| 8    | 5.71 | 0.714 | 7.47 | 0.934 | 8.00 | 1.000 |
| 10   | 9.10 | 0.910 | 8.50 | 0.850 | 11.37 | 1.137 |
| 12   | 8.80 | 0.734 | 10.86 | 0.905 | 14.29 | 1.191 |
| 18   | 12.52 | 0.698 | 12.05 | 0.669 | 20.45 | 1.136 |
| 24   | 12.06 | 0.502 | 17.03 | 0.710 | 18.33 | 0.764 |
| 30   | 13.91 | 0.464 | 18.02 | 0.601 | 21.88 | 0.729 |
| 36   | 14.95 | 0.415 | 17.42 | 0.484 | 22.72 | 0.631 |
| 42   | 13.55 | 0.323 | 18.69 | 0.445 | 21.89 | 0.521 |
| 48   | 12.96 | 0.270 | 18.19 | 0.379 | 20.95 | 0.436 |
| 54   | 14.67 | 0.272 | 18.51 | 0.343 | 22.39 | 0.415 |
| 60   | 14.16 | 0.236 | 18.59 | 0.310 | 21.92 | 0.363 |
| 66   | 14.52 | 0.220 | 18.33 | 0.278 | 20.90 | 0.317 |
| 72   | 14.86 | 0.206 | 16.96 | 0.236 | 18.17 | 0.252 |

Ethanol productivity reached the highest at initial stage of fermentation indicating that the *Saccharomyces cerevisiae* able to utilize sugar to produce bioethanol within a short time. However, the maximum bioethanol was only produced after 30 h of fermentation. The fermentation time was important since increasing time gave opportunity for the yeast to utilize more sugar. However, it was observed that increasing time to more than 36–48 h can cause declination of ethanol concentration due to the consumption of the accumulated ethanol by the organism. Similar phenomenon was reported by Kuhad et al. [8] in their work by using red sage as sugar sources.

At high concentration level, the production of bioethanol is limited by concentration of substrate and bioethanol itself. Sugar cannot be fully utilized and cause the production to be inefficient. The graph of glucose consumption rate at different substrate concentrations was plotted to calculate the rate of glucose consumption and cell growth. Fig. 2 and Fig. 3 show the glucose consumption and bioethanol production at different substrate concentrations.
Fig. 2: Glucose consumption in different substrate concentrations (60, 80 and 100%) via fermentation process

At the lowest substrate concentration (60%), the glucose consumption rate was 0.8747 g/L.h ($y = -0.8747x + 12.477$). This is slightly higher than glucose consumption rate at 100% substrate (0.8353 g/L.h) ($y = -0.8353x + 14.223$) and at 80% substrate (0.7337 g/L.h) ($y = -0.7337x + 11.435$). This should be evaluated together with bioethanol production rate to select the best oil palm trunk sap concentration that should be used in further study.

Fig. 3: Ethanol production in different substrate concentrations (60, 80 and 100%) via fermentation process.

Maximum bioethanol concentration was achieved by using 100% of OPT sap. This indicates that fermentation of OPT sap to bioethanol can be done without the need to be diluted. Low concentration of bioethanol was obtained at 60% and 80% since reduction of sugar amount occurred when OPT sap was diluted, which subsequently reduces the bioethanol production. The rate of bioethanol production is highest at 100% substrate concentration since its contain more sugar. High substrate concentration does not inhibit bioethanol production. At 60% substrate concentration, the rate of bioethanol of 0.645 g/L.h ($y = 0.645x + 1.5747$) was obtained while at 80%, 0.7441 g/L.h ($y = 0.7441x + 1.3663$) was obtained. At 100% substrate concentration, 1.033 g/L.h bioethanol productivity ($y = 1.033x + 0.7195$) was obtained.
4. Conclusion

In this work, the effect of different concentrations of OPT sap on bioethanol production has been investigated. OPT sap can be used effectively without dilution since no substrate inhibition was detected. Maximum bioethanol production was produced in fermentation of 100% OPT sap which is 1.033 g/L.h. This finding supported the claim made by other researchers which mentioned that oil palm trunk sap does not contain any inhibiting substances for bioethanol production [9, 10]. The use of waste, especially biomass can reduce the impact of energy demands on food-derived-ethanol. Further studies should be conducted to check the effect of chemical and physical factors which can affect bioethanol production from OPT sap.

5. Acknowledgement

This work was financially supported by research grant Tier 1 Vot U928 from Universiti Tun Hussein Onn Malaysia.

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