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An Evaluation of Oil Palm (Elaeis guineensis) Trunk Sap For Bioethanol Production

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Abstract: Oil palm trunk (OPT) generated from the replantation of oil palm trees at every 25-30 year interval has become a troublesome waste as it can be as a source of infection for young oil palm trees. This OPT contains an adequate amount of ready-to-use sugar in the form of sap which can be directly fermented to the bioethanol without pretreatment. Analysis of the OPT sap sugar content showed that sucrose is the dominant sugar followed by glucose and fructose. The fermentation process for bioethanol production from OPT sap was evaluated using Saccharomyces cerevisiae Kyokai no.7 in shake flask and 2 liter of bioreactor. After 48 h, the bioethanol concentration in the shake flask and bioreactor were 33.82 g/l and 48.86 g/l, respectively. Analysis on the practicability of bioethanol production from OPT sap showed that it was as good as other agricultural waste in the terms of production per hectare (1758 L of bioethanol per hectare of oil palm replantation). This result indicated that OPT sap waste may provide alternative potential raw material for bioethanol production.

Keywords— *bioethanol; elaeis guineensis; oil palm trunks; sap*

1. Introduction

Oil palm is an important commodity crop in Southeast Asia's countries such as Malaysia, Indonesia and Thailand [1]. In Malaysia, a total of 4.69 million hectares of land grown with the oil palm tree (Elaeis guineensis) and it is estimated that 7 million tons of the OPT are available per annum based on a 25-30-year life cycle [2]. These trunks are not fully utilized and were left as a troublesome waste in the plantation area. OPT contains sugar and starch which can attract microorganism to increase source of infection to new plants. These phenomena happened due to the presence of starch granules in the parenchyma lumen which promotes the growth of unwanted microorganism [3]. Considering OPT abundance, it is definitely suitable to be used as an alternative renewable fuel substrate for bioethanol production

Sap obtained from OPT can be considered as an important agricultural waste since it contains soluble sugar mainly sucrose, glucose and. Unlike many agricultural wastes, OPT sap can be directly fermented without any pretreatment to expose free sugar, thus it is an option of economic and cost effective raw material for bioethanol production. Ethanol can be produced from two main processes which are via synthetic process such as hydration of ethene, and via biological pathway such as fermentation. The economics of a hydrolysis process depend on the yield of the usable component such as glucose. Some of the options proposed to reduce costs of conversion of lignocellulosic to the ethanol are including eliminating pretreatment, increasing cellulose hydrolysis yield, enhancing enzyme activity and improving the fermentation yield [4]. Nevertheless, each pretreatment method has its own advantages and disadvantages that must be considered to be proceed for large scale commercial production. It is beneficial if it already contains a lot of fermentable sugar such as OPT sap, potato starch residue and sweet sorghum juice.

Others studies that using OPT sap as the raw material for bioethanol production involve a lot of pretreatment including sugar extraction, dilution and pH adjustment. The objective of this study is to examine the feasibility of OPT sap for direct bioethanol production through simple fermentation methods in order to eliminate most of the pretreatment procedures. The experiment to check the feasibility of using OPT sap as an alternative source for bioethanol production was directly carried out using. The fermentation is carry out by using OPT sap as the sole substrate without any addition of nutrient and pretreatment such as pH adjustment, saccharification and liquefaction.

The OPT sap used in this study was obtained from the whole trunk which showed the actual capability of the bioethanol production per oil palm tree. In addition, the practicability of OPT sap as an alternative substrate was also investigated by comparing with other published agricultural waste feedstock in terms of production of bioethanol per hectare.

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2. Materials and Methods

A. Oil Palm Trunk Sap

The 25 years old oil palm trees, felled for replantation purposes were freshly collected from RISDA Plantation in Pahang, Malaysia in May. The OPT was taken after removing the upper part of the trunk containing the fruit and frond as in Figure 1.

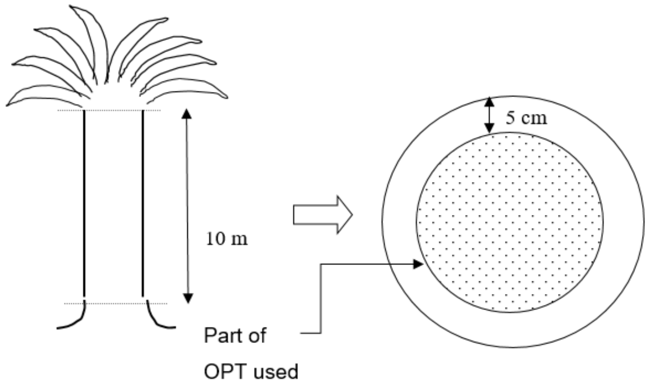

Figure1: Illustration of cutting procedure of OPT. all of the OPT sap was collected in a big container and mixed well before divided into 5 L bottles. It was kept under -20 ºC for storage and used within the three month the OPT sap was obtained. The sap media were filtered with 9.0 µm filter prior to use.

The OPT was cut into smaller pieces of 15cm length and 2cm width after removing the 5cm hard outer layer. The OPT pieces were then underwent mechanical pressing to optain sap within 12 hours. The sap was used for fermentation after heat sterilization by autoclave at 121ºC for 10 minutes. Sucrose, glucose and fructose in the OPT sap were determining by using HPLC.

B. Microorganism and preculture development

The microbial strain employed in this study is an industrial sake brewing strain, Saccharomyces cerevisiae Kyokai no.7 (ATCC 26422) obtained from the American Type Culture Collection. The strain was maintained by sub-culturing on YPD agar medium (in g/l: agar 20, yeast extracts 1.5, peptone 1.5 and dextrose 20) once every five weeks. The starter culture was prepared by inoculating previously sterilized liquid medium (in g/l: agar 10, yeast extracts 1.5, peptone 1.5 and dextrose 20) with a loopful of *S. cerevisiae Kyokai* no.7. The cells were grown aerobically at 30ºC, with an agitation rate of 150 rpm for 18 h.

C. Fermentation

The fermentation was started by transferring 10% v/v of inoculum into 500 ml shake flasks which contain 225 ml working volume containing OPT sap that makes the final working volume of 250ml. The fermentation was carried out for 48 h at 32ºC and 110 rpm. Using the same condition, the fermentation of OPT sap was also carried out in 2 liter Sartorius bioreactor with 1.5 liter working volume. Nitrogen gas was purged through both systems for 10 minutes to create an anaerobic condition. Samples were withdrawn aseptically from the fermentation broth periodically from 0 h to 48 h for the analysis of total sugar and bioethanol concentration. All experiments were repeated at least thrice.

D. Method of Analysis

High Performance Liquid Chromatography (HPLC) with a refractive index detector (RID) was used to determine sucrose, fructose, and glucose concentration. The column used was SUPERCOSIL LC-NH2. Acetonitrile and pure water at the ratio of 75:25 were used as the mobile phase at a flow rate of 0.6 ml/min.

Gas chromatography (Agilent Technologies 6890 Series) equipped with a flame ionization detector (FID) was set up to determine bioethanol concentration. The column used was an HP-INNOWax Polyethylene Glycol (30m x 250µm x 0.25µm nominal). The GC oven temperature was programmed with an initial temperature at 50ºC for 2 min, then remped at 20ºC/min to 170ºC. The temperature of the injector and detector were set at 250ºC. The carrier gas was helium at a constant flow rate of 45 ml/min. Ethanol and n-propanol standard with purity of 99% purchased from Sigma Aldrich were used as the external and internal standard for the quantitative analysis. Samples were prepared in the vial by filtering with 0.20 µm nylon filter.

3. Result

E. Sugar composition in the OPT sap

Table 1 show the concentration of individual sugar in OPT sap for this study.

TABLE I. INDIVIDUAL SUGAR CONCENTRATION IN OPT SAP

Sugar	***Concentration Gram/ liter***
Fructose	7.51
Glucose	16.99
Sucrose	73.42
Total sugar	101.77

All individual sugar can be expressed in an equivalent glucose concentration and sum up as total sugar using equation 1.

$$\text{Total sugar} = \text{glucose} + \text{fructose} + (1.0526 \times \text{sucrose}) \quad (1)$$

All calculation is in gram per liter. The data obtained was compared with previous study by other researchers shown in Table 2.

Analysis of the OPT sap composition for sugar content indicated that sucrose is the dominant sugar (73.42±5.54 g/l), followed by glucose (16.95±1.34 g/l) and fructose (7.55±0.64 g/l), which is similar to various studies by Yamada and Eze [5-6]. However, studies by Murai and Kosugi [7-8] observed glucose as the dominant sugar in their study instead of sucrose. It may be due to the different procedures used during collecting OPT sap. Total sugar is different about 10% from upper to lower part. It was observed that concentration of total sugar is more concentrated at lower part of OPT compared to upper part.

TABLE II. DOMINANT SUGAR OF OPT SAP IN VARIOUS STUDIES

Study	***Eze and Ogan, 1988 [6]***	***Kosugi et al., 2010 [8]***	***Yamada et al. 2010 [5]***	***This study***
Age of oil palm tree	-	25 year	25 year	25 year
Place	Nigeria	Penang, Malaysia	Kedah, Malaysia	Pahang, Malaysia
Time elapse	Directly from oil palm	Within 120 h	Within 24 h	Within 12 h
Range of total sugar (g/l)	106.3-127.6	20.1- 98.1	71.1-129.9	94.0 - 109.6
Major sugar	Sucrose	Glucose	Sucrose	Sucrose

The initial pH of fresh OPT sap was around 5.40-5.60. In contrast, works by Eze and Ogan [6] observed initial pH of 6.6 for pure sap. This differences can be related with time of analysis and how OPT sap was stored.

F. Bioethanol production from OPT sap

The feasibility of direct bioethanol production from OPT sap was evaluated in the shake flask and 2 liter bioreactor as a benchmark before starting a pilot scale-up study. Fig. 2 shows sugar consumption and bioethanol production in fermentation process at fixed time interval.

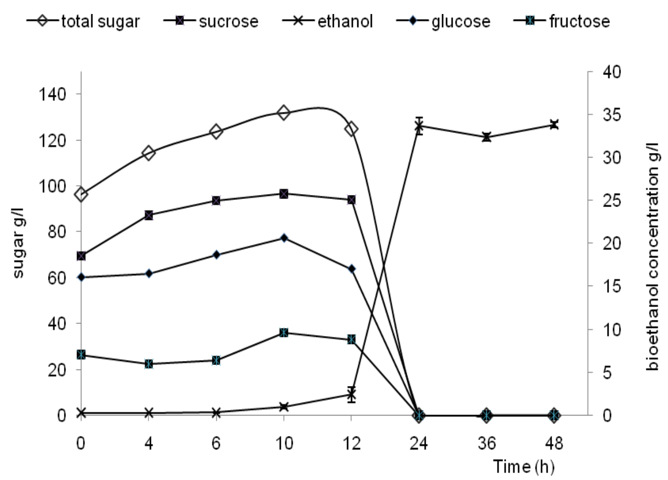

Fig. 2: Production of bioethanol from the fermentation of OPT sap in 500 ml of shake flask with total working volume of 250 ml. Fermentation was carried out at condition 32 ^{o}C and 110 rpm using *S. cerevisiae* Kyokai no. 7.

As shown in Fig. 2, at 0 h, no bioethanol was present in fermentation medium. In other words, ethanol was only being produced during the fermentation by *S. cerevisiae Kyokai* no.7.). This results also demonstrated that OPT sap can be directly used for fermentation of bioethanol without complicated pretreatment such as saccharification to release sugar or delignification.

The production of bioethanol was started at sixth hours of fermentation. During 12 to 24 h of fermentation, rapid increase in bioethanol production was observed. The stationary phase of bioethanol production was observed during 24-48 of fermentation. This results in nearly constant bioethanol concentration. The highest bioethanol concentration observed was around 33.82 g/l.

4. Discussion

The main difference in the procedure is the time elapsed taken after oil palm was felled. In this study, sap was freshly obtained from OPT which is less than 12 h after OPT was felled, while Kosugi [7] kept OPT less than 5 days before the squeezing process. Characterization study done on sap sugar has proved that sucrose was the dominant sugar in the OPT sap if it was processed under 15 h after oil palm was felled. However, after 15 h, the concentration of glucose becomes higher as the concentration of sucrose is decreasing. This suggests that the increase of glucose concentration is related to the decrease of the sucrose concentration [5].

During sap extraction from OPT in the field, sucrose inside the OPT can be degraded by wild microorganism into glucose and fructose which cause an increase of both sugars. This theory was supported by the analysis of fresh sap obtained directly by tapping the male inflorescence of oil palm, with the finding that sucrose is the dominant sugar in the oil palm sap [6].

Up to 65% of theoretical bioethanol yield was obtained from 101.80 g/l of total initial sugar. The total sugar increased as fermentation began and this might be caused by the degradation of starch in the OPT sap and the hydrolysis of cellulose and hemicelluloses [5]. Parenchyma of OPT contains extremely high starch content where it can be used by yeast for bioethanol production [9]. The sugar concentration dropped sharply in 12-24h when bioethanol was exponentially generated.

The experiment in 2 liter bioreactor had obtained 48.86 g/l of bioethanol and 0.480 g/g of bioethanol yield. This amount of bioethanol produced is about 93.93% of the theoretical yield. This is higher than the result obtained from our previous study on bioethanol prodution from OPT sap using baker's yeast *Saccharomyces cerevisiae* which only obtained 15.29 g/l of bioethanol [10]. However, result also can be varied due to different strain and different initial concentration of OPT sap used.

Despite the higher final ethanol concentration achieved in 2 L of fermentor, it was observed that fermentation started to produce ethanol 1-2 h late than in 500 ml of shake flask. This might be caused by the late adaptation experience by yeast due to the differences on geometric of fermentor, even though same ratio of inoculum was used. Change of scale resulting changes in physical environment experience by cells causing the metabolic response of the culture differ from one scale to another [11]. This is strongly related with transport process in fermentation medium.

Each hectare of oil palm plantation normally contains 140 oil palm trees with the average height of 9-12 meters each. An oil palm tree can generate 200 L of OPT sap, In other words, about 28000 L of OPT sap are available per hectare of replantation area. From this figure, it was projected that about 1758 L of bioethanol per hectare can be obtained using the fermentation data in 2 liter bioreactor (48.86 g/l of bioethanol). Table 3 shows the comparison of bioethanol production per hectare of oil palm replantation with other raw materials.

TABLE III. COMPARISON OF BIOETHANOL PRODUCTION CAPACITY USING DIFFERENT RAW MATERIAL

Raw Material	Bioethanol (liter/hectare)	Refernences
Forage sorghum (stalk juice)	770	[12]
Sweet sorghum (stalk juice)	924-1051	[12]
Wheat	1075-1730	[13]
OPT sap (stalk juice)	1758	This study
Corn	2011-3700	[13]
Sugar beet	5145	[13]
Sugar cane	6641	[13]
Forage sorghum (stalk juice)	770	[12]

The production of bioethanol from OPT sap was higher than the bioethanol production per hectare of forage and sweet sorghum stalk juice which is 770 L/hectare and 1075-1051 L/hectare, respectively [12]. Sweet sorghum stalk have a high sugar content and is grown primarily for silage and syrup production. Due to this fact, Kim [14] used sweet sorghum juice for bioethanol production as it contains ready sugar (glucose, fructose, sucrose), which make it does not require to pre-hydrolysis before the fermentation and also does not have any use as food.

Average projection of bioethanol production from OPT sap was lower than the bioethanol production from corn (2011 L/hectare), sugar beet (5145 L/hectare) and sugar cane (6641 L/hectare) [13]. However, this can still be improved in future by enhancing extraction process and employing optimization study to increase bioethanol production.

Locally grown oil palm waste is a good choice for a more cost effective production of bioethanol since it is inedible, inexpensive and abundant. These results also depicted that OPT sap is on par with other feedstocks especially with agricultural waste in terms of production per hectare.

Further investigation regarding the cost of bioethanol fermentation from OPT sap as part of an ongoing economic analysis is needed. The discarded outer layer from bioethanol mill can be send to plywood industry and the OPT sap can be used as a substrate for bioethanol production. After squeezing the OPT sap, the middle and inner layer can be dried further and processed as wood based product such as blackboard or softboard.

5. Conclusion

This study showed that it is possible to use OPT sap as a substrate for bioethanol production since it is successfully fermented to bioethanol with less pretreatment compared to other reported biomass. A maximum of 33.82 g/l bioethanol was produced from the fermentation of OPT sap in 500 ml of shake flask while similar fermentation in 2 liter bioreactor obtained up to 48.86 g/l of bioethanol.

From this figure, it was projected that about 1758 L of bioethanol can be produced from one hectare of oil palm replantation. This production showed that the bioethanol production from the OPT sap was competitive with other agricultural waste in terms of production per hectare.

The usage of OPT sap as a carbon sources for bioethanol production is favorable compared to the other biomass since it need no pretreatment to expose ready sugar. It is necessary to find a low cost medium, which contains suitable nutrient which can produce a good amount of bioethanol since the cost of media culture is one of the main factors that determine the economic viability of bioethanol production from agricultural waste. Further research should be done to optimize bioethanol production from OPT sap in a larger scale bioreactor. This study can be continued until downstream process where bioethanol is extracted and purified from OPT sap broth. This is important to estimate economical value of bioethanol production from OPT sap.

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