FERMENTATION OF OIL PALM TRUNK ACID HYDROLYSATE TO ETHANOL

H.H. Yeoh, K.O. Lim and M.D. Mashitah[1]

School of Physics, Universiti Sains Malaysia
[1]School of Chemical Engineering, Universiti Sains Malaysia (Perak Campus)

ABSTRACT

Studies on the feasibility of using oil palm trunk acid hydrolysates as a substrate for ethanol production using the yeast Saccharomyces cerevisiae were carried out in shake flask culture. The effect of pH and fermentation time on the rate of ethanol production were investigated. Results showed that with 3 hours fermentation period, the highest ethanol yield was obtained at pH 4.75. This yield was about 14% of the dry weight of the sample used; thus giving a fermentation efficiency of 94.7%.

1. INTRODUCTION

Due to concerns about the environment as well as the rapid depletion of fossil fuels, studies on alternative renewable sources of energy are being aggressively pursued. Studies on bioethanol, especially those in Brazil and the United States, have indicated that it has the potential to replace gasoline at a competitive price. In addition the use of bioethanol will not contribute towards global warming as there will be practically no net emission of carbon dioxide to the atmosphere[1].

It has been reported that in tropical and sub-tropical regions, the major raw materials used for ethanol production are sugar from sugar cane and its by-product, molasses; while in temperate regions, starch or starchy products from crops such as corn, sorghum, wheat, rye, barley and potatoes are used[2]. Since all these raw materials are food products that will increase in price when demand is high, other cheaper raw materials are preferred. A way out is to utilise lignocellulosic wastes or non-food organic materials as the raw materials for bioethanol production. The lignocellulosic wastes mentioned include the various types of agricultural and forestry residues, waste paper, municipal solid organic wastes and other industry organic wastes. Other than this, bioethanol can also be produced from plants that were planted specifically for bioethanol production[3].

Lignocellulosic biomass can be converted to ethanol directly or indirectly after its conversion to glucose. For the latter pathway, the lignocellulosic material is first converted to glucose either via acid or enzymatic hydrolysis.

It was reported that, among the various Malaysian woods investigated, such as oil palm trunk *(Elaeis guineensis)*, rubber wood *(Hevea brasiliensis)*, acacia *(Acacia mangium)*, batai *(Paraserianthes falcataria)* and yemane *(Gmelina arborea)*, oil palm trunk (OPT) was found to be the most suitable lignocellulosic material for sugar conversion, and thus ethanol production[4]. This is attributed to OPT's low lignin and high cellulose content. It was estimated that in the year 2000, 6.24 million tonnes of dry OPT will be available from replanting processes[5, 6].

In general, the conversion of lignocellulosic material to glucose and then ethanol, is governed by the equation below:

$$\underset{\text{cellulose}}{(C_6H_{10}O_5)_n} + nH_2O \longrightarrow \underset{\text{glucose}}{nC_6H_{12}O_6} \longrightarrow \underset{\text{ethanol}}{2nC_2H_5OH} + 2nCO_2$$

Ethanol production from glucose produced from enzymatic hydrolysis of OPT had been investigated by Putri Faridatul & Puad[2]. This paper reports on a study of the possibility of converting OPT to ethanol via sulphuric acid hydrolysis of the biomass and fermentation of the hydrolysate.

2. METHODS

2.1 Acid hydrolysis of OPT

One whole and freshly cut oil palm trunk (OPT) of about 25 years old was taken from an oil palm estate for the study. The tree was about 11.9 meters in height, with diameters of about 0.69 meter, 0.42 meter and 0.38 meter at the butt, middle and the top sections respectively. The trunk was sectioned into lengths of about 0.6 meter each. Samples were taken from various parts of each section of the trunk, cut into small pieces, dried in Moisture Analyser ASTM D3173 CMA II (model 144300, Boekel Industries Inc.) to achieve constant weight and then cooled to room temperature before they were taken out of the Moisture Analyser. The samples were subsequently ground to about 0.2 mm in size and thoroughly mixed.

0.50g of the dried and mixed OPT sample was put into a 250 ml conical flask and pretreated with a solution of 75% sulphuric acid at 50°C for 1 h. The ratio of acid to sample weight used was 15:1 (w/w) as this ratio was found to work well on fibres of the oil palm fruit, bagasse and rice husks[7]. Distilled water was then added to the pretreated material to reduce the concentration of the acid to 1.7%, as our previous study showed that the highest glucose yield was obtained at an acid concentration level of about 1.7%[8]. The mouth of the flask was then covered with an aluminium foil and autoclaved at 121°C for 30 minutes.

After hydrolysis, the sample was allowed to cool and later filtered using a piece of Whatman filter paper No.1. The filtered solution was then neutralised with a solution of 2.5M sodium hydroxide. The amount of glucose present in the neutralised solution was determined enzymatically (glucose enzymatique PAP 1200, Bio Merieux,

France). A spectrophotometer (Shimadzu UV-1201) calibrated at 505nm was used. Standard procedures were followed. The glucose yield was expressed as weight of glucose produced over original dry weight of sample used.

2.2 Fermentation of the OPT hydrolysate

0.0525g of the yeast *Saccharomyces cerevisiae* (0.01% (w/w) of hydrolysate) was added to a sterilised culture medium prepared from ingredients shown in Table 1. The yeast was cultured in an incubator shaker (Orbital Shaker Incubator, YIH DER, LM-510, Taiwan) at 32°C, 200rpm for 24 hours.

Table 1: Composition of culture medium.

Ingredient	Quantity (g)	Distilled water added (ml)
D-glucose	0.75	37.5
Peptone	0.375	15
Yeast extract	0.225	11.25

While waiting for the yeast to be cultured, the OPT hydrolysate as prepared in section (i) above was prepared for the fermentation process, by following the steps mentioned below. The pH of the hydrolysate was adjusted to the required value using a calcium hydroxide slurry (prepared by mixing one part of calcium hydroxide powder with two parts of water). Any gypsum formed from the neutralisation reaction was then filtered from the pH-adjusted hydrolysate using Whatman filter paper No.1. A volume of 525ml of the pH-adjusted hydrolysate was then poured into an Erlenmeyer flask and autoclaved at 121°C for 30 minutes.

Meanwhile, the nutrient medium was also prepared and autoclaved. The composition of the nutrient medium is as shown in Table 2.

Table 2 : Composition of nutrient medium

Ingredient	Quantity (g)	Distilled water added (ml)
Yeast extract	0.9	50
$(NH_4)_2SO_4$	1.5	
K_2HPO_4	3.3	50
$MgSO_4.7H_2O$	0.15	

After sterilisation the nutrient medium and the hydrolysate were allowed to cool to room temperature in a sterile environment under a laminar flow (Laminar Flow — Gelman Class 100 Series, Gelman — Singapore). When cooled, the nutrient medium and the OPT hydrolysate were then mixed with the cultured yeast. Nitrogen gas was sparged into the mixture at 4 psi for 15 minutes in order to ensure an anaerobic environment for the fermentation process to take place (Figure 1). Immediately after the mixing, about 10ml of the sample was withdrawn for analysis which included changes in pH, glucose content and ethanol yield. The procedure was repeated at every hour interval for 15 hours. The glucose content was tested enzymaticaly with a spectrophotometer (Shimadzu UV-1201) as mentioned in section (i), while the amount of ethanol produced was tested by using a calibrated gas chromatograph (model HP 5890 Series II, Hewlett Packard, USA).

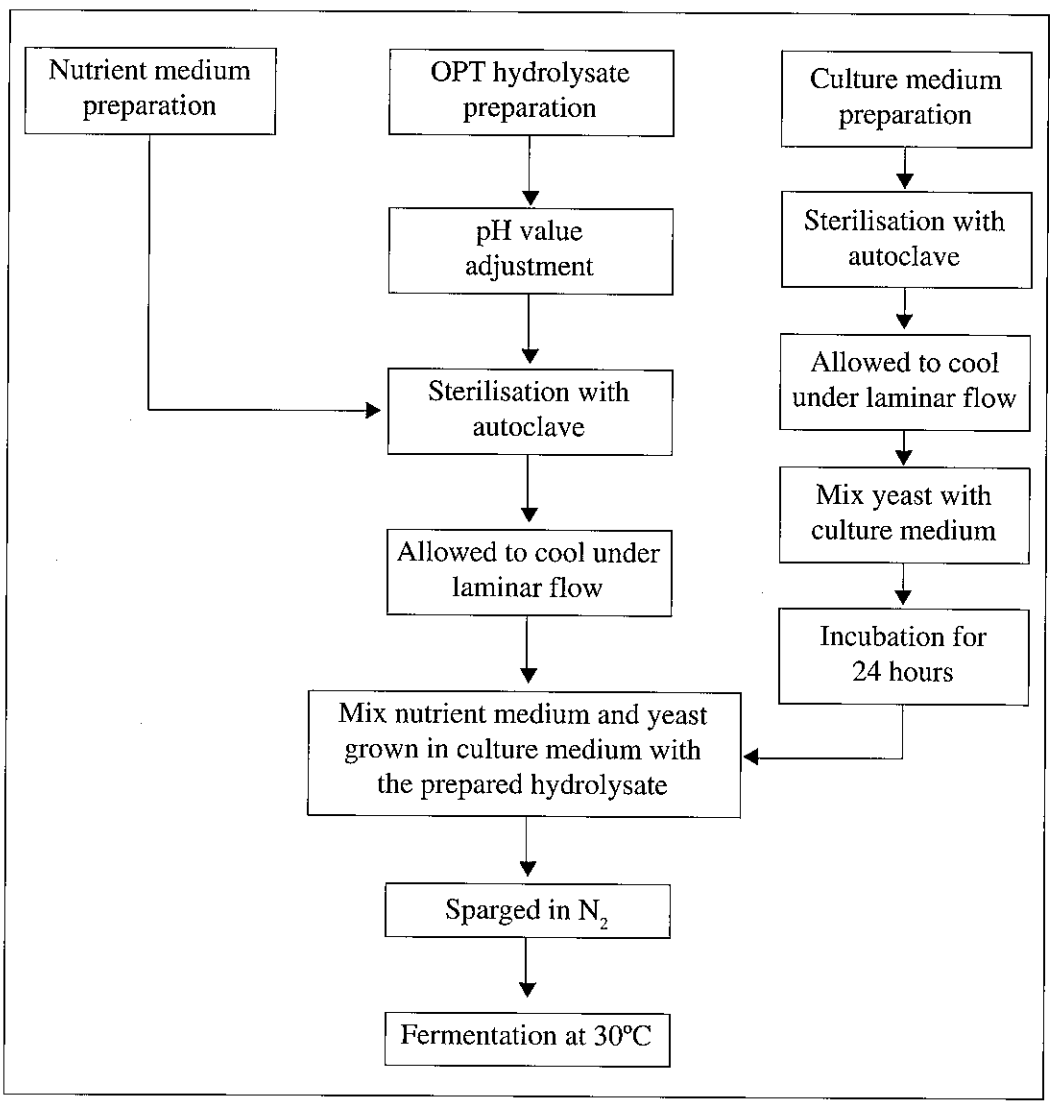

Figure 1: Flowchart of the preparation of OPT acid hydrolysate for fermentation.

3. RESULTS AND DISCUSSION

Although the procedure for acid hydrolysis differed from the reflux process (conducted at atmospheric pressure) reported previously[8], where 4 hours were needed instead of the 30 minutes required here, the glucose yield obtained from both procedures were comparable. The former yield was about 31%, while the latter produced a glucose yield of about 29 to 31%.

The results of ethanol fermentation studies using acid hydrolysate of OPT as a substrate and *Saccharomyces cerevisiae* are shown in Figures 2–4. As glucose was also added to the culture medium, the ethanol will also be produced from this added glucose source rather than the glucose content in the OPT hydrolysate alone. Thus, a control set of experiments was also conducted in which the OPT hydrolysate is replaced with distilled water for each of the OPT experimental run.

Figures 2(a), 3(a) and 4(a) show the results of the experimental runs before correction using results of the control sets. The ethanol yields from these runs were 21.2%, 22.5% and 20% of the sample dry weight, respectively. These values are too high compared with the theoretical value, whereby the maximum ethanol yield should be about 51% of the glucose content in the hydrolysate. As the glucose content in the hydrolysate was only about 28 – 30%, the maximum ethanol yield expected should be about 14 – 15%. The results of the control sets shown in Figures 2(b), 3(b) and 4(b) gave ethanol yields of approximately 7.2%, 8.5% and 6%, respectively. After subtracting the ethanol yields of the control sets from the yields of the experimental runs, the true ethanol yield from OPT hydrolysates are shown in Figures 2(c), 3(c) and 4(c) respectively. An average value of about 14% was found. This value falls within the expected theoretical range of the ethanol yield.

3.1 Effect of pH value on ethanol yield and fermentation rate

Referring to Figures 2(c), 3(c) and 4(c), the highest ethanol yield found at pH 2.54, 4.75 and 6.73 was about 14% of the OPT dry weight. The figures also show that ethanol yield seems not to depend on pH. According to Buzas *et al*[9], the optimum pH value for *S.cerevisiae* was between 4.2 – 4.8. However, Putri Faridatul & Puad[2] reported that there was no difference between the fermentation process conducted at pH 5.4 and 4.75. Wilkinson & Rose[10] agreed that there was no significant difference between fermentation yields conducted in the pH range between 2.4 –8.6. Thus, the results at the present study concur with most of the reported work.

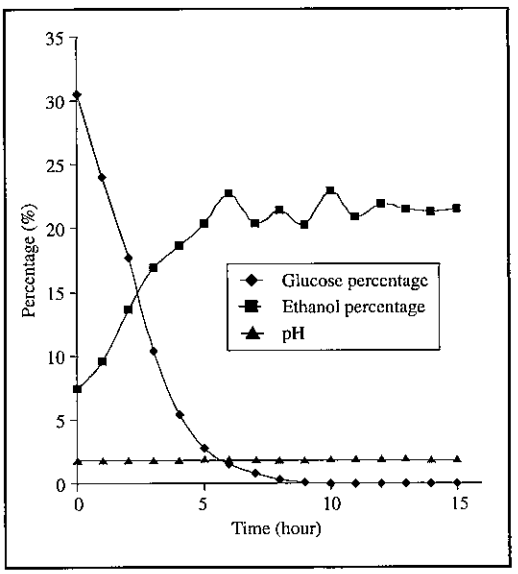

Figure 2(a): Changes of glucose and ethanol concentrations as well as pH as a function of fermentation time at initial pH = 1.88 (pH value shown is an absolute value not a %) [before correction with control values].

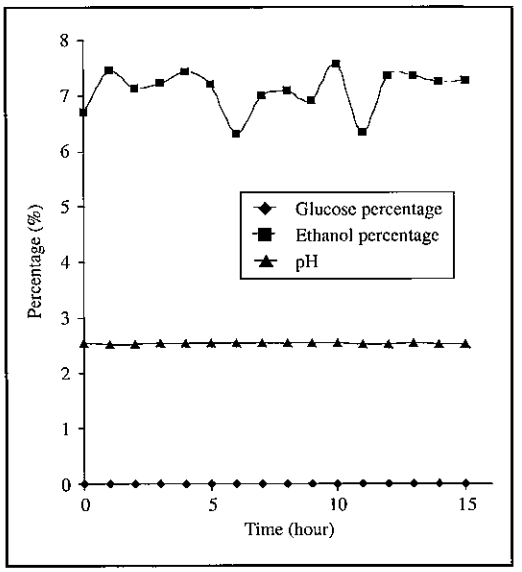

Figure 2(b): Changes of glucose and ethanol concentrations of controls as well as pH as a function of fermentation time at initial pH = 1.88 (pH value shown is an absolute value not a %).

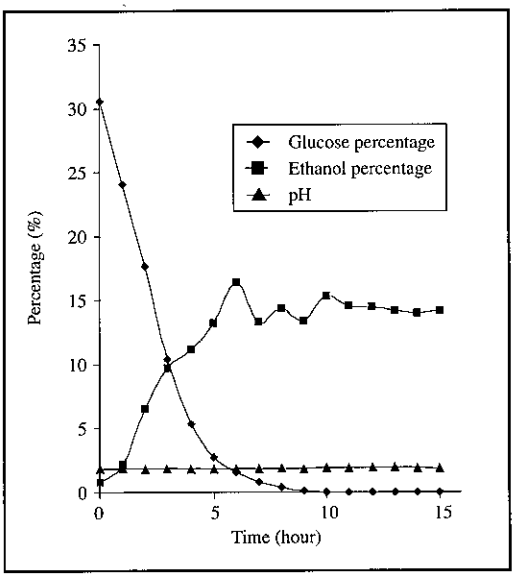

Figure 2(c): Changes of glucose and ethanol concentrations as well as pH as a function of fermentation time at initial pH = 1.88 (pH value shown is an absolute value not a %) [after correction with control values].

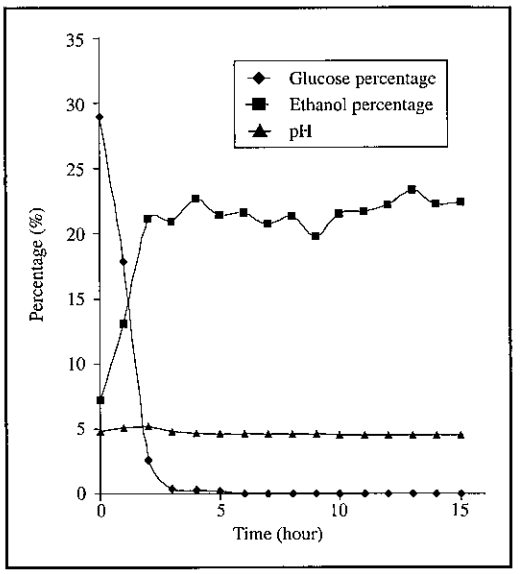

Figure 3(a): Changes of glucose and ethanol concentrations as well as pH as a function of fermentation time at initial pH = 4.75 (pH value shown is an absolute value not a %) [before correction with control values].

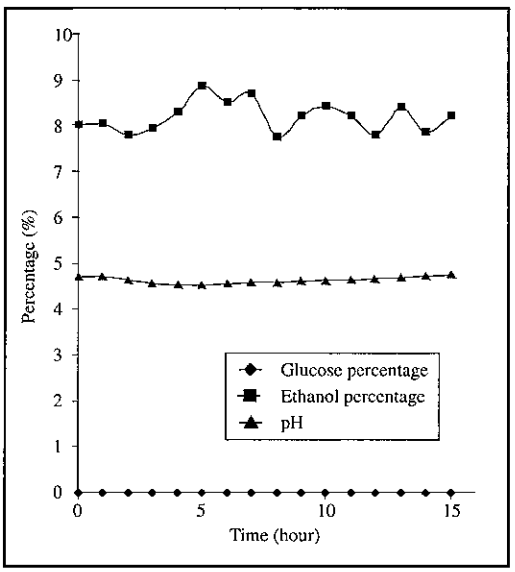

Figure 3(b): Changes of glucose and ethanol concentrations of controls as well as pH as a function of fermentation time at initial pH = 4.75 (pH value shown is an absolute value not a %).

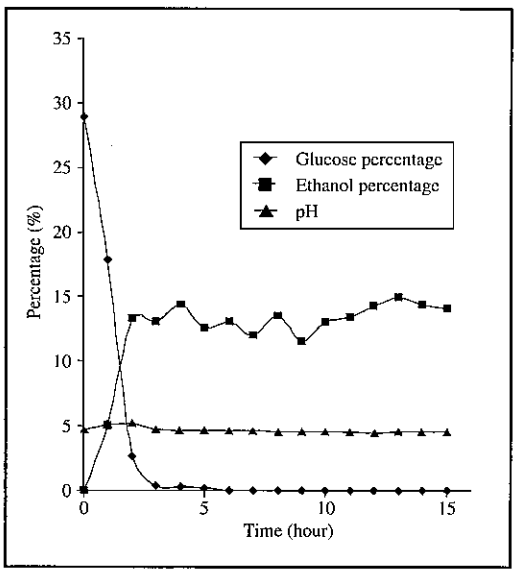

Figure 3(c): Changes of glucose and ethanol concentrations as well as pH as a function of fermentation time at initial pH = 4.75 (pH value shown is an absolute value not a %) [after correction with control values].

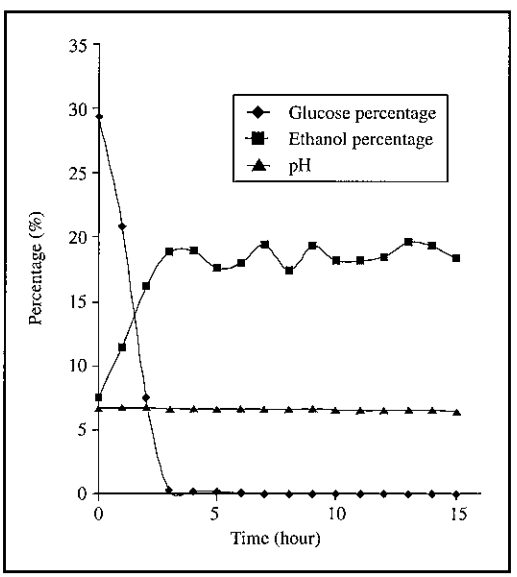

Figure 4(a): Changes of glucose and ethanol concentrations as well as pH as a function of fermentation time at initial pH = 6.73 (pH value shown is an absolute value not a %) [before correction with control values].

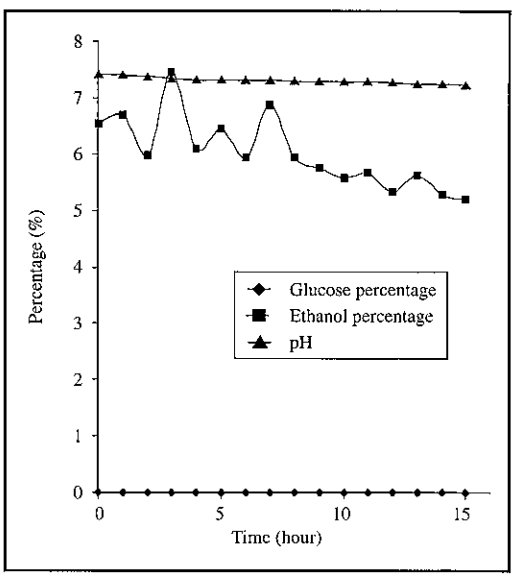

Figure 4(b): Changes of glucose and ethanol concentrations of controls as well as pH as a function of fermentation time at initial pH = 6.73 (pH value shown is an absolute value not a %).

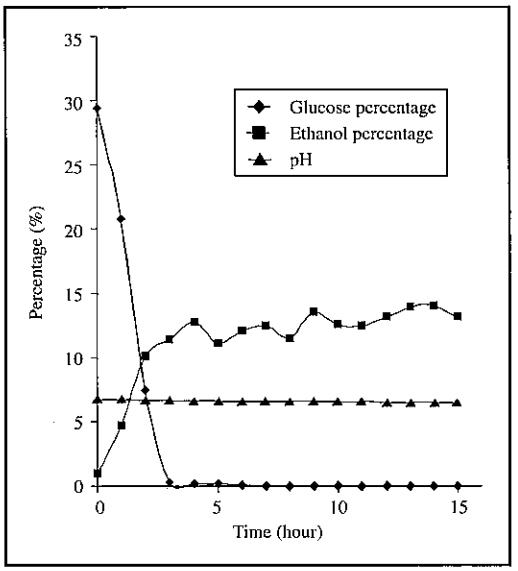

Figure 4(c): Changes of glucose and ethanol concentrations as well as pH as a function of fermentation time at initial pH = 6.73 (pH value shown is an absolute value not a %) [after correction with control values].

When the time taken for attaining maximum ethanol yield is considered, it was found that the fermentation process at a pH 2.54 took about 9 hours to convert all the glucose to ethanol. However, when the pH rose to 4.75 and 6.73, the time taken for ethanol production was reduced to 3 hours. Thus, among the 3 pH values studied, the fermentation rate at pH 2.54 was the slowest. Although the fermentation rates at pH 4.75 and 6.73 were not significantly different, it was found that the ethanol produced at the second hour of fermentation time at pH 4.75 was a little higher than that at pH 6.73. The results reported by Wilkinson & Rose[10], showed that the fermentation time required to reach highest ethanol yield at pH 3.0, 4.0, 5.0, 6.0 and 7.0 was 29 hours, 14.5 hours, 17.5 hours, 15.5 hours and 35 hours respectively. This is in agreement with the results obtained in the present study.

3.2 Fermentation efficiency

The efficiency of the fermentation process can be calculated as follows[11]:

$$\% \text{ fermentation efficiency} = \frac{\text{Actual alcohol produced}}{\text{Alcohol that could be produced theoretically}} \text{ X } 100$$

Theoretically, 1 gram of glucose will produce about 0.51 gram of ethanol. Since the initial glucose content in the hydrolysate was known, the theoretical ethanol yield can thus be calculated. The fermentation efficiencies for the fermentation process at different pH values are shown in Table 3.

Table 3: Fermentation efficiency at different pH values.

pH	2.54	4.75	6.73
Initial % glucose content	30.50	28.99	29.39
Theoretical % ethanol yield	15.56	14.78	14.99
Experimental % ethanol yield	14	14	14
Fermentation efficiency	90.0	94.7	93.4

From Table 3, it is noted that the efficiency of the fermentation process was highest at pH 4.75. This was followed by values at pH 6.73 and then at pH 2.54. However, the difference in fermentation efficiency among these experimental sets was not pronounced, especially at pH values of 4.75 and 6.73.

The efficiency values found in this study agreed with that reported by Kosaric *et al*[12] who stated that the fermentation process will normally produce yields of less than 90 – 95% of theoretical. This drop in yield was attributed to nutrient requirements that are related to the formation of new yeast cells as well as cell maintenance. Besides this, other side reactions occurring during the fermentation process may also use up 4–5% of the substrate.

The high fermentation efficiencies of OPT hydrolysate may be due to the nutrient sources contained in the OPT hydrolysate itself. Other than the nutrients added, the nutrient found in the OPT hydrolysate are as shown in Table 4.

Table 4: Nutrient contents in OPT hydrolysate before neutralisation.

Mn	**Ca**	**Ni**	**Fe**	**Mg**	**Zn**	**K**	**Na**
0.05	4.5	0.12	0.75	3.4	0.6	18	3

Note:
All values are in parts / million (ppm).
K and Na were determined by a flame photometer, while the other elements were determined using an atomic absorption spectrometer.

4. CONCLUSION

Although acid hydrolysates of OPT were reported to contain a lot of impurities that might cause negative effects on fermentation yield, this study had shown that OPT acid hydrolysates can be fermented to ethanol satisfactorily at pH 4.75 using *Saccharomyces cerevisiae*. A high fermentation efficiency of 94.7% was obtained and an ethanol yield of about 14% based on the dry weight of OPT was found after 3 hours of fermentation time.

5. ACKNOWLEDGEMENT

This work was supported by an IRPA grant (No. 305/pfizik/610502) from the Ministry of Science, Technology and Environment, Government of Malaysia.

6. REFERENCES

1. Tyson, K.S., Riley, C.J., & Humpreys, K.K. 1993. Fuel cycle evaluations of biomass-ethanol and reformulated gasoline. NREL/TP-463-4950, Golden Co., National Renewable Energy Laboratory.
2. Putri Faridatul Akmar & Puad Elham. 1991. Ethanol fermentation from oil palm trunk hydrolysate. In *Oil Palm Trunk and Other Palmwood Utilization. Proc. of a National Seminar.* Kuala Lumpur, Malaysia, 4-5 March, pp. 321-27.
3. Lynd, L.R., Cushman, J.H., Nichols, R.J., & Wyman, C.E. 1991. Fuel ethanol from cellulosic biomass. *Science*, 251, 13-18.
4. Tomimura, Y., Khoo, K.C. & Putri Faridatul Akmar. 1989. Enzymatic hydrolysis of some Malaysian woods. *Journal of Tropical Forest Science,* 1(3), 255-262.
5. Lim, K.O. 1986. The energy potential and current utilisation of agriculture and logging wastes in Malaysia. *Renewable Energy Review Journal,* 8,57-75.
6. Lim, K.O.1986. The future energy potential of replanting wastes in Malaysia. *Renewable Energy Review Journal,* 8(2), 76-85.
7. Liew, S.S. 1989. Hidrolisis asid bagas dan 'palm press fibre' kepada gula yang boleh difermentasikan. Final Year Dissertation, Food Technology, School of Industrial Technology, Universiti Sains Malaysia.
8. Lim, K.O.; Faridah Hanun Ahmaddin & S.Malar Vizhi. 1997. A note on the conversion of oil-palm trunks to glucose via acid hydrolysis. *Bioresource Technology,* 59, 33-35.
9. Buzas, Z.S., K. Dallmann & B. Szajani. 1989. Influence of pH on the growth and ethanol production of free and immobilised S.cerevisiae cells. *Biotechnology Bioengineering,* 34, 882-884.
10. Wilkinson J.F. & Rose., A.H. Fermentation processes. 1963. *In Biochemistry of Industrial Microorganisms,* ed. C. Rainbow & A.H. Rose. Academic Press London and New York, pp. 379-414.
11. Stark, W.H. 1954. Alcoholic fermentation of grain. In *Industrial Fermentations VI,* ed. A.U. Leland & J.H. Richard. Chemical Publishing Co., Inc., New York, pp.17-72.
12. Kosaric Naim, Andrzej Wieczorek, Gregory P. Cosentino, Robert J.Magee & Jiri E.Prenosil. 1983. Ethanol fermentation in biotechnology. In *Biomass, microorganisms for special applications, microbial products I, energy from renewable resources,* 3, ed. H.J. Rehm and G. Reed. Verlag Chemie Gmbh, D-6940 Weinheim.