Optimisation of Bioethanol Production from Oil Palm Trunk Sap

Jolius Gimbun[1*], *Nor Shahirah* Mohd Nasir[2], *Sumaiya* Zainal Abidin[1], *Chin Kui* Cheng[3] and *Maizirwan* Mel[4]

[1]Centre for Research in Advanced Fluid and Processes, Universiti Malaysia Pahang, Gambang 26300, Malaysia
[2]Malaysian Institute of Chemical and Bioengineering Technology, Universiti Kuala Lumpur, Alor Gajah, Malaysia
[3]Department of Chemical Engineering, Khalifa University, Abu Dhabi, United Arab Emirates
[4]Department of Chemical Engineering and Sustainability, International Islamic University Malaysia, Kuala Lumpur 53100, Malaysia

Abstract. This paper presents an optimization of bioethanol production from oil palm trunk sap (OPTS) fermentation. The OPTS was obtained from an old palm tree (30 years old), whereas ethanol fermentation was carried out using *Saccharomyces cerevisiae*. The sugar content in OPTS and fermentation mother liquor was determined using high-performance liquid chromatography (HPLC). The parameters such as initial pH, temperature, and agitation rate were optimised using response surface methodology (RSM) with rotatable central composite design (CCD). It was found that the highest yield of bioethanol (75.82%) was obtained at the initial pH (5.79), temperature (31.05 ºC), and agitation rate (164.38 rpm). The optimization model of OPTS fermentation to bioethanol developed in this work may provide useful guidance to obtain a high ethanol yield from OPTS.

1 Introduction

Malaysia and Indonesia are the two largest producers of palm oil, with a combined output that accounts for approximately 85% of global palm oil producers [1, 2]. At an interval of approximately 20–25 years, oil palm trees must be replanted due to the decrease in oil productivity of old trees and difficulty in harvesting their fruit [3, 4]. This means that about 64 to 80 million old palm trees will be felled annually in Malaysia, which can generate 15.2 million tonnes of oil palm trunks [3].

The hard outer layer of oil palm trunk can be used for plywood manufacturing [5, 6]. However, the softer inner part of oil palm trunk is often discarded. These trunks are rich in sugar and may be used for the production of bioethanol [1, 6-9]. In order for the palm oil industry to be sustainable [3], the felled oil palm trunk waste must be utilised. Moreover, the felled old palm trunk may become a breeding ground of oil palm eating pest like weevil if it left rotten on the plantation site. The weevil may infect the non-felled palm tree leading to

* Corresponding author: jolius@ump.edu.my

the tree death [10]. Thus, it is vital that the felled oil palm tree is removed from the plantation site before the re-planting can commence.

Previous study on bioethanol fermentation of oil palm trunk sap was performed by several researchers [1, 7-8, 11]. They reported extensive content of sugar, mainly glucose, sucrose and fructose from the sap. The aforementioned studies explore the effect of nutrient addition as well as the different yeast strains on the bioethanol yield from oil palm trunk sap. Bukhari and Loh [11] also studied the effect of single parameter such as temperature, pH and nitrogen sources on the bioethanol yield. However, limited study on the optimization of bioethanol production from oil palm trunk sap, hence this is the aim of this work.

2 Methodology

2.1 Chemicals and materials

A 30-year-old oil palm trunk (OPT) was obtained from FELDA Jengka, Pahang, Malaysia. Only the inner core was used in this work, which was chopped into smaller pieces of about 20 cm × 20 cm × 1 cm. The OPT sap was obtained using a heavy-duty sugar cane pressing machine. The sap was filtered to remove impurities before being used for fermentation.

The sulphuric acid (H_2SO_4) was purchased from Merck (Darmstadt, Germany). Yeast extracts, peptone, magnesium sulfate (MgSO4), and standard sugars for HPLC analysis such as glucose, sucrose, and fructose were procured from Fisher Scientific (Leicestershire, UK), while the galactose standard sugar and L-alanine ($C_3H_7NO_2$) were procured from Acros (New Jersey, USA). *Saccharomyces cerevisiae* (yeast) was purchased from AB MAURI (Balakong, Malaysia).

2.2 Pure culture and inoculum preparation

Saccharomyces cerevisiae culture was generated by dissolving yeast powder in nutrient broth containing 10 g yeast extract, 20 g peptone, and 2 g glucose per 900 mL and incubating for 24 hrs at 100 rpm and 30 °C. Yeast suspension was streaked over a nutrient agar plate containing 20 g agar, 20 g peptone, 10 g yeast extract, and 2 g glucose and incubated for 2-3 days at 30 °C. A loopful of cells from one colony was added to 100 mL of sterile nutritional broth in a 250 mL shake flask. At 30 °C and 150 rpm, the strain was incubated for 18 hours. After 14 hours, the yeast growth curve reached a stationary phase, which was used as a standard starting concentration. Using a calibrated UV-Vis spectrophotometer U-1800 (Hitachi, Japan) at 600 nm, the cell concentration was standardised to 0.2-0.4 g/L (OD = 1.5-2.0).

2.3 OPTS fermentation

The *S. cerevisiae* suspension was centrifuged before being transferred into a 250 ml conical flask containing 100 ml of sterilised OPTS, $MgSO_4$ and $C_3H_7NO_2$. The supernate was discarded and the precipitate alone (i.e., the yeast) was transferred to the fermentation media. The optimum fermentation conditions for maximum bioethanol production were determined using the response surface methodology (RSM). A five-level-three-factor central composite design (CCD) was used to study the influence of the most significant process variables, namely the initial pH, temperature, and agitation rate, following the work by Liu and Shen [12]. The CCD and RSM were performed using Design Expert Software Version 7.1.6. A design with 19 runs was set based on computer generated process variables, including 5 replicate central points and $\alpha = 2$. The variables and the selected levels for the fermentation

process were: pH (4–7); temperature (25–40 ºC); agitation (110–250 rpm) as shown in Table 1. Each run was repeated in triplicate.

2.4 Sample analysis

The analysis of sugar content and ethanol concentration from the sample was performed using an Agilent 1200 HPLC equipped with a Rezex ROA column (150 x 7.80 mm) and a refractive index detector. The chromatography grade sulphuric acid (0.005 N) was used as the mobile phase and the flow rate was set at 0.5 ml/min. The column temperature was set at 60 °C and the RI detector temperature at 40 °C. The injection volume was 10 µL. The peaks for standard sucrose (4.8-5.4 min), galactose (5.3-5.6 min), glucose (5.6-6.3 min), fructose (6.3-6.8 min) and ethanol (12.2-13.2 min) were found in the chromatogram. The concentration of sugar and ethanol was calculated using an eight-point calibration curve.

Table 1. Variation of level for variables.

Independent variable	**Symbol**	**Variation levels**				
		-α	-1	0	+1	+α
Initial pH	A	2.5	4	5.5	7	8.5
Temperature (°C)	B	17.5	25	32.5	40	47.5
Agitation rate (rpm)	C	40	110	180	250	320

3 Results and discussion

The relationship between independent variables and response is fitted to a quadratic model. A statistically significant quadratic model was obtained between the variables and the response. The analysis indicated that the p-value and F-value of the quadratic model were 0.0017 and 8.75, respectively, which confirmed that the model is highly significant with low probability. As can be seen, the predicted values were well matched with experimental values with an R^2 value of 0.8975 and 89.75% of the response was well described by the model. In the lack of fit test, the fit value of 0.1043 indicates that the lack of fit is not significant and also shows that the model is considerably fit.

$$Ethanol\ yield\ (\%) = 77.06 + 2.08A - 1.94B - 2.01C - 0.47AB + 3.11AC - 3.39BC - 3.84A^2 - 3.28B^2 - 1.70C^2 \quad (1)$$

Initial pH (A) was the most significant factor that largely affected the bioethanol yield, followed by agitation rate (C) and temperature (B). The two-level interactions between initial pH and agitation rate (AC) and between temperature and agitation rate (BC) showed a significant effect on ethanol yield. In a similar manner, all the second-order effects showed significant results, including A2, B2, and C2. The two-level interaction between initial pH and temperature (AB) was the only model with a non-significant term that had a p-value greater than 0.05. Consequently, the ranking of the models can be evaluated based on the significance of the factors, which resulted as follows: A2>B2>BC>AC>A>C2>C>B>AB. The quadratic model in terms of coded variables and actual variables is shown in Equations (1), with initial pH (A), temperature (B), and agitation rate (C).

The difference between the actual response and the predicted response is called the residual. The normal probability plot of the residuals is shown in Figure 1(a). Figure 1(b) shows the interaction between factors A and B while holding factor C constant at 180 rpm towards bioethanol yield. The interaction between the initial pH and temperature (AB) shows

that bioethanol yield increases when the temperature and pH are increased from 25 to 40 °C and 4.0 to 7.0, respectively. The response showed a steady increase until it reached the maximum yield of 75.81% at pH 5.5 and temperature at 25 °C. The increasing pH value at a low temperature (25 °C) showed a higher bioethanol yield, whereas at a higher temperature (40 °C), bioethanol yield was lower. However, the slope showed that the increasing pH after 5.5 causes a slight decrease in yield.

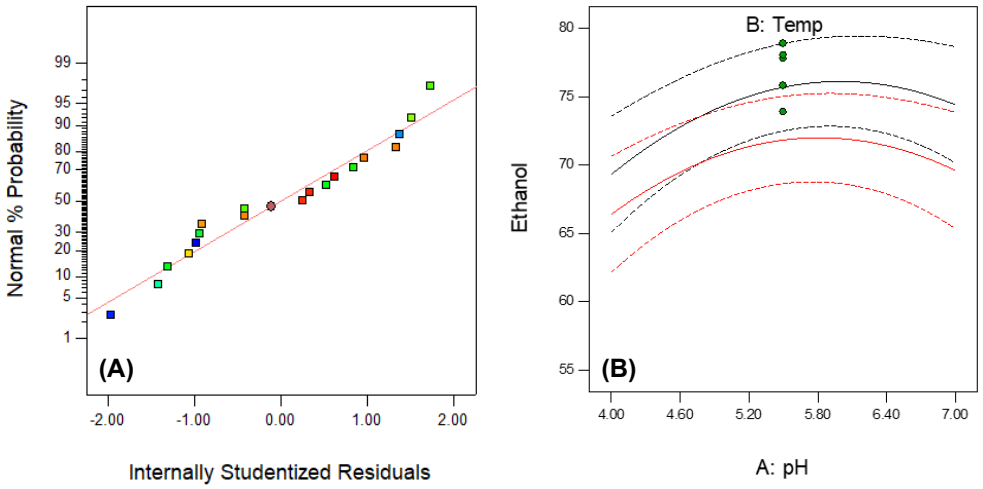

Fig. 1. A) Normal probability plot of the residuals, B) Effect of pH and agitation on ethanol yield

Yeast growth is dependent on cell maintenance energy, which makes the fermentation temperature play an important role in cell performance, and it in turn improves the product yield. Therefore, the change in temperature from low to high made a huge difference in bioethanol yield. The yield coefficient decreased when using temperatures outside the optimum temperature value because this process can increase the requirements for cell maintenance [13].

The interaction between initial pH and agitation rate (AC) while holding temperature (B) constant at 32.5 °C toward bioethanol yield is shown in Figure2(a). The interaction between initial pH and agitation rate (AC) demonstrated that bioethanol yield increased when agitation rate and pH changed from 110 to 250 rpm and 4.0 to 7.0, respectively. The cross section between the slope of pH and the agitation rate confirmed that the interaction model is significant. The response yield showed a linear increase with the increment of pH value at a higher agitation rate (250 rpm), whereas a decreasing slope pattern was shown at a lower agitation rate (110 rpm). The medium with a different pH can cause a change in the enzyme activity as well as in the reaction rate. pH is known to significantly influence bioethanol fermentation by *Saccharomyces cerevisiae* by affecting all cellular processes such as yeast growth, byproduct formation, and fermentation rate. This effect is due to the presence of H^+ ions in the liquid environment [14-15].

Figure 2(b) illustrates the interaction between factors B and C while holding factor A at pH 5.5 on the response of bioethanol yield. Referring to the interaction factor of temperature and agitation (BC), the response yield showed a linear decrease with the increment of temperature from 25 to 40°C at a higher agitation rate (250 rpm), whereas an increasing slope pattern was shown at a lower agitation rate (110 rpm). The interaction model is significant because there is a cross section between the slope of the temperature and the rate of agitation.

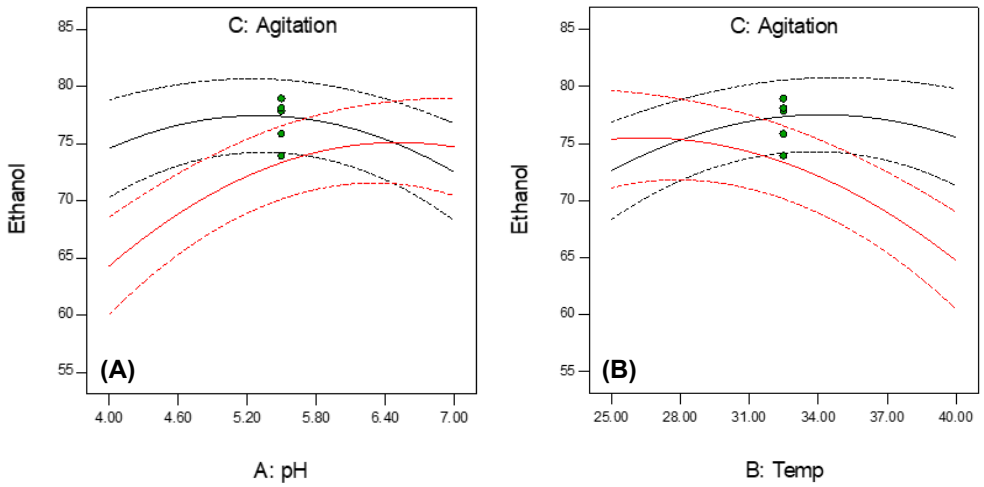

Fig. 2. A) Effect of pH and agitation on ethanol yield, B) Effect of temperature and agitation on ethanol yield

The bioethanol yield (78.89%) at a higher agitation rate (180 rpm) was higher compared to the yield (66.32%) at a lower agitation rate (110 rpm). Agitation rate is beneficial to the growth and performance of yeast by providing adequate mixing, which improves the mass transfer and heat transfer characteristics for the substrates, products, by products, and oxygen in the bioreactor.

All the process variables, including initial pH, temperature, and agitation rate, were set up within the range to attain the optimum bioethanol yield. In addition, the bioethanol yield was set to its maximum level. There are four solutions shown as the optimum points for OPTS fermentation. However, the first solution was chosen due to its high desirability value of 0.95, which is closer to the maximum desirability value of 1.0. The model shows that the highest bioethanol yield of about 77.67% can be achieved at pH, temperature, and agitation rate of about 5.79, 31.05 °C, and 164.38 rpm, respectively.

4 Results and discussion

A response surface methodology was employed to determine the optimum conditions for bioethanol yield from the fermentation of OPTS. The optimum conditions for OPTS fermentation using *Saccharomyces cerevisiae* were achieved at 31.05 °C, 164.38 rpm and pH 5.79, which yielded a bioethanol content of about 77.67%. The bioethanol yield is mostly affected by the pH and temperature in the fermentation vessel. Meanwhile, the effect of agitation is least significant. The data obtained from experiment shows a good fit with a second order model with R^2 value of 0.8975. The model developed in this work may be used to optimise the bioethanol yield from OPTS fermentation owing to the good fit between the model (eq. 1) and the experimental data.

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