

PAPER • OPEN ACCESS

## Optimization of process parameters for bioethanol production from oil palm frond juice by *Saccharomyces cerevisiae* using response surface methodology as a tool

To cite this article: S H M Zani *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **702** 012003

View the [article online](#) for updates and enhancements.

# Optimization of process parameters for bioethanol production from oil palm frond juice by *Saccharomyces cerevisiae* using response surface methodology as a tool

S H M Zani<sup>1</sup>, F M Asri<sup>1</sup>, N S Azmi<sup>2</sup>, H W Yussof<sup>1</sup> and M A K M Zahari<sup>1,3\*</sup>

<sup>1</sup>Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak Gambang, 26300 Kuantan, Pahang, Malaysia

<sup>2</sup>Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak Gambang, 26300 Kuantan, Pahang, Malaysia

<sup>3</sup>Bio Aromatic Research Centre of Excellence, Universiti Malaysia Pahang, Lebuhraya Tun Razak Gambang, 26300 Kuantan, Pahang, Malaysia

\*E-mail: [ahmadkhushairi@ump.edu.my](mailto:ahmadkhushairi@ump.edu.my)

**Abstract.** The aim of this research was to investigate the effect of temperature, medium initial pH and rotation rate on the production of bioethanol from OPF juice without nutrient and nitrogen source supplementation using *Saccharomyces cerevisiae* Kyokai No. 7 (ATCC 26622). A five-level-three-factor central composite design (CCD) was employed in this study and the central point of each process variable was chosen based on the best condition obtained from the one-factor-at-time (OFAT) method. The parameters ranges were set as follows; medium initial pH (5-9), temperature (27.5-37.5°C) and rotation rate (80-120 rpm). Bioethanol and residual sugars concentration were determined using High Performance Liquid Chromatography (HPLC). The optimum conditions for bioethanol production from OPF juice were achieved at medium initial pH (6.62), rotation rate (96.51 rpm) and temperature (33.03°C). Based on the validation experiment, the optimum bioethanol yield was  $0.50 \pm 0.02$  g/g sugars and this value was in close agreement with the model prediction where the difference was only 4.17%. Under the optimal conditions, the bioethanol yield obtained was 47.06% higher compared with non-optimized condition. The promising yield obtained in this study suggests that OPF juice can be used as a renewable and complete fermentation feedstock for bioethanol production.

## 1. Introduction

In Malaysia, recent developments in palm oil industry have heightened the need for production of value-added product and sustainability environment. Zwart [1] reported that there was a dramatic increase in the production of dry weight oil palm biomass in the oil palm plantation. In 2011, the generation of oil palm biomass such as trunks, fronds, and empty fruit bunches and other biomass fractions were estimated at 80 million tons [1, 31]. These resources however, present an attractive raw material for the newly emerging and potentially profitable renewable energy and bio-based chemicals market [2]. Recently, researchers have shown an increased interest in the use of oil palm trunks sap [3-5] and lignocellulosic biomass [6-8, 31-32] as an alternative to edible food sources such as corn, sugarcane and sugar beets [8-9] for the production of bioethanol. This was due to the fact that bioethanol production from corn, sugarcane and sugar beets has posed a threat to the food supply, and the cost of these raw



Content from this work may be used under the terms of the [Creative Commons Attribution 3.0 licence](https://creativecommons.org/licenses/by/3.0/). Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

materials accounts for up to 40% to 70% of the production cost [8]. In addition, tough competition between edible food sources for human consumption and production of bioethanol raise attention for researcher to find an alternative feedstock [2].

Oil palm frond (OPF) is a by-product of the cultivation of oil palm trees is a promising raw material as OPF is the most generated biomass from the oil palm plantation [31]. Previously, it was reported that OPF juice contained renewable sugars such as glucose, sucrose and fructose, which have great potentials as renewable carbon source for the production of poly(3-hydroxybutyrate), P(3HB) [10]. Further evaluation on the potential of OPF juice as fermentation feedstock was tested for bioethanol production by *Saccharomyces cerevisiae* (Baker's yeast) [2]. It was reported that the bioethanol yield obtained from OPF juice without nitrogen source supplementation was slightly lower compared with the fermentation supplemented with nitrogen source under non-optimized condition [2]. Even though, higher bioethanol yield could be obtained by nitrogen source supplementation, it was not recommended at industrial scale production since the addition of nitrogen source and other nutrient in fermentation medium could contribute to high production cost. In order to make the production of bioethanol feasible for industrial application, it is crucial to have high bioethanol production yield without any nutrient and nitrogen source supplementation. One of the alternatives is by optimizing the physical parameters including medium initial pH, temperature and rotation rate which could affect the bioethanol production. It was reported that the optimization study of the fermentation parameters; temperature, rotation rate and pH will contribute to high fermentation efficiency [6, 11-13, 22, 30]. Raikar [22] proposed, pH value has significant influence on alcoholic fermentation. pH of the broth influence bioethanol production in terms of bacterial contamination, yeast growth, fermentation rate and by-product formation [30]. In terms of temperature, lowering the temperature resulted in decrease of ethanol yield meanwhile increase of extremely high temperature resulted in decrease of ethanol yield and enzymes to be easily denatured [6, 30]. Rodmui et al. [11] proposed, agitation is important in fermentation process to enhance cell mass and ethanol productivity. In summary, it had been reported that most of fermentation process employed *S. cerevisiae* for bioethanol production were carried out at temperature between 30°C to 37°C, whereas pH in the range of 4.25 to 6.0 and fermentation process were performed with agitation speed in the range of 50 to 200 rpm [6, 11-13, 22, 30].

In this study, bioethanol production from OPF juice by *Saccharomyces cerevisiae* Kyokai No. 7 (ATCC 26622) without nutrient and nitrogen source supplementation was optimized *via* response surface methodology (RSM) with central composite design (CCD). Prior to that, one-factor-at-time (OFAT) method was employed to screen the best condition of parameters affecting the production of bioethanol. The effects of physical parameters including medium initial pH, temperature and rotation rate were evaluated to improve the bioethanol fermentation performance. This was followed by optimization of fermentation process for bioethanol production by using response surface methodology (RSM) to explore the response pattern. A five-level-three-factor central composite design (CCD) was employed in this study and the central point of each process variable was chosen based on the best condition obtained from the OFAT method.

The OFAT method for parameter design was implemented to obtain the best possible condition. In OFAT method, information on one-factor in each experimental trial was gathered and repeated for all respected factors [14]. Optimization process is defined as a way to enhance the performance of any system or process by obtaining the maximum benefit and investigating suitable parameters which give best response [15]. OFAT is a conventional method in which influences one factor at a time on an experimental response is studied. Only one parameter is changed, others are kept fixed [15]. Wahid and Nadir [16] proposed that OFAT do not require an advanced statistical knowledge in terms of execution and data analysis. An exact optimum can be studied using RSM which illustrates quadratic surfaces such as maximum, minimum, ridge and saddle [17]. This method of optimization is recommended due to its desirability function which gives the optimum performance levels for one or more responses.

## 2. Materials and Methods

### 2.1 Preparation of oil palm frond juice

In this study, fresh OPF (petiole part without leaves) were collected from the oil palm plantation at Felda Lepar Hilir 1, Gambang, Pahang, Malaysia during the dry season in the month of May. The OPF juice was collected by crushing the fresh OPF petioles using a conventional sugarcane press machine following a method described earlier by Zahari et al. [10]. It was centrifuged at  $15,000 \times g$  for 15 min and the supernatant was filtered using a mixed cellulose ester membrane filter with the pore size between 3 and 5  $\mu\text{m}$  (Cole Parmer, Illinois, USA) and stored at  $-20^\circ\text{C}$  for storage purposes before being used for fermentation [10].

### 2.2 Chemicals and yeast strains

The sulfuric acid ( $\text{H}_2\text{SO}_4$ ), sodium hydroxide (NaOH) and yeast extract were obtained from R&M Marketing Essex (UK). Bacteriological peptone was obtained from Oxoid Ltd. Basingstoke (Hampshire, England). Meanwhile, standard sugars for HPLC analysis such as glucose, sucrose and fructose were obtained from Fisher Scientific (Leicestershire, UK). An industrial strain of *Saccharomyces cerevisiae* (*S. cerevisiae*) Kyokai No. 7 (ATCC 26622) was obtained from American Type of Culture Collection (ATCC).

### 2.3 YPD (Yeast extract peptone dextrose)

Yeast (*S. cerevisiae* Kyokai No. 7 (ATCC 26622)) was grown and maintained on YPD agar following method described earlier by Kosugi et al. [3] and Ho and Powel [13] with some modification composed of 20 g/L technical agar, 20 g/L dextrose anhydrous, 20 g/L bacteriological peptone and 10 g/L yeast extract. The prepared media were then autoclaved at  $121^\circ\text{C}$  for 15 minutes. The YPD agar was poured into sterile Petri dishes and left for solidification. YPD agar also can be prepared by using 28 g/L of nutrient agar. Strains were maintained and sub-cultured into fresh Petri dishes every 5 weeks.

### 2.4 Inoculum preparation

A 3-loop full of microorganism (*S. cerevisiae* Kyokai No. 7 (ATCC 26622)) from the plate was transferred into the growth medium for a culture contained: 5 g/L glucose, 10 g/L peptone, 5 g/L yeast extract and 200 mL distilled water. The culture was grown in a 250 mL Erlenmeyer flask containing 100 mL growth medium on a shaker incubated at  $30^\circ\text{C}$  and 200 rpm for 24 h to reach the exponential phase following the method described earlier by Chin et al. [6] and Bakri et al. [18] The cell concentration was standardized to 0.2-0.4 g/L (OD = 1.5-2.0) determined using a calibrated UV-vis spectrophotometer U-1800 (Hitachi, Japan) at 600 nm. All of the procedures were carried out aseptically and analysis was run in duplicate.

### 2.5 Screening of process variable using one-factor-at-time (OFAT) method

Preliminary experiment was carried out in the first place to study the effect of sterilization on bioethanol production by employing two set of experiments using autoclaved and non-autoclaved OPF juice as a fermentation substrate and the result obtained from this study was used for subsequent experiment. Both of the experiments were conducted in a rotary shaker (150 rpm) under anaerobic condition at  $30^\circ\text{C}$  for 48 h without pH adjustment. Samples were withdrawn every 6 or 12 h from the broth for bioethanol and residual sugars determination.

For the first part of this study, the effect of different parameters on bioethanol production from OPF juice was screened by using one-factor-at-time (OFAT) method. The OPF juice was filtered using 9.0  $\mu\text{m}$  mixed cellulose ester membrane filter to remove the unwanted particles as described by Norhazimah [19]. OPF juice (100% v/v) which comprises of glucose, sucrose and fructose was used as the carbon source and nutrients throughout the study period. Pre-cultured yeast cells (10% v/v) were inoculated into a 250 mL Erlenmeyer flask containing 100 mL of autoclaved OPF juice following the previous method described by Zahari et al. [2] and Kosugi et al. [3] without any nutrient or nitrogen source supplementation. In order to study the effect of medium initial pH on bioethanol production, the initial pH value of OPF juice was adjusted to pH 5.0-9.0 using 2 M NaOH prior to autoclave. Another set of

experiment was conducted to study the effect of rotation rate on bioethanol production by investigating several rotation rates at 0, 50, 100, 150 and 200 rpm. For the effect of temperature, various temperatures in the range of 27.5-37.5°C were investigated. Fermentation was run for 24 h under anaerobic condition, and all experiments were conducted in duplicates. Samples were harvested at the end of the fermentation period for bioethanol and residual sugars determination.

### 2.6 Optimization of process variable using Response Surface Methodology (RSM)

Meanwhile, for the second part of this study, the effect of three independent variables (pH, temperature and rotation rate) on the bioethanol yield was optimized using a factorial Central Composite Design (CCD) of Response Surface Methodology (RSM). A five-level-three-factor CCD was employed in this study and the three most significant process variables namely the medium initial pH, temperature and rotation rate. The CCD and RSM were performed using commercial software, Design Expert Version 7.1.6 (Statease Inc., Minneapolis, Minn., U.S.A.). A total design of 20 runs were set based on computer generated process variable including 6 replicate central points and  $\alpha = 2$ . The central point of each parameter studied in optimization experiment was selected based on the results obtained from OFAT experiment and the parameters ranges were set as follows; medium initial pH (5-9), temperature (27.5-37.5°C) and rotation rate (80-120 rpm) as shown in table 1.

**Table 1.** Independent variables and their coded and actual levels used in the RSM studies for optimizing the fermentation conditions of bioethanol production using OPF juice.

Independent variable	Unit	Symbol	Coded level				
			$-\alpha$	-1	0	1	$+\alpha$
Temperature	°C	A	22.5	27.5	32.5	37.5	42.5
Initial medium pH		B	3	5	7	9	11
Rotation rate	rpm	C	60	80	100	120	140

For the optimization study, fermentation was run for 24 h under anaerobic condition and all experiments were conducted in duplicates. Samples were withdrawn at the end of the fermentation period and the cells were separated from the fermentation broth by centrifugation at 5,000 rpm for 10 min. The cells were discarded, whereby the supernatant were filtered in vial by using syringe filter 0.2  $\mu\text{m}$  prior for analysis using HPLC.

### 2.7 Statistical analysis

Response surface methodology (RSM) was statistically analyzed by Design-Expert, Version Version 7.1.6 software (Statease Inc., Minneapolis, Minn., U.S.A.). The coefficients can be obtained through multiple regression analysis. Estimation of coefficients with levels higher than 95% ( $p < 0.05$ ) were included in the CCD models. The bioethanol yield can be expressed as a function of independent variables by a second order polynomial equation:

$$Y = \beta_0 + \sum \beta_0 x_j + \sum \beta_{jj} x_j^2 + \sum \beta_{jk} x_j x_k \quad (1)$$

where, Y is the response (bioethanol yield),  $\beta_0$ ,  $\beta_j$ ,  $\beta_{jj}$  and  $\beta_{jk}$  represent the regression coefficient for intercept, linear, quadratic and interaction terms, respectively. The responses obtained were statistically evaluated by using analysis of variance (ANOVA) and the model was built based on the variables with confidence levels more than 95%.

### 2.8 Analytical methods

The biomass concentration was determined by using a UV-Vis spectrophotometer (OD = 600 nm) (Hitachi, Japan). Samples for quantitative analysis were centrifuged at 5,000 rpm for 20 minutes to obtain the supernatants. The supernatants were filtered through 0.22  $\mu\text{m}$  membrane filter for the determination of residual sugars and bioethanol concentration. Sugars and bioethanol were quantified

through high performance liquid chromatography (HPLC) (Agilent 1200 series, U.S.A). A Rezex ROA organic acid H<sup>+</sup> (300×7.8 mm) column and RI detector was used for the separation. The chromatography grade 0.005 N H<sub>2</sub>SO<sub>4</sub> was used as mobile phase and the flow rate was set at 0.6 mL/min. The column temperature was set at 60°C and RI detector temperature at 40°C. The injection volumes of 10 µl were applied. The components were identified by comparing their retention times with those of authentic standards under analytical conditions and quantified by external standard method [10].

### 3. Results and Discussion

#### 3.1 Sugars composition of oil palm (OPF) juice

The results of HPLC analysis on sugars concentration in OPF juice is shown in table 2.

**Table 2.** Initial sugars concentration contained in the oil palm frond (OPF) juice.

Types of sugar	Sugars concentration (g/L)	
	This work	Zahari et al. (2012)
Glucose	44.16	53.95
Sucrose	11.25	20.46
Fructose	1.46	1.68
Total	56.87	76.09

The total sugars concentration in OPF juice used in this study was 56.87 g/L. Glucose was found to be dominant sugar (44.16 g/L) followed by sucrose (11.25 g/L) and fructose (1.46 g/L). The result obtained in this study was almost similar with the findings reported by Zahari et al. [10]. They have reported that glucose was the dominant sugar in OPF juice followed by sucrose and fructose. However, Zahari et al. [10] showed higher total sugars concentration (76.09 g/L) compared to this study where sugars composition was reported as; glucose (53.95 g/L), sucrose (20.46 g/L) and fructose (1.68 g/L). This might be due to the different location of vegetation of the oil palm tree used in this study, which could affect the sugars concentration in the OPF. The OPF in this work was obtained from the oil palm plantation in Gambang, Pahang, meanwhile Zahari et al. [10] obtained their sample from Serdang, Selangor. Another possible explanation which could contribute to the low sugars content in OPF juice in this study could be due to the time of harvest of fresh OPF during the dry season in the month of May. According to Yusof Basiron, chief executive officer of the Malaysian Palm Oil Council (MPOC), moderate amount of rain may provide a good condition to induce the growth of oil palm tree [20] and subsequently influence the sugars content in the OPF as well.

#### 3.2 Preliminary experiment

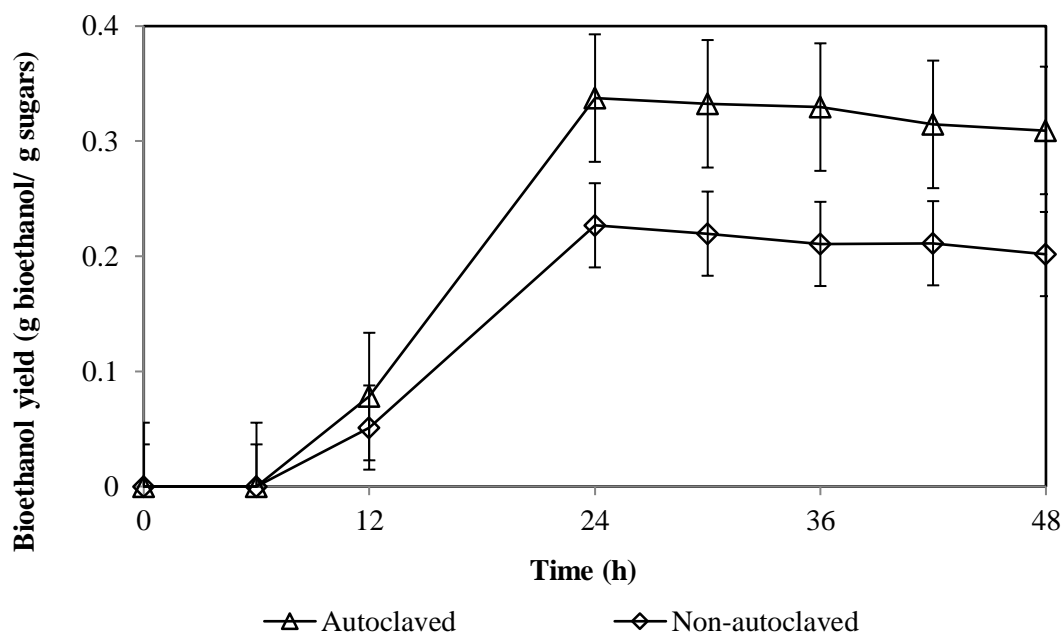
##### 3.2.1 Effect of sterilization

OPF juice is readily fermentable to produce bioethanol because it contains mixture of sugars and rich in minerals and nutrients which are essential for bacterial growth during fermentation. Bioethanol concentrations and total sugars consumed were analyzed using high performance liquid chromatography (HPLC), whereby the bioethanol yield (g g<sup>-1</sup>) was calculated based on experimental bioethanol produced and expressed as g bioethanol per total g of sugar utilized (equation (2)):

$$\text{Bioethanol yield} \left( \frac{\text{g bioethanol}}{\text{g sugars}} \right) = \frac{\text{Bioethanol concentration} \left( \frac{\text{g}}{\text{l}} \right)}{\text{Total sugars consumed} \left( \frac{\text{g}}{\text{l}} \right)} \quad (2)$$

To investigate the effect of sterilization on bioethanol production, two sets of experiment was conducted, where autoclaved (sterile) and non-autoclaved (non-sterile) OPF juice were used as a

substrate for fermentation. In order to achieve this, yeast, *S. cerevisiae* Kyokai No. 7 (ATCC 26622) was cultured into autoclaved and non-autoclaved OPF juice and the result was depicted in figure 1.



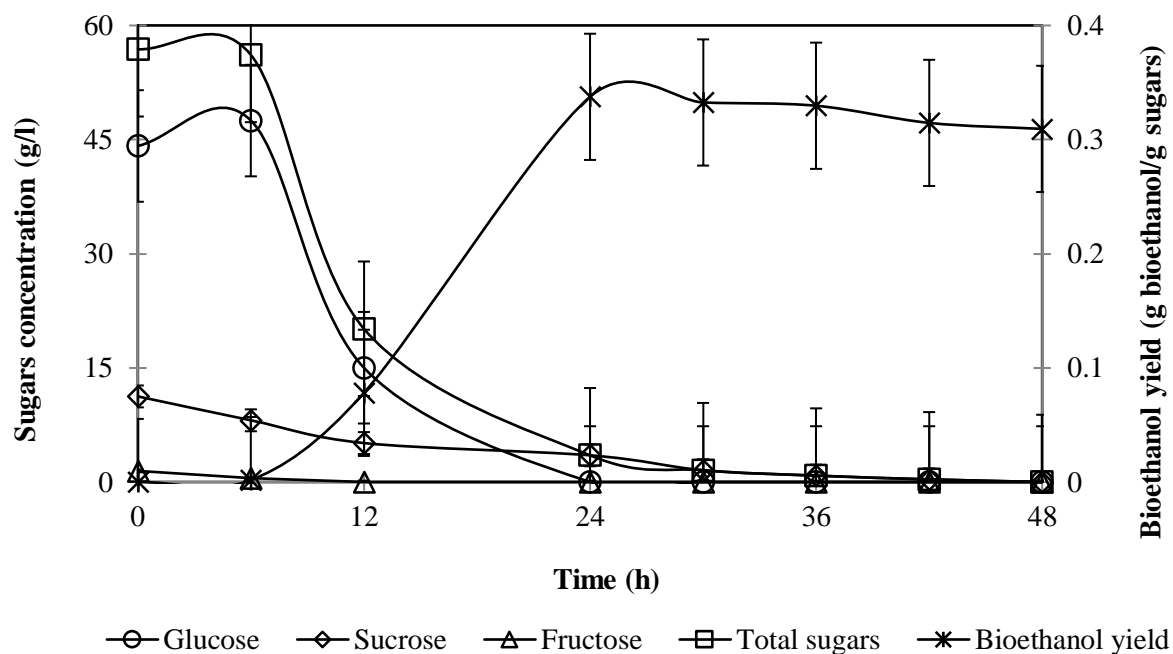
**Figure 1.** Comparison of bioethanol yield by *S. cerevisiae* Kyokai No. 7 (ATCC 26622) supplemented with autoclaved and non-autoclaved OPF juice. (Experiments were conducted in a rotary shaker (150 rpm) under anaerobic condition at 30°C for 48 h without pH adjustment).

As shown in figure 1, it was noted that highest yield of bioethanol at 0.34 g bioethanol/ g sugars was obtained after 24 h of fermentation period when sterilized (autoclaved) OPF juice was used as fermentation substrate. For the non-sterilized (non-autoclaved) OPF juice, only 0.23 g bioethanol/ g sugars were obtained within the same fermentation period. It was observed that the total bioethanol yield obtained was slightly higher using sterilized OPF juice as a fermentation feedstock. It is worth to mention that, by autoclaving the OPF juice, any unwanted microorganisms which can cause contamination are inhibited or killed. This result is in agreement with other findings whereby heat sterilization may affect bioethanol production from oil palm trunk (OPT) sap by using the similar yeast strain [19]. In the report, it was mentioned that the maximum bioethanol concentration in heat sterilized sap was 29.96% higher than the fermentation in cold sterilized sap, and this was two times higher than the fermentation in non-sterile sap. In addition to that, higher bioethanol yield was obtained in fermentation using sterilized OPF juice might be due to some complex oligosaccharides in OPF juice was also hydrolysed to monosaccharides during autoclaving, resulted in more reducing sugars [33-35]. The reducing sugars produced was further utilized by *S. cerevisiae* Kyokai No. 7 (ATCC 26622) to produce bioethanol hence increased the yield.

### 3.2.2 Sugars consumption and bioethanol production profile.

Fermentation profile for bioethanol production from sterilized (autoclaved) OPF juice using *S. cerevisiae* Kyokai No. 7 (ATCC 26622) is shown in Figure 2. The bioethanol yield increased proportionally with reducing sugar content from fermentation broth. The highest bioethanol yield of 0.34 g bioethanol/ g sugars was obtained after 24 h of fermentation period. Prolonged time of incubation up to 48 h did not contribute to the increase in production of bioethanol. Whereby, it was slightly decreased to only 0.31 g/g sugars of bioethanol yield, which accounts for approximately 8.82% decrease. In Figure 1, there is a clear trend of decreasing in the production of bioethanol after 24 h of incubation might cause due to the decreased of sugars concentration level in the fermentation broth. During the fermentation, equal molarity of CO<sub>2</sub> and bioethanol was produced hence reduction of sugar consumption leads to weight lost in CO<sub>2</sub>. This explained the reduction in bioethanol production in a longer period of

time [21]. From this explanation, for the subsequent experiment, the fermentation was conducted for 24 h of incubation period to evaluate the effects of several physical parameters on bioethanol production from OPF juice by *S. cerevisiae* Kyokai No. 7 (ATCC 26622).



**Figure 2.** Sugars consumption and bioethanol production profile by *S. cerevisiae* Kyokai No. 7 (ATCC 26622) supplemented with OPF juice.

Figure 2 also demonstrated the profile of sugars consumption by *S. cerevisiae* Kyokai No. 7 (ATCC 26622) in the fermentation broth throughout the incubation period. Overall, sugars in OPF juice was completely consumed by the yeast at the end of fermentation period including sucrose. During the first 6 h until 12 h, the sugars concentration decrease rapidly as bioethanol was produced. Our finding revealed that the concentration of fructose, sucrose and total sugar started to decrease during the first 6 h and then rapidly decreased afterward. This finding highlights the increase in glucose concentration can be attributed to the breakdown of sucrose to its monomer by the presence of invertase during the fermentation as sucrose is a disaccharide composed of glucose and fructose. The present finding also supported by previous studies which concluded that *S. cerevisiae* has the ability to produce invertase enzyme namely periplasmic invertase in which used for conversion of sucrose to glucose and fructose at the yeast cell during the fermentation process [2, 5].

### 3.3 Screening of parameters affecting bioethanol production from OPF juice

#### 3.3.1 Effect of medium initial pH

The medium initial pH is a key factor which has significant influence on fermentation [22] [23]. All organism and cellular processes are affected by pH; this is mainly due to the concentration of H<sup>+</sup> ions in the liquid environment. The cells grow and perform fermentation best within a certain pH range. In this study, the effect of medium initial pH on bioethanol production from OPF juice was conducted by adjusting the initial pH value of OPF juice prior to autoclaving between 5.0 and 9.0 with an increment of 1.0. As shown in table 3, bioethanol yield was found to be the highest when the medium initial pH was adjusted at pH 7.0 compared to others after 24 h of fermentation period. The highest bioethanol yield obtained in this experiment was 0.39 g/g sugars. Thus, pH may be an important factor to achieve maximum bioethanol yield. Optimum pH is essential for bioethanol yield to avoid maximum acidic or basic condition of medium hence retard the metabolic of yeast and cell growth [24].



**Table 3.** Bioethanol yield at different initial medium pH with rotation rate and temperature were set at 150 rpm and 30°C, respectively.

Initial medium pH	Bioethanol concentration (g/L) <sup>a</sup>	Bioethanol yield (g/ g sugars)
5	19.79	0.35
6	20.14	0.35
7	22.10	0.39
8	18.40	0.32
9	16.77	0.29

<sup>a</sup>Determination by HPLC from filtered supernatant after 24 h of incubation period.

### 3.3.2 Effect of rotation rate

Rotation of an incubator shaker is necessary for constant mixing of the medium components to provide uniform oxygen transfer rates. Rotation also played significant role in improving bioethanol concentration and yield [12]. The effect of rotation rate is fundamental to obtain successful fermentation by providing adequate mixing, mass transfer and heat transfer [11]. Besides assisting mass transfer between two different phases of the medium, it also enables uniform suspension of microbial cells in homogenous nutrient medium. table 4 shows bioethanol production by *S. cerevisiae* Kyokai No. 7 (ATCC 26622) using OPF juice as the fermentation feedstock at different rotation rate of 0, 50, 100, 150 and 200 rpm.

**Table 4.** Bioethanol yield at different rotation rate with initial medium pH and temperature were set at 7.0 and 30°C, respectively.

Rotation rate (rpm)	Bioethanol concentration (g/L) <sup>a</sup>	Bioethanol yield (g/ g sugars)
0	19.00	0.33
50	19.47	0.34
100	23.00	0.40
150	22.10	0.39
200	19.86	0.35

<sup>a</sup>Determination by HPLC from filtered supernatant after 24 h of incubation period.

After 24 h of incubation period, rotation rate at 100 rpm gave the best bioethanol yield of 0.40 g/ g sugars compared to the other rotation rate. Thus, maximum productivity in microbial fermentation was achieved at optimum rotation rate. Mittal [25] proposed that rotation creates shear forces by causing morphological changes and disruption of cell structure. High rotation rate is not suitable for successful fermentation as it could contribute to the effect of hydrodynamic stress which can cause leakage of intracellular compounds [18]. However, rotation is needed to improve cell mass and bioethanol activity. Therefore, low rotation rate may contribute to low bioethanol production due to less nutrient consumption by yeast cells in static condition [12]. Optimum rotation rate will enable symmetrical fermentation system hence accelerating nutrient consumption by yeast.

### 3.3.3 Effect of temperature

In this study, the influence of different temperature on the bioethanol fermentation by *S. cerevisiae* Kyokai No. 7 using OPF juice was studied with regard to bioethanol production. Temperature is one of the most significant parameters that contribute to yeast growth and fermentation performance. *S. cerevisiae* Kyokai No. 7 is a type of yeast which is mesophilic in nature, thus able to withstand temperature up to 48°C. However, Ho and Powel [13] suggested the preferable temperature for *Saccharomyces* yeast is between 25 to 35°C and at a temperature up to 43°C, yeast cells began to lose their capability to be superior ethanologenic yeast strains. Table 5 shows the bioethanol yield at various temperatures from 27.5 to 47.5°C.

**Table 5.** Bioethanol yield at different temperature with initial medium pH and rotation rate were set at 7.0 and 150 rpm, respectively.

Temperature (°C)	Bioethanol concentration (g/L) <sup>a</sup>	Bioethanol yield (g/ g sugars)
27.5	18.15	0.32
30.0	22.10	0.39
32.5	23.11	0.41
35.0	18.85	0.33
37.5	16.95	0.30

<sup>a</sup>Determination by HPLC from filtered supernatant after 24 h of incubation period.

Highest bioethanol yield was obtained at temperature of 32.5°C (0.41 g bioethanol/ g sugars) and thus regarded as an optimum temperature for production of bioethanol using OPF juice by *S. cerevisiae* Kyokai No. 7. Bioethanol yield however gradually decreased at 37.5°C and this event was previously reported by Fakruddin et al. [26] in which the production of bioethanol by strains *Saccharomyces unisporous* (P), *S. cerevisiae* (C) and (T) gradually decreased.

### 3.4 Optimization of bioethanol production employing Response Surface Methodology (RSM)

Based on the OFAT experiment, it was observed that the best condition for bioethanol production from OPF juice by *S. cerevisiae* Kyokai No. 7 was obtained at the following parameter's conditions; medium initial pH (7.0), temperature (32.5°C) and rotation rate (100 rpm). These conditions were then selected as the central point for optimization study using CCD. Studies were carried out to establish the range of parameters such as medium initial pH, temperature and rotation rate to be optimized. A design matrix corresponding to the yield of bioethanol was subjected to regression analysis to study the effect of these parameters. The RSM experimental design matrix with three factors at five levels and the experimental results are presented in table 6.

**Table 6.** The experimental results for bioethanol yield for the central composite design.

Standard Run	Factor A Temperature (°C)	Factor B Initial medium pH	Factor C Rotation rate (rpm)	Response Bioethanol yield (g bioethanol/ g sugars)
1	27.5	5	80	0.35
2	37.5	5	80	0.37
3	27.5	9	80	0.30
4	37.5	9	80	0.30
5	27.5	5	120	0.27
6	37.5	5	120	0.35
7	27.5	9	120	0.27
8	37.5	9	120	0.30
9	22.5	7	100	0.22
10	42.5	7	100	0.26
11	32.5	3	100	0.31
12	32.5	11	100	0.26
13	32.5	7	60	0.35
14	32.5	7	140	0.31
15	32.5	7	100	0.49
16	32.5	7	100	0.46
17	32.5	7	100	0.49
18	32.5	7	100	0.48
19	32.5	7	100	0.46
20	32.5	7	100	0.49

Bioethanol yield was used as a response and was arranged into design expert experiment based on standard run. As shown in table 6, the average value of all bioethanol yields was around 0.22–0.49 g bioethanol/ g sugars.

### 3.4.1 ANOVA and model development

ANOVA is known as analysis of variance which offers an excellent technique to determine the process variables that gives significant impact on process quality and their possible interaction. ANOVA which includes  $F$ -value,  $p$ -value,  $R^2$  and lack of fit was applied to determine suggested model that fit with experimental data.  $R^2$  is known as coefficients of determination to ensure the quality of fit for the model. The  $p$ -values of less than 0.05 were indicated as statistically significant. The significant terms showed whether the parameters studied affects fermentation process. The model and individual coefficient will be more significant if the results show a larger magnitude of  $F$ -value and a smaller  $p$ -value. The relationship between independent variables and response can be performed from analysis of quadratic model as shown in table 7.

Source	Sum of Squares	Degree of Freedom	Mean Square	$F$ -Value	$p$ -value, Prob>F
Process order: Quadratic					
Model	0.15	9	0.017	48.74	< 0.0001
A-Temp	0.0027	1	0.0027	7.90	0.018
B-pH	0.0045	1	0.0045	13.06	0.0047
C-Speed	0.0027	1	0.0027	7.90	0.018
AB	0.00061	1	0.00061	1.76	0.21
AC	0.0010	1	0.0010	2.90	0.11
BC	0.00061	1	0.00061	1.76	0.21
A <sup>2</sup>	0.094	1	0.094	271.43	< 0.0001
B <sup>2</sup>	0.063	1	0.063	181.03	< 0.0001
C <sup>2</sup>	0.037	1	0.037	108.87	< 0.0001
Residual	0.0034	10	0.00034		
Lack of Fit	0.0024	5	0.00048	2.22	0.20
Pure Error	0.0010	5	0.00021		
Cor. Total	0.15	19			
Standard Deviation		0.019	$R^2$		0.97
Mean		0.35	Adjusted $R^2$		0.95
C.V. %		5.27	Predicted $R^2$		0.86
PRESS		0.021	Adequate Precision		20.57

**Table 7.** Analysis of variance (ANOVA) for quadratic model

\*Lack of Fit is not significant relative to the pure error

The mathematical model derived from the experimental results for bioethanol yield (Y) was shown in Equation (3):

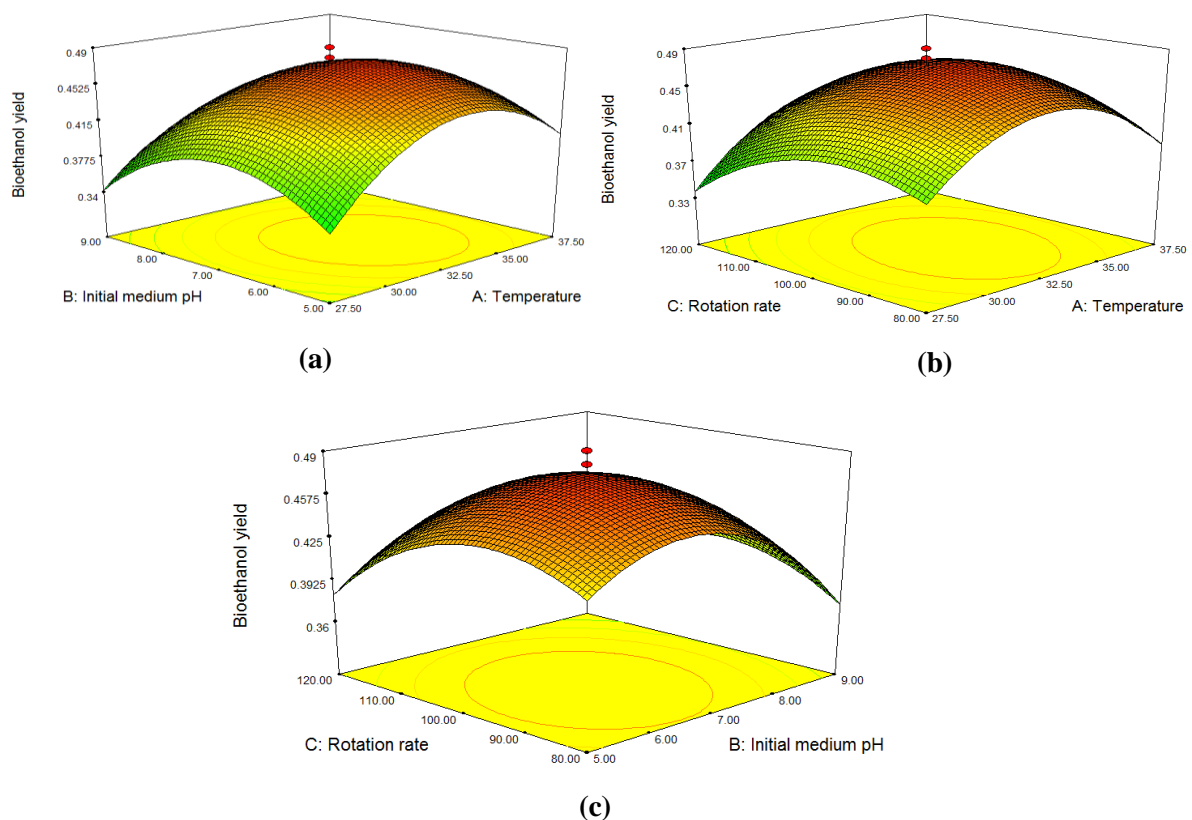
$$Y = +0.47 + 0.013 \times A - 0.017 \times B - 0.013 \times C - 0.0088 \times A \times B + 0.011 \times A \times C + 0.0088 \times B \times C - 0.061 \times A^2 - 0.05 \times B^2 \quad (3)$$

where Y is bioethanol yield, A is temperature, B is medium initial pH and C is rotation rate. The quadratic model was selected to provide the best fit with the experimental results.

The model presented in table 7 exhibits a high determination coefficient ( $R^2 = 0.97$ ), explaining 97.77% of the variability in the response, as well as a high value of the adjusted determination coefficient (adjusted  $R^2 = 0.95$ ), suggesting a high significance of the model. A very low probability ( $p < 0.0001$ ) obtained from the regression analysis of variance (ANOVA) demonstrated that the model was significant. In this study, all the linear model terms including temperature (A), medium initial pH (B) and rotation rate (C) have significant effect, as the p-values calculated for this factor was less than 0.05. Therefore, changes in this parameter could significantly impact the bioethanol production from OPF juice fermentation. The most significant effect is the linear term of medium initial pH (B), followed by rotation rate (C) and temperature (A). All the two level interactions including temperature and rotation rate (AC), temperature and medium initial pH (AB) as well as medium initial pH and rotation rate (BC) were indicated as significant. In a similar manner, all the second order effects showed the significant results including  $A^2$ ,  $B^2$  and  $C^2$ . Generally, the lack of fit p-value of 0.20 implied that the lack of fit is not significant relative to the pure error. The non-significant lack of fit is positive because it demonstrates a good fit of the model to the data. A good fit means that the generated models adequately explained the variation of data.

### 3.4.2 Response surface plot

Response surface plots based on equation (3), with the relationships between the response and variables, are presented in Figure 3 (a–c). The plots were constructed by plotting the response (bioethanol yield) on the Z-axis against any two dependent variables while maintaining the other variables at their optimal values.



**Figure 3.** Response surface plots depicting the interaction between variables in the production of bioethanol from OPF juice; (a) temperature ( $^{\circ}\text{C}$ ) and initial medium pH, (b) temperature ( $^{\circ}\text{C}$ ) and rotation rate (rpm), (c) initial medium pH and rotation rate (rpm).

Figure 3(a) depicts the interaction between temperature and the medium initial pH (AB) while holding factor C (rotation rate) at 100 rpm. As shown in Figure 3(a) it was observed that bioethanol yield increased when the temperature was changed from 27.5 to 37.5°C as medium initial pH increased from 5.0 to 9.0. In this study, the bioethanol yield decreased by lowering the temperature to 27.5°C with similar effects by increasing the temperature to 37.5°C. Similar event was reported by Chin et al. [6], whereby temperature affected the enzyme activity which explained the facilitation of chemical reactions within the yeast. In contrast, high bioethanol yield was observed at moderate temperature and medium initial pH ranges. Based on the optimum result suggested by the Design-Expert, Version 7.1.6 software, bioethanol yield was relatively high at temperature of 33.03°C and medium initial pH of 6.62. Adnan et al. [27] reported that bioethanol production from glycerol by *Escherichia coli* SS1 was greatly influenced by pH and an optimum pH value of 7.61 was identified. Further increases in the pH resulted in lower bioethanol production. The initial pH is an important factor that influences the NADH to NAD<sup>+</sup> ratio, which greatly affects the metabolic flux under anaerobic conditions [27]. Hence, to obtain optimal bioethanol production, it is necessary to control the medium initial pH under optimum conditions. In general, yeast is able to grow and efficiently ferment substrates into bioethanol at pH values of 3.5–6.0 and temperatures of 28 – 35°C. The optimum medium initial pH (6.62) and temperature (33.03°C) obtained in this work is within the range of those reported in the literature especially for *Saccharomyces cerevisiae*.

The interaction between temperature and rotation rate (AC) while holding medium initial pH (B) at 7.0 towards bioethanol yield in terms of 3D is shown in Figure 3 (b). As shown in Figure 3 (b), the bioethanol yield decreased at higher rotation rate (120 rpm) as the temperature was increased from 27.5 to 37.5°C. In contrast with lower rotation rate (80 rpm), it shows an increasing trend when temperature was increased. The lower bioethanol yield (0.31 g/ g sugars) showed at higher rotation rate (140 rpm) compared to central point rotation rate value (100 rpm) which has much higher bioethanol yield (0.49 g/ g sugars). Rotation rate is known to have an important role in ensuring uniform adequate mixing, mass transfer and heat transfer within the fermenter in medium components [5]. The effects of rotation rate are required for successful fermentation process to improve product yields. The advantages of rotation toward performance and growth of microorganism cells could improve the mass transfer on substrates, products or by-products and oxygen. Better mixing process has the capability to maintain adequate supply of sugars and nutrients to the cells as well as to maintain the concentration gradient between interior and exterior cells in fermentation broth [5].

Meanwhile, Figure 3 (c) depicts the interaction between the medium initial pH and rotation rate (BC) while holding factor A (temperature) at 32.5°C. The interaction between medium initial pH and rotation rate (BC) demonstrated that bioethanol yield decreased when rotation rate changed from 80 to 120 rpm as medium initial pH increased from 5.0 to 9.0. At higher rotation rate (120 rpm), the response yield illustrated a linear decrease with increasing pH value. In contrast, lower rotation rate (80 rpm) showed a pattern of increasing slope. The medium with pH variations may lead to the changes in enzyme activity as well as changes in reaction rate. pH plays a significant role in bioethanol fermentation by *Saccharomyces cerevisiae* as pH affects the growth of yeast, by-product formation and fermentation rate due to the concentration of H<sup>+</sup> ions in the liquid environment [5-6, 29].

In summary, high temperature still showed the production of bioethanol, however, slightly decreased with time of incubation. Changes in the medium initial pH might lead to the changes in the fermentation pathway. Hence, based on the result obtained; medium initial pH showed the highest significant effect towards the production of bioethanol. Minimal rotation rate was required to produce maximum bioethanol yield to ensure uniform mixing and consumption of nutrition. Therefore, it is necessary to enhance optimal temperature, medium initial pH and rotation rate to accelerate cell activities, thus achieve high bioethanol yield.

### 3.4.3 Confirmation of model prediction

The reproducibility of the model was tested by performing the fermentation under the optimal conditions obtained from the CCD. This validation was also used to verify the accuracy of the model. The bioethanol production model suggested that optimum bioethanol yield could be achieved at medium

initial pH of 6.62, temperature of 33.03°C and rotation rate of 96.51 rpm. The predicted bioethanol yield under these optimum conditions was 0.48 g bioethanol/ g sugars. Three replicates of the batch fermentation using OPF juice without nutrient supplementation under the optimized conditions were conducted in shake flask to confirm the model validity. Maximum bioethanol yield of 0.50 ±0.02 g/ g sugars was obtained from the confirmation test. These experimental findings were in close agreement with the model prediction, with a difference of only 4.17%. Hence, it is confirmed that the model developed from the response surface methodology could reliably predict bioethanol yields. According to Adnan et al. [27], differences between experimental and predicted values of less than 10% confirm the validity of a model. The yield obtained in this study was 47.06% higher compared with the bioethanol produce under non-optimized condition (0.33 g bioethanol/ g sugars). These findings suggest that in general, medium initial pH, temperature and agitation speed may indeed play an important role in the bioethanol production by *S. cerevisiae* Kyokai No. 7 (ATCC 2662) utilizing OPF juice as a complete medium. In addition to that, even without the supplementation of nitrogen source into the fermentation medium, the bioethanol yield obtained in this study was almost comparable to those reported by Zahari et al. [2]. They have reported that 0.49 g/g sugars of bioethanol yield were obtained from OPF juice supplemented with 4 g/L of peptone and yeast extract (nitrogen source). The evidence from this study suggests that OPF juice can be used directly as the fermentation medium for bioethanol production at industrial scale.

#### 4. Conclusion

The present work demonstrated that OPF juice can be a potential complete fermentation feedstock for the production of bioethanol. The optimal fermentation conditions for bioethanol production were determined using the methods of OFAT and response surface analysis. Experimental results indicated that the temperature exert significant effects on bioethanol yield. The maximum bioethanol yield of 0.50 g/ g sugars was obtained under the following optimum condition; medium initial pH (6.62), rotation rate (96.51 rpm) and temperature (33.03°C). Compared to the predicted maximum bioethanol yield of 0.48 g/ g sugars, only a small error exists between the predicted value and the actual experimental value. In conclusion, this study demonstrated that high bioethanol yield could be obtained by culturing *S. cerevisiae* Kyokai No. 7 at the optimized condition using OPF juice as the sole renewable fermentation feedstock. Under the optimal conditions, the bioethanol yield obtained was 47.06% higher compared to non-optimized condition.

#### Acknowledgements

The authors would like to acknowledge Universiti Malaysia Pahang (UMP) and the Ministry of Higher Education (MOHE) for the scholarship and financial support by providing the research grant, FRGS (RDU140139) and RAGS (RDU121406). The authors also would like to acknowledge the Institute of Postgraduate Studies of UMP for motivating the author to publish this work and PhD student, Kamaliah Abdul Samad for supplying the raw material.

#### References

- [1] Zwart D 2013 *Journal of Oil Palm and Environment* **4**(5) 41-62
- [2] Zahari M A K M, Abdullah S S S, Roslan A M, Ariffin H, Shirai Y and Hassan M A 2014 *Journal of Cleaner Production* **65** 252-60
- [3] Kosugi A, Tanaka R, Magara K, Murata Y, Arai T, Sulaiman O, Hashim R, Hamid Z A A, Yahya M K A, Yusof M N M, Ibrahim W A and Mori Y 2010 *Journal of Bioscience and Bioengineering* **110**(3) 322-25
- [4] Norhazimah A H and Faizal C K M 2013 *Research Journal of Chemistry & Environment* **17**(10) 90–93
- [5] Shahirah M N N 2014 *Master Thesis* (Universiti Malaysia Pahang Malaysia)
- [6] Chin K L, H'ng P S, Wong L J, Tey B T and Paridah M T 2010 *Bioresource Technology Journal* **10** 3287-3291
- [7] Mabee W E, McFarlane P N and Saddler J N 2011 *Biomass and Bioenergy* **35** 4519–4529
- [8] Pothiraj C, Arumugam R and Gobinath M 2014 *Bioresources and Bioprocessing* **1** 27

- [9] Rudolf A, Karhumaa K and Hagerdal B H 2009. In: *T. Satyanarayana, G. Kunze (eds.), Yeast Biotechnology, Diversity and Applications* (New York: Springer Science. Publishing Co.) 489-513
- [10] Zahari M A K M, Zakaria M R, Ariffin H, Mokhtar M N, Salihon J, Shirai Y and Hassan M A 2012 *Bioresource Technology Journal* **110** 566-71
- [11] Rodmui A, Kongkiattikajorn J and Dandusitapun J 2008. *Kasetsart Journal, Nat Sci.* **42** 285-93
- [12] Yan L, Tiansheng Q, Naikun S, Mingzhe G, Yanling J and Hai Z 2009 *Chinese Journal of Applied and Environmental Biology* **15**(4) 563-67
- [13] Ho D H N and Powel C 2014 *International Journal of Renewable Energy and Environmental Engineering* **2**(1) 1-6
- [14] Frey D, Engelhardt F and Greitzer E M 2003 *Research Engineering Design* **114** 65-74
- [15] Bezerra M A, Santelli R A, Oliveira E P, Villar L S and Escalera L A 2008 *Talanta.* **76**(5) 965-77
- [16] Wahid Z A and Nadir N 2013. *World Applied Sciences Journal 21 (Mathematical Applications in Engineering)* **21** 56-61
- [17] Sakkas V A, Islam M A, Stalikas C and Albanis T A 2010 *Journal of Hazardous Material* **175** 33-44
- [18] Bakri Y, Mekaeel A and Koreih A 2011 *Brazilian Archives of Biology and Technology* **54**(4) 659-64
- [19] Norhazimah A H 2012 *Master Thesis* (Universiti Malaysia Pahang, Malaysia)
- [20] Basiron Y 2011 *Bloomberg Businessweek*, <http://www.bloomberg.com/news/articles/2011-02-24/palm-oil-output-in-malaysia-to-gain-as-weather-boosts-yields-group-says>. Accessed July 10, 2019
- [21] Krishnamurthy R, Animasaun D A, Ingalhalli R S and Ramani N D 2014 *International Journal of Pure & Applied Bioscience* **2**(22) 174-80
- [22] Raikar R V 2012 *International Journal of Environmental Sciences* **3**(2) 776-783
- [23] Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S and Kong H 2012 *Biomass and Bioenergy* **47** 395-401
- [24] Willaert R and Nedovic V A 2006 *Journal of Chemical Technology and Biotechnology* **81** 1353-67
- [25] Mittal G S 1992 *Lancaster: Technomic*
- [26] Fakruddin M, Islam M A, Ahmed M M and Chowdhury N 2013 *International Journal of Renewable and Sustainable Energy* **2**(4) 133-139
- [27] Adnan N A A, Suhaimi S N, Aziz S A, Hassan M A and Phang LY 2014 *Journal of Renewable Energy* **66** 625-633
- [28] Alam M Z, Kabbashi N A and Hussin S N I S 2009 *Journal of Industrial Microbiology & Biotechnology* **36** 801-808
- [29] Pramanik K 2003 *Journal of the Chinese Institute of Chemical Engineers* **34** 4) 487-42
- [30] Azhar S H M, Abdulla R, Jambo S A, Marbawi H, Gansau J A, Faik A A M and Rodrigues K F 2017 *Biochemistry and Biophysics Reports* **10** 52-61
- [31] Ahmad F, Zhang Z, William O S D and O'Hara I M 2019 *Renewable and Sustainable Energy Reviews* **109** 386-411
- [32] Hossain N, Zaini J and Mahlia T M I 2017 *International Journal of Technology* **1** 5-18
- [33] de Moura F A, Macagnan F T and da Silva L P 2014 *International Journal of Food Science and Technology* **50**(2) 275-81
- [34] Bougrier C, Delgenès J P and Carrère H 2008 *Chemical Engineering Journal* **139** 236-244
- [35] Hafid H S, Rahman N A, Md Shah U K, Baharudin A S 2015 *Journal of Environmental Management* **156** 290-98