A Factorial Analysis Study on Enzymatic Hydrolysis of Fiber Pressed Oil Palm Frond for Bioethanol Production

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Abstract. Different technologies have been developed to for the conversion of lignocellulosic biomass to suitable fermentation substrates for bioethanol production. The enzymatic conversion of cellulose seems to be the most promising technology as it is highly specific and does not produce substantial amounts of unwanted byproducts. The effects of agitation speed, enzyme loading, temperature, pH and reaction time on the conversion of glucose from fiber pressed oil palm frond (FPOPF) for bioethanol production were screened by statistical analysis using response surface methodology (RSM). A half fraction two-level factorial analysis with five factors was selected for the experimental design to determine the best enzymatic conditions that produce maximum amount of glucose. FPOPF was pre-treated with alkaline prior to enzymatic hydrolysis. The enzymatic hydrolysis was performed using a commercial enzyme Cellic CTec2. From this study, the highest yield of glucose concentration was 9.736 g/L at 72 hours reaction time at 35 °C, pH 5.6, and 1.5% (w/v) of enzyme loading. The model obtained was significant with p-value <0.0001. It is suggested that this model had a maximum point which is likely to be the optimum point and possible for the optimization process.

1. Introduction
According to Malaysian Innovation Agency in year 2011, the palm oil sector correspondingly generates the largest amount of biomass waste that is estimated around 80 million dry tons in 2010. It is expected to increase around 100 million dry tons by 2020 [1]. This huge amount of biomass waste may leads to serious disposal problem. Therefore, many studies have been conducted in turning this biomass waste into fermentable sugars by various methods and pretreatment and as a substitute for non-renewable energy sources. Oil palm frond (OPF) is currently been considered as one of the most sustainable lignocellulosic biomass (LCB) sources which are valuable and profitable to the agriculture industry. Presently OPF is used in local beef and dairy industry as source of fiber or as an ingredient in a complete fodder for ruminant animals in terms of pallets, silage or cubes [2]. Recent study showed

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that OPF was able to produce renewable sugars by simply pressing fresh OPF to obtain the juice [3]. The remaining from the pressing process, which is fiber pressed oil palm frond (FPOPF) was normally disposed. Due to its abundant availability and low cost materials, FPOPF has the potential as an alternative and renewable LCB sources for the production of many value-added products such as bioethanol. Alkaline pretreatment is the most effective pretreatment in increasing biomass digestibility by reducing lignin content. Sodium hydroxide (NaOH) was used in alkaline pretreatment because it can causes swelling of the biomass structure by increasing the internal surface of the cellulose and decreasing degree of polymerization that allows the lignin to broken down [4]. The use of enzymes in the hydrolysis of LCB is more effective that acid hydrolysis because enzymes are highly specific, can work at mild process condition and does not produce substantial amounts of unwanted byproducts [5]. However, most of the previous research focused on using purified enzyme which is expensive rather than commercial industrial enzymes. This study aimed to find the best condition in the enzymatic hydrolysis of pretreated FPOPF to produce glucose using CelliC CTec2. A half fraction two-level factorial analysis with five factors was studied for their effect to the enzymatic hydrolysis. This study is crucial for the optimization stage later because each main effects had the tendency to affect one another as the experiment runs.

2. Materials and Method
Oil palm frond (OPF) was obtained from a local palm oil plantation at Kuantan, Pahang. The OPF was pressed to remove juice by using sugarcane press machine. Then, fiber pressed oil palm fronds (FPOPFs) were dried under the sun for three days and then mechanically shredded into pieces using grinder. Subsequently, the shredded FPOPF were sieved to obtain fractions with a particle size less than 1 mm. Dried FPOPF was stored in sealed plastic bag at room temperature. Enzymatic hydrolysis was carried out using CelliC CTec2, a commercial enzyme preparation kindly provided by Novozymes (Denmark).

2.1 Biomass characterization
Characterization of FPOPF was done according to the analytical procedure by [6][7][8]. Extractives were determined in two steps of Soxhlet extraction. Next, the chlorination method (ASTM standard) was performed to determine the cellulose and hemicellulose content by removing the lignin [7][8]. The hemicellulose content was determined by subtracting the holocellulose content with the α-cellulose. Ash content was determined by using muffle furnace at 575 ± 25°C for 24 ± 6 hours (NREL standard). The sample was then cooled inside a desiccator before the measurement of sample mass using analytical balance.

2.2 Alkaline pretreatment
FPOPF sample was soaked in NaOH solutions with concentration 4.42% (w/v). The sample was treated at 100°C for an hour [9]. Then, the treated OPF was washed thoroughly with de-ionized water until turned to neutral before dried in the oven at 105°C and stored. The dried sample was used for enzymatic hydrolysis.

2.3 Two-level factorial analysis experimental setup
To identify the factors that affect the enzymatic hydrolysis, several factors were tested earlier and obtained from literature study. Five independent variables which are agitation speed (A), percentage (w/v) of enzyme loading (B), pH (C), temperature (D) and time (E) were selected for factorial designs of $2^5$-1 in order to screen their effect on the response of glucose yield as shown in Table 1. All experiments were carried out in triplicate and the averages were taken as responses.

2.4 Enzymatic hydrolysis
Enzymatic hydrolysis was carried out in 250 mL Erlenmeyer flask using 1.5 grams of pretreated FPOPF in the presence of sodium acetate buffer at different pH (pH 4 – pH 5.6) and enzyme loading
(1.5% – 6% w/v). The enzyme loading was calculated based on a gram of Cellic CTec2 was loaded for every 20 gram of substrate. The reaction mixture (30 mL) was incubated at different temperature (35°C – 65°C), reaction time (3 – 72 hours), and agitation speed (50 rpm – 200 rpm). Then, the hydrolysates were filtered for further analysis.

2.5 Glucose analysis
The hydrolysate was analysed by 3,5-dinitrosalicylic acid (DNS) method with glucose as a standard. 3 mL of sample was added to 3 mL of DNS reagent and placed in boiling water bath for 10 min. The mixture was cooled to room temperature and 1 mL of potassium sodium tartrate was added to maintain the colour of mixture. Then, reducing sugar was analysed using UV-Vis spectrophotometer with absorbance at 575 nm.

### Table 1. Experimental design of factor screening process using $2^{5-1}$ factorial design with respond.

| Run | Agitation speed, rpm | Enzyme loading, % | pH | Temp., °C | Time, hour | Glucose, g/L |
|-----|----------------------|-------------------|----|-----------|------------|--------------|
| 1   | 50                   | 1.5               | 5.6 | 65        | 72         | 7.372        |
| 2   | 50                   | 1.5               | 5.6 | 35        | 3          | 7.414        |
| 3   | 200                  | 6.0               | 4.0 | 65        | 3          | 0.850        |
| 4   | 200                  | 1.5               | 5.6 | 35        | 72         | 9.736        |
| 5   | 50                   | 6.0               | 4.0 | 35        | 3          | 0.276        |
| 6   | 200                  | 1.5               | 4.0 | 35        | 3          | 1.608        |
| 7   | 50                   | 1.5               | 4.0 | 65        | 3          | 0.251        |
| 8   | 50                   | 1.5               | 4.0 | 35        | 72         | 3.949        |
| 9   | 200                  | 6.0               | 5.6 | 35        | 3          | 7.482        |
| 10  | 200                  | 1.5               | 5.6 | 65        | 3          | 8.167        |
| 11  | 50                   | 6.0               | 5.6 | 35        | 72         | 7.647        |
| 12  | 200                  | 6.0               | 4.0 | 35        | 72         | 3.337        |
| 13  | 50                   | 6.0               | 5.6 | 65        | 3          | 8.153        |
| 14  | 50                   | 6.0               | 4.0 | 65        | 72         | 4.624        |
| 15  | 200                  | 1.5               | 4.0 | 65        | 72         | 4.179        |
| 16  | 200                  | 6.0               | 5.6 | 65        | 72         | 8.629        |

### Table 2. Characterization of raw FPOPF and pretreated FPOPF.

| Characterization | Raw FPOPF, % | Pretreated FPOPF, % |
|------------------|--------------|---------------------|
| Extractives      | 10.68        | 4.84                |
| Cellulose        | 25.19        | 45.42               |
| Hemicellulose    | 24.50        | 14.01               |
| Lignin           | 35.58        | 25.53               |
| Ashes            | 2.59         | 1.94                |
| Moisture content | 1.45         | 8.26                |

3. Results and Discussion

3.1 Biomass characterization
The result on the composition of FPOPF is shown in Table 2. The composition of extractives, hemicellulose, lignin and ashes in raw FPOPF were higher than the pretreated FPOPF. The differences of lignin composition from 35.58% to 25.53% showed that delignification was occurred during the alkaline pretreatment. The cellulose content in pretreated FPOPF was nearly double than raw FPOPF. It is proved that the alkaline pretreatment process has altered the structure of biomass by removing the lignin in order to make cellulose more accessible to the enzymes and later converted to fermentable sugars [10].
3.2 Factor affecting enzymatic hydrolysis

The effect of agitation speed, percentage (w/v) of enzyme loading, pH, temperature and time were evaluated using factorial analysis that consists of 16 run as shown in Table 1. The highest glucose concentration was obtained at 9.736 g/L where the hydrolysis conditions at 72 hours reaction time with temperature of 35°C, pH 5.6, and 1.5% (w/v) of enzyme loading. This finding was similar to the work by [11]. Thus, the presence of a sufficient amount of enzyme was important in order to obtain higher glucose yield during enzymatic hydrolysis. According to [12], the enzyme cost significantly contributed to the cost of biomass conversion process. Thus, by minimizing the enzyme dosage accordingly, this can reduce the cost of biomass conversion process. Eventually, in this present work, 1.5% (w/v) of enzyme loading was enough to perform a cellulose conversion during enzymatic hydrolysis. So, the cost of glucose in bioethanol production can be further reduced.

In percentage of contribution, the most important factor which gives the highest percentage contribution for enzymatic hydrolysis is pH with 82.18% followed by reaction time of 9.25%. Meanwhile, temperature shows the lowest contribution which is 0.024%. Other variables that less contributed to the effect of the enzymatic hydrolysis were agitation speed and percentage of enzyme loading (w/v) by having a value of 0.73% and 0.11% respectively.

From the ANOVA of this experimental design, the model obtained was significant as suggested by the model F value at 229.97 and low probability value (p-value <0.0001). The $R^2$ value obtained in this model was 0.9962, which is in good agreement with the adjusted $R^2$ value of 0.9919. The high $R^2$ value of 0.9962 indicates that the model was well adapted to the response. Equation 1 shows the response surface quadratic model for glucose production which can be presented in terms of coded factors as in the following equation:

$$\text{Glucose} = 5.23 + 0.27X_1 - 0.10X_2 + 2.85X_3 + 0.049X_4 + 0.95X_5 - 0.32X_1X_2 + 0.39X_2X_4 - 0.68X_3X_5$$

(1)

Where, $X_1 =$ agitation speed, $X_2 =$ enzyme loading, $X_3 =$ pH, $X_4 =$ temperature and $X_5 =$ time

Based on the empirical model, the coefficients of $X_1$ to $X_5$ are small compared to the interception. This gives an indicator of the existent of the design plateau as the other coefficient for the factors are lower than the interception at 5.23. This plateau showed that the design had a maximum point which is likely to be the optimum point and possible for the optimization process later.

4. Conclusion

The best condition for the enzymatic hydrolysis process of FPOPF using Cellic CTec2 was at pH 5.6, 35°C, enzyme loading of 1.5%, at 72 hours and 200 rpm. The model obtained had a maximum point which is likely to be the optimum point and possible for the optimization process later. Enzymatic hydrolysis using the commercial enzyme is looking more promising for the industrial application in the production of bioethanol. More work need to done in recycling the enzyme to further reduce the biomass conversion process.

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6. References

[1] Malaysian Innovation Agency (MIA) 2011 National Biomass Strategy 2020: New Wealth Creation for Malaysia’s Palm Oil Industry. Available at: http://www.
innovation.my/pdf/Imbas/National_Biomass_Strategy_Nov_2011_FINAL.pdf (accessed January 2013)

[2] Wan Zahari M, Abu Hassan O, Wong H K and Liang J B 2003 Journal of Anim. 16 625-634

[3] Zahari M A K M, Zakaria M R, Ariffin H, Mokhtar M N, Salihon J, Shirai Y and Hassan M A 2012 Journal of Bioresource Technology 110 566–71

[4] Taherzadeh M J and Karimi K 2008 Int. J. Mol. Sci. 9 1621-1651

[5] Zheng J, Choo K, Bradt C, Lehoux R and Rehmann L 2014 Biotechnology Reports 3 99-107

[6] Ehrman T 1994 Standard Method for the Determination of Extractives in Biomass. National Renewable Energy Laboratory (NREL)

[7] Wise L E, Murphy M and D’Addieco A A 1946 Paper Trade Journal 122 35–43

[8] Teramoto Y, Lee S H and Endo T 2009 Journal of Bioresource Technology 100 4783–4789

[9] Sukri S and Rahman R 2014 Romanian Biotechnological Letters 19 9006–9018

[10] Siti-Normah M D S, Sabiha-Hanim S, Noraishah A, 2012 Journal of waste.org 6 1391-1395

[11] Chen M, Xia L and Xue P 2000 Int Biodeter Biodegr. 59 85–89