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# Production of Fermentable Sugars by Oil Palm Fronds (OPF) Through Fungal Solid State Bioconversion

Khadijah Hanim Abdul Rahman<sup>1,2\*</sup>, Norainon Maulad Nashir<sup>1</sup>, Nor 'Izzah Zainuddin<sup>1</sup>

<sup>1</sup>Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Malaysia Perlis, Kampus Uniciti Alam, 02100 Sg.Chuchuh, Padang Besar, Perlis.

<sup>2</sup>Center of Excellence for Biomass Utilization, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia

\*E-mail: khadijahhanim@unimap.edu.my

**Abstract.** Oil palm fronds (OPF) are an abundant lignocellulosic waste generated from the oil palm field. In this study, efficient saccharification processes of oil palm fronds (OPF) through fungi bioconversion were explored. The objective of this study is to investigate the effectiveness of *Aspergillus niger* (*A.niger*), *Phanerochaete chrysosporium* (*P. chrysosporium*) and *Aspergillus terreus* (*A. terreus*) UniMAP AA-1 in producing fermentable sugar as well as the important parameters that can affects the bioconversion process. Solid-state fermentation process was carried out with the total working volume of 20 g at 30°C for 7 days. Throughout this study, *A. terreus* UniMAP AA-1 was found as the most potential microorganisms in converting cellulose into glucose compared to the other microorganisms, which were *A. niger* and *P. chrysosporium*. Optimization experiment of fermentable production from *Aspergillus terreus* UniMAP AA-1 was designed using Central Composite Design (CCD). CCD identified optimum condition for inoculum size, which was 4 % (w/v) and pH 8 in optimization study using Response Surface Methodology (RSM) and maximum fermentable sugar produced was 3.6732 mg/mL. The data obtained from this study may applicable for scale-up process of fermentable sugars production from OPF in the future.

## 1. Introduction

Currently, fermentable sugar production from lignocellulosic biomass is a significant research focuses in the production of renewable biofuels and other bioproducts. Malaysia is one of the most important agricultural countries in the world with exports including palm oil, cocoa and rubber. Higher increase in demand and supply, substantial amounts of lignocellulosic agriculture wastes are created after downstream processes [1][2]. In reality, most of the agricultural wastes generated came from oil palm fields [3]. From the data of lignocellulosic biomass produced in Malaysia in the year 2012, 83 million tonnes of solid wastes and 60 million tonnes of liquid wastes were generated from oil palm plantations. From the statistics, 75% of solid wastes generated is made up of oil palm fronds (OPF) [4]. Based on the data, OPF was considered as solid agricultural waste that abundantly available in oil palm plantations [5]. Instead of burning the OPF, a proper method of disposing and decaying the OPF are

<sup>1</sup> To whom any correspondence should be addressed.



needed. Conversion of OPF for the production of fermentable sugar is suggested as attractive approach instead of burning that can contribute to other environmental issues [6][7].

OPF are composed of cellulose, hemicellulose and lignin. About 35 to 50% cellulose, 25 to 30% hemicellulose and 20 to 25% lignin presents in the lignocellulosic biomass [8]. Due to the cellulosic and hemicellulosic content, OPF can be regarded as a source for sustainable and renewable feedstocks for the fermentable sugar production [9]. The lignocellulosic biomass requires hydrolysis process for the conversion of cellulosic/hemicellulosic components into fermentable sugars that can be utilized by microorganisms. Hydrolysis is defined as a process to convert complex polysaccharides in the raw feedstocks to simple sugars. The factors affecting the cellulose hydrolysis are accessible surface area available of the waste materials and crystallinity of cellulose fibre [10][11]. The composition of lignocellulose complex defines the accessibility of enzymes produced by microorganisms to its fibre components.

Pretreatment process plays a substantial role in the lignocellulosic conversion in order to weaken the structure of lignin and disrupting the crystalline structure of cellulose and hemicelluloses. The important of disrupting the lignin structure is to improve the enzyme breakthrough during the hydrolysis process and increase the surface area to enhance the production of fermentable sugars for biofuels production [12][13]. Cellulolytic enzyme produced by fungi can be utilised to breakdown the cellulose into its constituent sugars through enzymatic hydrolysis in solid state fermentation. Although cellulose might be hydrolysed by non-enzymatic methods, the method of microbial enzymatic hydrolysis application is much economic compared to the alternative methods (acid hydrolysis) because it is carried out in milder reaction conditions, higher product yields and less energy requirement [14][15][16]. Several parameters are required to be optimized as to intensively apply the enzymatic approach. The optimum condition applied may result higher production of reducing sugar produced.

The ultimate goals of this study is to determine the effectiveness of *A. niger*, *P. chrysosporium* and *A. terreus* strain UniMAP AA-1 in producing fermentable sugars. The optimize parameters were determined using the most potential fungal strain, *A. terreus* UniMAP AA-1 using RSM by CCD software. This optimization determined the optimum condition needed for each scope of the parameters used to bring on high fermentable sugar production.

## 2. Materials & Methods

### 2.1 Collection of Raw Material and Preparation of Substrate

The OPF were obtained from Kampung Gajah, Perak. The OPF were cut into small pieces, undergo drying and grinding process to increase the surface area for alkaline pretreatment reaction. The processed raw materials were washed thoroughly using distilled water and were placed in an oven at 60°C for 24 hours. The dried OPF were stored in an air-tight container and kept at room temperature until further use.

### 2.2 Pretreatment of Substrate

The OPF were further treated with 2 % (w/v) of sodium hydroxide (NaOH). In this procedure, 50 g of OPF were soaked in 500 mL of 2 % (w/v) NaOH at 30 °C for 4 h, followed by filtering and rinsing it with distilled water until it was completely free of alkali. The sample was then dried in an oven at 60°C for 24 hours and stored in the air-tight container at room temperature.

### 2.3 Preparation of Inoculums

Microorganisms used to ferment the OPF were *A. niger*, *A. terreus* strain UniMAP AA-1 and *P. chrysosporium*. All strains were obtained from School of Bioprocess Engineering Culture Collection, Universiti Malaysia Perlis. All culture plates were incubated at 30 °C for 7 days sporulation. The 7 days old spores were used as inoculums for fermentation. The inoculums were prepared by washing the growing culture with 25 ml sterile distilled water into the petri dish. The spore suspensions were

rubbed using hockey sticks and the contents were filtered using filter paper. The microbial suspension was then stored at 4 °C until further use.

#### 2.4 Growth Media Preparation

Growth media were prepared as a supplement for fungi growth. Growth media solution contains 0.2 % of  $\text{KH}_2\text{PO}_4$ , 0.5 % of  $\text{NH}_4\text{NO}_3$  and 0.1 % each of  $\text{NaCl}$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ . The solution was autoclaved for prior usage.

#### 2.5 Solid State Fermentation

All three types of fungi undergo solid state fermentation with constant condition of pH value of 5, growth temperature of 30 °C, moisture content of 60 % and inoculum size of 15 % with the total working volume of 20 g. All of the flasks were done in triplicate. The process was conducted in aseptic conditions to avoid any contamination to occur. Sampling was done for consecutively 7 days as to determine the growth profile of each microorganism and maximum reducing sugar production.

#### 2.6 Extraction of Fermentable Sugar

Crude fermentable sugar from the fermented OPF was extracted by a simple contact method. The fermented OPF were suspended in sterile distilled water and were incubated for 1 hour at room temperature in a rotary shaker at 150 rpm. The content of the flask were filtered using filter paper. The filtrates were further centrifuged for 30 minutes at 5000 rpm for 4 °C. Supernatant from the centrifugation process were analysed by Dinitrosalicylic (DNS) method.

#### 2.7 Optimization of Process Parameters

Optimization study was conducted through Central Composite Design (CCD) to find the optimum condition of the involved parameters in the fermentable sugar production. Inoculum size and pH were optimized and examined at low level (-1) and high level (+). Central point of 5 was applied in the design and based on the range, 13 runs were obtained as shown in Table 1. Substrate concentration was fixed to 30% (w/v) while moisture content percentage depends on the inoculum sizes used. Samples were incubated at 30 °C for 5 days.

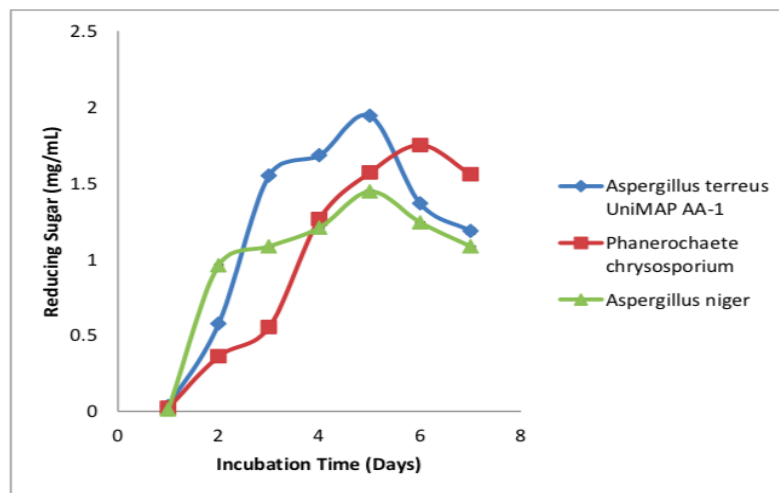
#### 2.8 Validation of the Model

In order to verify the adequacy of the developed CCD model, confirmations run were performed. The goals for each parameters was set to be in the range level while the fermentable sugar as the response was set to be at the maximum level.

### 3. Results and discussion

#### 3.1 Growth Profile Determination

Based on the growth profile in Figure 1, *A. terreus* strain UniMAP AA-1 showed the highest production of fermentable sugar compared to *P. chrysosporium* and *A. niger* at day 5. In this study, *A. niger* achieved its exponential phase faster than *P. chrysosporium* and *A. terreus* strain UniMAP AA-1. Towards the end of the growth *A. niger* consumed back the sugar produced thus made the production of sugar became lower. The sugar production of *P. chrysosporium* was lower than *A. terreus* strain UniMAP AA-1 because at the beginning of growth, *P. chrysosporium* secretes ligninase enzymes before secretes the cellulase enzymes. Due to that, the production of sugar is lower compared to *A. terreus* strain UniMAP AA-1 [17]. Other than that, *P. chrysosporium* and *A. niger* could not produce high reducing sugars as good as *A. terreus* strain UniMAP AA-1 presumably because their growth were also poor on the substrates and could not produce the enzymes needed effectively in order to degrade the lignocellulosic materials into sugars [18].



**Figure 1** Growth profile of fermentable sugars production by *Aspergillus niger*, *Aspergillus terreus* strain UniMAP AA-1 and *Phanerochaete chrysosporium*.

### 3.2 Optimization of Process Parameters Affecting Bioconversion

Using *A. terreus* UniMAP AA-1 as the most potential microorganisms, results obtained from CCD studies is presented in Table 1. The highest yield of fermentable sugar was observed at Run 2 with 3.6732 mg/mL. The lowest production was observed at Run 11 with the amount of fermentable sugar of 1.0546 mg/mL. ANOVA for the response surface is shown on the Table 2. Values of P less than 0.05 indicates that the model are significant. Since the  $p$ -value of the model is 0.0002, it implies that the model is significant. Corresponding with lower  $p$ -value, interaction terms  $A^2$  (Inoculum size) and  $B^2$  (pH) also are highly significant compared to others variable. Moreover, Lack of Fit test was employed in this optimization to determine the fitness of the model. Lack of Fit also is the variation of data around the fitted model. In this optimization, the model appeared to be adequate with no significant Lack of Fit of 0.2756.

The model also portrayed a good determination of coefficient which is 0.9520 which explained that 95.2 % of the variables (inoculum sizes and pH) attributed to the production. The high value of adjusted  $R^2$  that is 0.9177 also indicates a high significance of the model. In addition, high value of  $R^2$  also indicated better correlation between actual and predicted value. Coefficient of Variation (CV) obtained for this optimization is 11.71 % thus indicates a good reliability of the experiment. CV indicates the degree of precision. The more high value of CV shows that the reliability of experimental is lower [19]. Adequate precision ratio obtained also shows an adequate signal where its value is 12.944 that is more than ratio of 4 in this design.

**Table 1** : Optimization of fermentable sugar using CCD

Run	Factor 1 A: Inoculum Sizes (% w/v)	Factor 2 B:pH	Yield of Fermentable Sugar (mg/ml)
1	4.00	8.00	3.3357
<b>2</b>	<b>4.00</b>	<b>8.00</b>	<b>3.6732</b>
3	4.00	8.00	3.0885
4	4.00	8.00	3.3357
5	6.00	10.00	1.6433
6	1.17	8.00	2.0135
7	2.00	6.00	1.7905
8	6.83	8.00	2.2822
9	4.00	10.83	1.2163
10	4.00	5.17	1.0948
<b>11</b>	<b>2.00</b>	<b>10.00</b>	<b>1.0564</b>
12	4.00	8.00	3.4537
13	6.00	6.00	2.1670

**Table 2** : Optimization of fermentable sugar using CCD

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value	
Model	10.14	5	2.03	27.77	0.0002	Significant
A	0.23	1	0.23	3.09	0.1222	
B	0.15	1	0.15	2.02	0.1983	
AB	0.011	1	0.011	0.15	0.7086	
A <sup>2</sup>	2.47	1	2.47	33.84	0.0007	
B <sup>2</sup>	8.30	1	8.30	113.63	<0.0001	
Residual	0.51	7	0.073	-	-	
Lack of Fit	0.30	3	0.099	1.87	0.2756	Not
Pure Error	0.21	4	0.053	-	-	Significant
Cor Total	10.65	12	-	-	-	

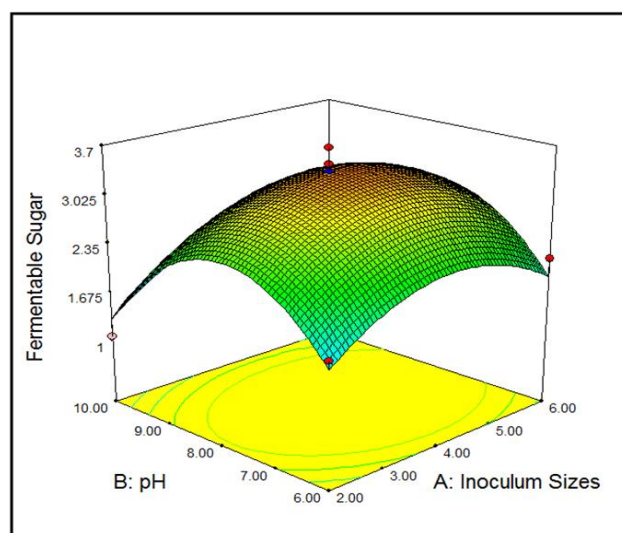
(Note : Variables are denoted in alphabetical order in which A : Inoculum Size (%)(w/v) and B : pH

Figure 2 showed the response surface and contour plot between inoculum sizes (A) and pH (B) towards the fermentable sugar production. Based on Figure 2, it showed that the amount of fermentable sugar increased to 3.6732 mg/mL when the inoculum sizes was increased from 2 % to 4 % (w/v) and pH of 6 to 8. Research done by Tanuja *et al.* [20] also has obtained its maximum fermentable sugar of 0.344 mg/mL using *Bacillus subtilis* and pretreated saw dust as a substrate. The optimum condition applied was 2 % (w/v) of inoculum size with pH of 8.

In comparison with this research, 3.6732 mg/mL fermentable sugar was produced at the optimum condition, where inoculum size is 4 % (w/v) with pH 8 were applied. This research shows some increment of fermentable sugar produced compared to research done by Tanuja *et al.* [20]. However, the yield of fermentable sugar started to decrease to 1.643 mg/mL when both condition were at

maximum in which inoculum size of 6 % (w/v) with pH at 10. This results shows that increment in inoculum size and pH both negatively affects the amount of fermentable sugars produced which is from 3.4537 mg/mL to 1.643 mg/mL. Based on Anusith *et al.* [21], an increase in inoculum sizes would ensure a rapid proliferation and biomass synthesis. However, overcrowding number of spores in the inoculum may deplete the necessary nutrient needed for the metabolite production. Optimum inoculum size for the reducing sugar production will ensure a balance between proliferating biomass and availability of nutrients that supported production of reducing sugar.

Range of pH that is not favorable by the microorganisms also can alter the enzyme characteristics. Optimization study of reducing sugar that has been done by Singhanian *et al.* [22] stated that variation of pH made the charge of the substrate and ionic components of the substrates changes thus affecting the enzyme activity. Due to that, only low amount of enzyme can be used to convert the cellulose into glucose thus reduced the amount of fermentable sugar produced.



**Figure 2** 3D Plot of Interaction between Inoculum Sizes (A) and pH (B) on Fermentable Sugar Production

### 3.3 Validation of the Model

The experimental value gives the amount of fermentable sugar of 2.8432 mg/mL while the predicted value is 2.96348 mg/mL. Percentage error obtained from this validation is 4.06 %. However, the value of experimental is still lower than predicted value thus makes this result as a good agreement. Therefore, this result confirms the validation of the model. The error occurred might due to the nature of the microorganisms and its biochemical reaction [23].

## 4. Conclusions

*Aspergillus terreus* UniMAP-AA1 was found to be the effective microorganism in this research compared to the other two microorganisms. Process parameters such as inoculum sizes and pH were then being optimized using RSM under CCD. During optimization, the highest production of fermentable of 3.6732 mg/mL were identified to be at 4 % (w/v) inoculum size and pH 8. Therefore, it was proven that oil palm fronds (OPF) have the potential to substitute other types of raw materials since it is so abundant in our country and can be used for the production of value added products.

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