Factors affecting cellulase production by Aspergillus fumigatus SK1 from solid state fermentation of oil palm empty fruit bunches using application of 2-level factorial design

Mohamadreza Soleimaninanadegani, M.S. Madihah and S.K. Ang

Abstract

A study was conducted to screen parameters affecting the production of cellulase by Aspergillus fumigatus SK1 on solid state fermentation of oil palm empty fruit bunch using the application of 2-level factorial design. Through the two-level full factorial design (2LFD), the experimental design was employed to screen the significant environmental factors for cellulase (CMCase, FPase and β-glucosidase) production. Full factorial design of four factors with six center point was conducted which consist of 22 runs. All data obtained were then used as input to the Design Expert software for further analysis, according to steps outline. ANOVA analysis showed that the models were significant (p < 0.0001) for CMCase, FPase and β-glucosidase. Inoculum size, temperature, moisture and ammonium sulphate were identified as significant factor affecting cellulase production on solid state fermentation. Statistical analysis revealed that the linear model is significant with $R^2$ value of 0.9569, 0.9867 and 0.9873 for CMCase, FPase and β-glucosidase production, respectively. Cellulase production was found to be high at the optimal condition were: inoculums size 109, moisture 74%, temperature 39 and ammonium sulphate 3.5 g/L. The obtained optimal process parameters predicted and verified by confirmation experiments with less than 5% error.

Keywords: Oil palm empty fruit bunch (OPEFB); Cellulase; 2-Level factorial design; Solid state fermentation

1 Introduction

In Malaysia, oil palm is one of the most significant agro-products, so after palm oil refining process a biomass resource such as oil palm empty fruit bunch (OPEFB) generate as an inexpensive lignocellulosic source material. In addition, these waste materials utilized as a combustion fuel for process heating generating steam- and electrical power in the oil refining process (Lam and Lee 2011). However, utilization of OPEFB as a significant substrate could reduce the waste pollution, which could make magnificent potential to achieve useful products such as enzyme (Villena and Gutiérrez-Correa 2006). Nowadays, cellulase has deduced substantial momentousness due to in various industry comprising food products, animal feeds, wastewater treatment process and fuel production (Ögel et al. 2001). Cellulase comprises of three different major composites which consisted of CMCase (Endoglucanase), FPase (Exoglucanase), β-glucosidase (Sun and Cheng 2002). OPEFB is composed of 17.6 % (w/w) lignin, 45 -50% (w/w) cellulose and 25-35% (w/w) hemicellulose (Sun and Cheng 2002). Solid state fermentation (SSF) delivers multitude advantages in processing of biodegradation in waste of agriculture fields, to compare with another fermentation system such as submerge fermentation (SmF); SSF require lower energy consumption, lower potential of bacterial contamination, simple technology and equipment for fermentation and fungal spores (Hölker et al. 2004). SSF evaluated as a vast system producing enzyme with high yield than SmF, and also appropriate for thermolabile products (Muller dos Santos et al. 2004). The difference in process control between SSF and SmF is mainly due to the use of solid substrates with very low moisture content in system. (Suryanarayan 2003). Although cellulase production has been widely carried out in several reports and using different agro-industry residues such as sugar cane bagasse, rice husk, rice straw, wheat straw, maize straw and other food processing waste under solid state fermentation (Silva et al. 2005; Lee et al. 2010; Chen et al. 2008; Jeya et al. 2009), thus, it is necessary to study on the production of cellulase by the microorganism under solid state fermentation conditions, which can degrade cellulose of the OPEFB with high productivity. However, the production of cellulase by Aspergillus fumigatus SK1 has been reported to be highly affected on oil palm trunk (OPT) and fermentation conditions (Ang et al. 2013). Therefore, the main objective of this study was to screening the optimal condition and significant parameters in cellulase production on OPEFB involved in solid-state fermentation, in order to enhance cellulase production by Aspergillus fumigatus SK1. In addition, the effects of four significant variables on CMCase, FPase and β-glucosidase production were evaluated in 2-Level Factorial Design (2LFD), and the study verified and confirmed the model to assess the coefficients.
2 Materials and methods

2.1 Microorganisms and inoculum preparation

The fungus, Aspergillus fumigatus SK1, used in this study for cellulase production obtained from the stock culture of biorefinery laboratory of Biotechnology and Medical Engineering Department, Universiti Teknologi Malaysia UTM. Inoculum was prepared by maintaining the fungus on potato dextrose agar (Difco) plates at room temperature for 7 days then the fungus grown well. Spores were harvested using 1% (v/v) Tween-80 and collected by centrifuging at 4000 rpm for 20 min (4°C). The spores were diluted in order to obtain spore inoculum of 10^7-10^9 spores/g of OPEFB.

2.2 Substrate and physical pretreatment

OPEFB was used as a substrate in this study, which obtained from Department of Biotechnology, Universiti Putra Malaysia (UPM), Serdang. The final moisture was defined by commercial moisture analyser (MX50, AD Weighing Co., Ltd., Japan) to determine the OPEFB water contain and adjust the standard curve for moisture, which is one of the selected factors in this study. And OPEFB in this study used based on untreated substrate.

2.3 Medium for cellulase production

Mandel and Weber medium was used for production of cellulase from Aspergillus fumigatus SK1. The medium composition was (g/L): KH_2PO_4, 2.0; (NH_4)_2SO_4, 1.4; MgSO_4, 0.3; CaCl_2, 0.3; peptone, 0.75; urea, 0.3; carboxymethyl cellulose, 10; 2 ml of Tween 80; and trace elements, 1.0 ml with pH 5. Trace elements composition includes (g/L): FeSO_4, 5.0 × 10^{-3}; MnSO_4.7H_2O, 1.4 × 10^{-3}; ZnSO_4.7H_2O, 1.6 × 10^{-3}; and CoCl_2, 2.0 × 10^{-5}. The production medium was then autoclaved at 121 °C, 1.03 bar for 20 min.

2.4 Cellulase production under solid state fermentation

The 2-Level Factorial Design (2LFD), was applied to modeling in solid state fermentation, which carried out in 250 ml Erlenmeyer flasks, each having 15 g of OPEFB, moistened adjust to medium (pH 5.5) to attain the final substrate-to-moisture ratio according to 2 LFD design. The flasks were sterilized by autoclaving at 121 °C (15 psi), and thereafter cooled to room temperature and inoculated with desired volume of inoculum according to design 2 LFD (0.5% w/w). The spores distribute well throughout the substrate and incubated. The fungal fermented OPEFB was aseptically removed from flasks after an appropriate interval, suspended in 25 mL of cold 0.05 M sodium acetate buffer and vortex gently at maximum speed for 2 min to extract cellulase enzymes. The combination was centrifugation at 4000 rpm for 30 min at 4 °C to obtain the supernatant which contained the crude enzymes. The combination of crude cellulase was used accordingly to determine cellulase activity (endoglucanase activity, exoglucanase activity and β-glucosidase activity), reducing sugar concentration and protein concentration. All experiments and analysis were carried out in triplicate. Degradation in different conditions was measured according to 2-Level Factorial Design. Table 1 shows the experimental variables studied.

Table 1: Variables in real values, for screening by the 2-level factorial

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>low level (-)</th>
<th>High level (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Initial level of inoculum</td>
<td>10^{-9}</td>
<td>10^{-7}</td>
<td></td>
</tr>
<tr>
<td>B Moisture</td>
<td>%</td>
<td>65</td>
<td>80</td>
</tr>
<tr>
<td>C Temperatures</td>
<td>°C</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>D Ammonium sulphate</td>
<td>g/L</td>
<td>1.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>

2.5 Analysis procedure

The total cellulase (CMCase, FPase and β-glucosidase), were determined by using the method described by Wood et al. (1988). Endoglucanase activity (CMCase) was measured as using a reaction mixture containing 0.5 ml of 2% (W/V) carboxymethyl cellulose in 0.05 M sodium acetate buffer (pH 5.0) and with0.5 ml suitably diluted crude enzyme for 30 min at 50°C to develop the reaction between CMCase in sample and reaction mixture. After cooling, absorbance was read at 540 nm. Endoglucanase and exoglucanase were carried out in sodium acetate buffer (0.05 M, pH 5), with 0.5 ml and 1 ml suitably diluted crude enzyme respectively. Exoglucanase (FPase) assay was carried out by Whatman number 1 filter paper strip (1 cm × 6 cm, 50 mg) incubating in 0.5 mL suitably diluted crude enzyme with 1 ml sodium acetate buffer (0.05 M, pH 5) for 60 min at 60°C. The reaction was terminated by adding 1 mL dinitrosalicylic acid (DNS) and 2 drops of 0.1 M sodium hydroxide by subsequently placing the test tubes in a water bath at 100°C for 15 min. After cooling the tubes, the intensity of the color was read at 540 nm in UV-VIS spectrophotometer (Miller 1959). β-glucosidase production was determined by measuring the p-nitrophenol released after incubating the mixture of 0.5 ml of crude enzyme with 1.6 mL of sodium acetate buffer (0.05 M, pH 5), 1 mL of p-nitrophenyl- β-d-glucoside (pNPNG) for 30 min at 70°C, followed by addition of 2 mL glycine buffer (0.4 M, pH 10.8). One unit of endoglucanase and exoglucanase (FPase) activity correspondent to 1 μmole of glucose released per minute. Protein content was determined by the method of using bovine serum albumin as a standard (Lowry method). Determination of reducing sugar using 3,5-dinitrosalicylic acid (DNS method) is based on the capability of glucose as reducing sugar which can reduce the oxidized form of DNS reagent. The reduced form of DNS reagent is measured using spectrophotometry at 540 nm wavelength (Lowry et al. 1951).

2.6 Experimental design

Design and optimal of model for the improvement and design of product often involved various variables that likely to be significant in design of expert. Full factorial design (2k) is a common experimental design used in the screening experiment (Myers and Anderson-
Cook 2009). In factorial designs, many factors have been extensively used in the manufacturing industry as a means of high yield output for a given input of resources (Borror et al. 1997). The main strategy of using design of expert in this study is to use the design to identify the most important factors and levels of the factors that determine responses and then to use these factors in normal production (Calleri de Milan et al. 1992).

### 2.7 Two-level factorial design

A factorial model is composed of a list of coefficients multiplied by associated factor. A factorial design model can be presented as: \( \text{Response} = b_0 + b_1A + b_2B + b_3C + b_4D + b_{12}AB + b_{13}AC + \ldots \) Where \( b_n \) is the coefficient associated with the \( n \)th factor, and the letters, A, B, C, . . . represent the factors in the model. Four variables, which were expected to have an effect on OPEFB enzymatic degradation, were identified by early experiments. The variables having most significant effect on enzymatic degradation of OPEFB were then identified using a 2LFD. The variables that were included in the screening experiment and their settings are listed in Table 1. Each independent variable was investigated at a high (+1) and a low (1) level. A \( 2^4 \) full factorial experimental design based on four independent variables, namely Initial level of inoculums, (A) Moisture (B), Temperatures (C) and Ammonium sulphate (D) were investigated by two-level full factorial design and ANOVA to identify the important factors that exerted a significant effect on the cellulase (Bairagi et al. 2007). Table 2 shows the design matrix covering four variables to evaluate their effect on enzymatic degradation of OPEFB; it also gives the response evaluated as reducing sugar (g/L). The runs were randomized for statistical reasons. The variables having major effects on enzymatic degradation of OPEFB were identified on the basis of confidence levels above 95% (\( P < 0.05 \)). The significant factors and interactions identified from the half normal plot analysis were chosen for generating the first-order model for the response of texture after the effects and interactions were evaluated.

The significance of linear effects of the four variables was evaluated by variance analysis (ANOVA). The coefficient of determination of \( R^2 \) (pronounced r-square) and adjusted \( R^2 \) coefficient was used for evaluation of the fit of the model. The statistical significance of the second-order model equation was determined by a significant F-value and an insignificant lack-of-fit F-value (Myers and Anderson-Cook 2009). The modeling and statistical analysis was performed using by Design Expert® Software, Version 6.0 (Stat-Ease Inc. Minneapolis). The predicted optimal value was confirmed by the experiment using the selected optimum values of the five variables.

### 3 Results and discussion

#### 3.1 Two-Level factorial design

2LFD was used as a screening method to determine which of the four variables affect on CMCase, FPase and \( \beta \)-glucosidase production in SSF system (Table 1). Variables having the most significant effects on the cel-
lulase production on OPEFB under 22 experiments in SSF system. Screening design was used to detect the factors or independent variables that had a higher impact on the response variable—CMCase, FPase and β-glucosidase. Table 3 shows the predicted levels of cellulase production from along with experimental data. The half normal plot can be used to determine the significant factors affecting the response. The factors points that lie along the line are negligible and the rest of the factors and their cross-interaction give significant effect towards the response (Bairagi et al. 2007).

The effect of ammonium sulphate (factor D) in CMCase production (Fig 1) and effect of temperature (factor C) in FPase and β-glucosidase (Fig 2, Fig 3) obviously falls far away from the line, adding understanding that it represents a strong signal. This result correlates well with the P value <0.0001. The effect of inoculum (factor A), moisture (factor B), temperatures (factor C) and ammonium sulphate (factor D) was a significant variable are provided in Fig 1, Fig 2 and Fig 3 for all responses except factor A for β-glucosidase. Next, the data shows that the interaction effect of factors of CMCase plot are: ABD, ABC, AD, BCD and CD. Moreover, in FPase plot factor BC, AC, ABD, BCD, CD, BD and in β-glucosidase plot factor BCD, BD, AC, AB, ACD and BC are all significant with different degrees of importance. Regression coefficient, $R^2$ value of 0.9569, 0.9867 and 0.9873 suggests model adequacy and depict that the models are effective and can be accepted for CMCase, FPase and β-glucosidase production, respectively. With such regression coefficient, it shows that equation (1), (2) and (3) represent the true relationship between the variables in this fermentation process. The $R^2$ value of 0.9569, 0.9867 and 0.9873 were in reasonable agreement with the adjusted $R^2$ value of 0.8808, 0.9733 and 0.9684 for CMCase, FPase and β-glucosidase production, respectively. The vicinity of adjusted $R^2$ to $R^2$ means a good adjustment of the theoretical values of the experimental data from the model (Bahrin et al. 2012). Hence, without further amendment of the reduced model for FPase, CMCase and β-glucosidase production based on regression model provides an excellent explanation of the relationship between the independent variables and the response. ANOVA for CMCase, FPase and β-glucosidase model was obtained in Table 3, Table 4, Table 5, respectively. ANOVA was used for statistical analysis and regression model. It shows that the P-value obtained was <0.0001, which indicated a smaller p-value compared to the desired significant level of $P = 0.05$, therefore, models are desirable as it indicates that the terms in the model have a significant effect on cellulase production on palm oil empty fruit bunch in this model. Table 3, Table 4 and Table 5 shows the resulting ANOVA table for the improved model for response length. However, the backward elimination procedure in Design Expert software, it was automatically reduced the terms that are not significant in the model.

Temperature is one of the vital environmental factors which could influence enzyme activity and is essential for a fermentation process (Rastogi et al. 2010).
Table 3: Regression analysis (ANOVA) for the CMCase production from in solid state fermentation SSF

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>F-value</th>
<th>P &gt; F</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>252.114</td>
<td>10</td>
<td>25.211</td>
<td>22.190(^a)</td>
<td>&lt; 0.0001(^b)</td>
<td>0.9569</td>
</tr>
<tr>
<td>Residual</td>
<td>11.361</td>
<td>10</td>
<td>1.136</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>1.515</td>
<td>5</td>
<td>0.303</td>
<td>0.153</td>
<td>0 · 9696</td>
<td></td>
</tr>
<tr>
<td>Pure error</td>
<td>9.846</td>
<td>5</td>
<td>1.969</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>total 263.496</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)F-value is significant. \(^b\)Model is significant, with P > F less than 0.05.
\(^c\)Model is fit due to insignificant F-value. Standard deviation is 1.25. Adjusted R\(^2\) = 0.8808

Table 4: Regression analysis (ANOVA) for the FPase production from in solid state fermentation SSF

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>F-value</th>
<th>P &gt; F</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2.832</td>
<td>10</td>
<td>0.283</td>
<td>74 · 022(^a)</td>
<td>&lt; 0.0001(^b)</td>
<td>0.9867</td>
</tr>
<tr>
<td>Residual</td>
<td>0.038</td>
<td>10</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>0.027</td>
<td>5</td>
<td>0.005</td>
<td>2.585</td>
<td>0 · 1603</td>
<td></td>
</tr>
<tr>
<td>Pure error</td>
<td>0.01</td>
<td>5</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>total 3.059</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)F-value is significant. \(^b\)Model is significant, with P > F less than 0.05.
\(^c\)Model is fit due to insignificant F-value. Standard deviation is 0.062. Adjusted R\(^2\) = 0.9733

Table 5: Regression analysis (ANOVA) for the β-glucosidase production from in solid state fermentation SSF

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>F-value</th>
<th>P &gt; F</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>322.11</td>
<td>12</td>
<td>26.842</td>
<td>52 · 001(^a)</td>
<td>&lt; 0.0001(^b)</td>
<td>0.9873</td>
</tr>
<tr>
<td>Residual</td>
<td>4.13</td>
<td>8</td>
<td>0.516</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>2.37</td>
<td>3</td>
<td>0.79</td>
<td>2.246</td>
<td>0 · 2007</td>
<td></td>
</tr>
<tr>
<td>Pure error</td>
<td>1.76</td>
<td>5</td>
<td>0.351</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>total 515.17</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)F-value is significant. \(^b\)Model is significant, with P > F less than 0.05.
\(^c\)Model is fit due to insignificant F-value. Standard deviation is 0.72. Adjusted R\(^2\) = 0.9684

It was observed that when the culture temperature increased to an optimum level of 45°C an enhancement in CMCase activity was achieved (Ang et al. 2013). Correspondingly, at higher temperature, the thermal denaturation of enzymes of the metabolic pathway could result in decreased enzyme production (Rajoka 2004). Temperature is important as it provides thermal denaturation of enzymes of the metabolic pathway for release energy for the reactants to reach transition state and consequently increases the rate of degradation of OPEFB.

3.2 Regression analysis

It is possible also to predict the outcome with a simple equation that uses the overall average modified up or down depending on the level for each factor (Abdul-Wahab and Abdo 2007). This equation is called the coded equation since the (+1) and the (-1) value are used to represent high and low levels, respectively. The data obtained was found to best regression fit model. Responses obtained from the experiments were analysed using analysis of variance (ANOVA). The regression equation for CMCase, FPase and β-glucosidase production (U/g) obtained are as in equation (1), (2) and (3) respectively, which correlated with the factors can be written as:

\[
Y_1 = +8.08 -0.77 \cdot A +1.52 \cdot B +0.83 \cdot C +2.97 \cdot D +0.66 \cdot A \cdot D +1.26 \cdot B \cdot C -0.45 \cdot C \cdot D -0.66 \cdot A \cdot B \cdot C +0.67 \cdot A \cdot B \cdot D +0.49 \cdot B \cdot C \cdot D \quad (1)
\]

\[
Y_2 = +0.53 +0.12 \cdot A -0.081 \cdot B -0.32 \cdot C -0.10 \cdot A \cdot C +0.15 \cdot B \cdot C -0.032 \cdot B \cdot D +0.054 \cdot C \cdot D -0.093 \cdot A \cdot B \cdot D +0.075 \cdot B \cdot C \cdot D +0.068 \cdot A \cdot B \cdot C \cdot D \quad (2)
\]

\[
Y_3 = +14.24 -0.010 \cdot A +1.62 \cdot B +2.35 \cdot C +1.97 \cdot D -0.92 \cdot A \cdot B +1.17 \cdot A \cdot C +0.31 \cdot A \cdot D -0.66 \cdot B \cdot C +1.43 \cdot B \cdot D +0.067 \cdot C \cdot D +0.91 \cdot A \cdot C \cdot D -1.57 \cdot B \cdot C \cdot D \quad (3)
\]

A = Inoculum; B = Moisture (%); C = temperature (°C) and D = Ammonium sulphate (g/L)

Where Y1, Y2 and Y3 represents CMCase, FPase and β-glucosidase concentration (U/g) while A, B, C and D represents inoculum, moisture (%), temperature (°C) and ammonium sulphate (g/L), respectively. These regression models were generated by the Design Expert® Software for accurate models, after the models had been considered all the variables. The predicted levels of CMCase, FPase and β-glucosidase enzyme obtained from enzymatic production on OPEFB in SSF at each experimental point using equation (1), (2) and (3) are shown in Table 2 along with experimental data.

3.3 Response analysis

Using Design-Expert software statistical analysis of the data is performed. The results came through a half-normal plot in the ranks of the absolute value of various effects are determined. Four potential independent
variables, namely inoculum (A), temperature (B), moisture (C) and ammonium sulphate (D) were investigated by ANOVA to identify the important factors in cellulase production in SSF. The half normal plot can be used to determine the significant factors affecting the response. This plot will determine the ranks of the absolute value of various effects. The factors points that lie along the line are negligible and the rest of the factors and their cross-interaction give significant effect towards the response. (Jain et al. 2011). Lack of fit indicates how well the chosen model fits the data. With fewer than five or six replicates, the lack of fit test has a very low power because too few centre points inflate the error (Ba¸s and Boyacı 2007). According to half normal plot analysis (Fig 1, Fig 2, Fig 3), the insignificant effects fall along the straight line extending from the origin if the observed effects and interactions are due to solely to the experimental error and are normally distributed. Any effects considerably to the right side of this line were considered to be statistically significant. The assumption of linearity and normality were checked by residual plot analysis of CMCase, FPase and β-glucosidase as shown in Fig 4, Fig 5 and Fig 6, respectively. The residual plots of the model are randomly distributed along the line of identity. This result gives good indication of excellent predictions of maximum response alongside constant variance and adequacy of the linear regression model (Jo et al. 2008).

As a feature under diagnostic plots in Design Expert serve a quick view of any abnormality of the response from the reduce model. Most of the plots displayed studentised form of residuals which showed how well the model satisfied the assumptions of analysis of variance (Fig 7, Fig 8 and Fig 9). The sufficiency of the primary model should be checked before the conclusions from the analysis of variance are adopted. Based on statistical tests, examination of residuals was used for investigation of serious violation of the basic assumptions and
Figure 8: Plot of studentized residuals versus predicted response of FPase enzyme from OPEFB enzymatic degradation

Figure 9: Plot of studentized residuals versus predicted response of FPase enzyme from OPEFB enzymatic degradation

Model adequacy. Residual versus predicted plot for CMCase, FPase and β-glucosidase production are normally distributed and the equality of variance does not seem to be violated. There is no severe indication of non-normality, nor are there any verification pointing to possible normal plot and the equality of variance assumption does not seem to be violated.

Model checking using the normal plot of residuals indicates that it is also normally distributed and it resembles a straight line. Most of the plots displayed normal plot and residual versus predicted form of residuals which depict how satisfaction of models as well the assumptions of analysis of variance. Plots residual versus experimental run order, we’re used to test for any confounding variables that might influence the cellulase production during the experiment. In fact, the vast analysing plots do not indicate a serious problem in all responses data of cellulase production as results.

3.4 Optimal designs

The experiments showed the effect of different parameter interaction to produce cellulase enzyme in SSF system. Thus, in order to achieve a combination wherein maximal of cellulase was occurring, optimization was carried out using a numerical option of the Design expert software. The design expert in the optimization section provides optimal designs with the different desirability factors which calculated based on the best fit models for CMCase, FPase and β-glucosidase production as an alternative solution (Table 6). First solution shows the highest desirability 93.5% and its alternative solution with different desirability.

The minimum and maximum must be provided for each parameter included. A weight can be assigned to each goal to adjust the shape of its particular desirability function. By combining desirability functions, can identify an overall desirability. A Linear Programming model seeks to maximize this function. The goal seeking begins at a random starting point and proceeds up the steepest slope to a maximum. The settings required to optimize different designs starting from several points in the design space improve the chances of finding the best desirability among all the probability (Saha et al. 2010; Senthilkumaar et al. 2006). Fig 10 shows the ramps of various factors. Table 4, disclosed that the overall desirability values are high in the region of CMCase, FPase and β-glucosidase production.

3.5 Confirmation experiments

To support the optimized data given by numerical modelling under optimized conditions, the confirmation experiments were conducted with the optimum values of the process. The parameters as suggested by the model (inoculums:10^9, moisture: 73.94, temperature: 39.11 and ammonium sulphate: 3.50). Finally, for their validation, triplicate confirmatory experiments were conducted using the optimized parameters. The experimentally obtained values of CMCase, FPase and β-glucosidase were 11.801 U/g, 0.668 U/g and 15.366 U/g, respectively. The results are closely related to the data obtained from optimization analysis, resulting in a very good agreement. The difference between the experimental result and model predicted values is less than 5% of all the four responses. Comparisons between the experimental result and model predicted values confirmed that the models are accurately adequate for predicting the responses. The nature of oil palm empty fruit bunches is limited by the great bulk of material, slow degradation in the field (Celik et al. 2010). In addition, aeration limitations were probably the reasons for the longer degradation time in the flasks study. However, the OPEFB must be disposed of in order to make way for the next process.

4 Conclusion

The purpose of this investigation was to screening the factors that affecting the cellulase (CMCase, FPase
Table 6: Desirability of inoculum size, moisture, temperature and ammonium sulphate to the responses of CMCase, FPase and β-glucosidase production

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Moisture</th>
<th>Temperature</th>
<th>Ammonium sulphate</th>
<th>CMCase</th>
<th>FPase</th>
<th>β-glucosidase</th>
<th>Desirability</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.71</td>
<td>80</td>
<td>44.43</td>
<td>3.5</td>
<td>14.556</td>
<td>0.38</td>
<td>20.295</td>
<td>0.883</td>
</tr>
</tbody>
</table>

Figure 10: Desirability ramp for numerical optimization of four three responses based on four variable, namely the inoculums, temperature, moisture and ammonium sulphate

and β-glucosidase) production by Aspergillus fumigatus SK1 using 2-level full factorial design which consisted of twenty-two experiments through the solid state fermentation. The six experiments organized of the central point for each stage and the remaining sixteen experiment of design. The maximum, CMCase, FPase and β-glucosidase for twenty–two experiments were detected at 15th, 20th, and 10th run respectively. By using 2LF, a maximum level of CMCase, FPase and β-glucosidase of 21.1729 U/g, 1.608 U/g and 22.216 U/g, respectively were produced at 15th, 20th, and 10th run respectively. The effect of factor A, B, C and factor D were found to be significant factors that could affect the process of enzymatic degradation of OPEFB. Finally, recommended parameters as a solution report for optimal condition of CMCase, FPase and β-glucosidase. Further research is needed to explore the full potential of enzyme production by the central composite design (CCD) to identify the optimum conditions for fermentation of enzymatic degradation of OPEFB by Aspergillus fumigatus SK1 in SSF by analyzing the relationships among a number of parameters that affect the overall process.

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