IMPROVED CELLULASE PRODUCTION BY *Aspergillus terreus* USING OIL PALM EMPTY FRUIT BUNCH FIBRE AS SUBSTRATE IN A STIRRED TANK BIOREACTOR THROUGH OPTIMIZATION OF THE FERMENTATION CONDITIONS

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Response surface methodology (RSM) was performed to evaluate the effects of dissolved oxygen tension (DOT) and initial pH on the production of carboxymethyl cellulase (CMCase), filter-paper hydrolase (FPase), and β-glucosidase by *Aspergillus terreus* in a 2 L stirred tank bioreactor. Delignified oil palm empty fruit bunch (OPEFB) fibre was used as the main substrate under submerged fermentation. Growth of *A. terreus* and the production of three main components of cellulase were optimized by central composite design (CCD) design. Statistical analysis of results showed that the individual terms of these two variables (DOT and pH) had significant effects on growth and the production of all components of cellulase. Maximum growth (13.07 g/L) and cellulase activity (CMCase = 50.33 U/mL, FPase = 2.29 U/mL and β-glucosidase = 15.98 U/ml) were obtained when the DOT and initial culture pH were set at 55% and 5.5, respectively. A high proportion of β-glucosidase to FPase (8:1) in cellulase of *A. terreus* could be beneficial for efficient hydrolysis of cellulosic materials. The use of OPEFB as a main substrate would reduce the cost of fermentation for the production of cellulase.

Keywords: Oil palm empty fruit bunch; Cellulase; Aspergillus terreus; Dissolved oxygen tension; Response surface methodology; Submerged fermentation

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INTRODUCTION

Lignocellulosic wastes refer to plant biomass wastes that are composed of cellulose, hemicellulose, and lignin. The lignocellulosic biomass, which represents the largest renewable reservoir of potentially fermentable carbohydrates on earth, is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries (Mtui and Nakamura 2005). The cellulase enzyme plays an important role in natural biodegradation processes, in which plant lignocellulosic materials are degraded by a wide variety of fungi, bacteria, actinomycetes, and protozoa (Peciulyte 2007). The processes related to the hydrolysis of lignocellulosic materials are usually applied in the chemical industry for the production of fermentable sugars, ethanol, organic acids, detergents, and other chemicals (Howard et al. 2003). In addition, cellulases find significant application in

the pulp and paper industry, textile industry, animal feed and food industry, and also in processing cellophane and rayon (Peciulyte 2007). Cellulase is potentially valuable for industrial saccharification of cellulosic biomass (Chinedu et al. 2008). The rising attention in the conversion of lignocellulosic materials into bulk chemicals and biofuels as a means of reducing energy deficiency has intensified the search for highly active and specific cellulases (Chinedu et al. 2008; Howard et al. 2003). Fungal cellulases are extracellular and inducible, and they normally include three main components known as carboxymethylcellulase (CMCase), filter-paper hydrolase (FPase), and β-glucosidase (Hanif et al. 2004). These enzymes synergistically break cellulose into fragments having two or three glucose units (Willey et al. 2008).

The rate of cellulase production is influenced by environmental conditions, components of nutrient medium that might act as inducers or repressors, cell density, and growth rate (Umikalsom et al. 1998). The effect of agitation intensity or shear rate on the cellulase-producing activity of fungal microorganisms has been well investigated (Lejeune and Baron 1995; Mukataka et al. 1988). But little attention has been paid to the effect of dissolved oxygen tension (DOT) in the culture throughout the fermentation at a fixed agitation speed on the synthesis of all three major components of cellulase. In many cases of cellulase production in stirred tank bioreactors, the DOT was not allowed to drop under a critical level, which was about 20 to 40% of saturation (Chaudhuri and Sahai 1993; Lejeune and Baron 1995).

An oil palm empty fruit bunch (OPEFB) is a type of lignocellulosic residues that typically contain 50% cellulose, 25% hemicellulose, and 25% lignin in their cell wall (Alam et al. 2005). The OPEFB fibre is a suitable renewable raw material for bioconversion into value-added products because it is easily accessible, abundant locally, and rich in lignocellulose. To the best of our information, no report has been found on cellulase production by *Aspergillus terreus* in a stirred tank bioreactor. A mathematical method such as response surface methodology (RSM) is a useful statistical method for studying the effect of factors influencing the response by varying them simultaneously using a limited number of experimental data (Neter et al. 1996).

The main objective of this study was to evaluate the effect of dissolved oxygen tension (DOT) level and initial culture pH on growth of *A. terreus* and cellulase production in 2 L stirred tank bioreactor using OPEFB as substrate. These two factors were subsequently optimized using RSM for improvement of all the three main components of cellulase (CMCase, FPase, and ß-glucosidase) production by *A. terreus*.

MATERIALS AND METHODS

Microorganism

The fungus, *A. terreus,* used in this study for cellulase production was isolated from a sample collected from the oil palm empty fruit bunch (OPEFB) compost from a local factory (Sri Ulu Langat, Dengkil, Selangor, Malaysia). Details of the method of isolation and identification of this fungus have been described in our previous paper (Shahriarinour et al. 2011a). *A. terreus* was grown on potato dextrose agar (Difco) at 30 °C for 7 days to allow the development of spores and then stored at 4 °C until use in inoculum preparation.

Medium and Inoculum Preparation

The basal medium as proposed by Mandels and Weber (1969) was used for cellulase production. In all experiments, yeast extract (8 g/L) and delignified OPEFB fibre (13.9 g/L) were added as a major nitrogen and carbon source to the basal medium, respectively. The delignified OPEFB fibres were prepared by treating the OPEFB fibres using physico-chemical and biological treatment as described in our previous study (Shahriarinour et al. 2011). For inoculum preparation, spores were harvested from the PDA slants using a sterile 0.01% (v/v) Tween 80 solution with the aid of wire loop. The spore suspension containing an average of 6×10^7 spores/mL was used as an inoculum in all the fermentations.

Fermentation

All fermentation experiments were carried out in a 2 L stirred tank bioreactor (B. Braun, Biostat B, Melsungen, Germany). Two six-bladed Rushton turbine impellers with a diameter (D) of 52 mm mounted on the agitator shaft were used for agitation. The bioreactor was equipped with temperature and dissolved oxygen controllers. Air was supplied to the culture through a single air sparger (0.1 mm internal diameter). During the fermentation, agitation speed (N) was fixed at 225 rev/min (impeller tip speed=$2\pi Nr$ =0.613 m/s), and DOT in the culture broth was controlled using a sequential cascade control of airflow rate. The maximum and minimum set points of permitted airflow rates were 1.5 L/min and 0.1 L/min, respectively. A polarographic dissolved oxygen probe (Ingold, Urdorf, Switzerland) was used to measure the DOT level in the culture. The output of the oxygen master controller works directly on the set point input value of the airflow controller. In all cases, DOT was successfully controlled within ± 2% of the specified set points (31, 40, 60, 80, and 88% saturation). The initial pH values (4.7, 5, 5.5, 6, and 6.2) of the culture were adjusted to appropriate values either by the addition of 1 N HCl or 1 N NaOH. The temperature within the bioreactor was controlled at 29 °C. Spore suspension (15 mL) was inoculated into the bioreactor containing 1.5 L medium. During the fermentation, samples were withdrawn at regular time intervals for analysis.

Crude Enzyme Extraction

After an appropriate time of incubation, the cultures were harvested at 24 h intervals and centrifuged at 18,500 × g (RTH 250 Rotor, Sorvall RT7 Plus) at 4 °C for 15 min. The supernatant was then analyzed for soluble protein and extracellular enzyme activities.

Analytical Procedure

A chemical method based on the measurement of acetylglucosamine was adopted to estimate the growth of A. *terreus*, since the physical separation of mycelium from the OPEFB fibres for measurement was not possible (Khan and Strange 1975). The increase of acetylglucosamine concentration in the medium was measured with a spectrophotometer (Model Shimadzu, UV-1601 PC) at 650 nm. The glucosamine concentration in the mycelia of *A. terreus* was found to be proportional to the mycelial weight and remained constant throughout the growth phases of the fungus.

Carboxymethylcellulase (CMCase) activity was determined by measuring spectrophotometrically the reducing sugar produced from 2% (w/v) carboxymethyl-

cellulose, while filter-paper-hydrolysing (FPase) activity was determined by estimating the reducing sugar liberated from filter paper (Wood and Bhat 1988). Both reactions were carried out in 0.05 M sodium acetate buffered at pH 5 and incubated at 50 °C. The reaction time was 30 min and 60 min for CMCase and FPase, respectively. One unit of CMCase or FPase activity was defined as 1 µmol reducing sugar released/mL enzyme/min. β-glucosidase was determined using the method described by Wood and Bhat (1988). In this method, *p*-nitrophenol released from *p*-nitrophenyl-β-D-glucopyranoside (Fluka) was measured using a spectrophotometer (Shimadzu, UV-1601 PC). One unit of β-glucosidase activity is defined as 1 µmol *p*-nitrophenol liberated/mL of enzyme/min, while the specific activity is defined as units/mg protein. Protein content was determined by the method of Bradford using bovine serum albumin as a standard (Bradford 1976).

Experimental Design for the Determination of Optimum Dissolved Oxygen Tension and Initial Culture pH

A five-level with two variables central composite rotatable design was adopted in this study. The design required 13 experiments including 4 factorial points, 4 axial points, and 5 replications at the central point (DOT 60%, pH 5.5). Five replications of the central point provided sufficient degrees of freedom for estimating the purely experimental error. The independent variables used for the optimization of growth of *A. terreus* and cellulase production using RSM were DOT (31% to 88%) and initial culture pH (4.7-6.2). The variables were coded according to equation (1),

$$X_{i=}\left(x_i - x_i^*\right)/(\Delta x_i) \quad (1)$$

where X_i is the coded value of i^{th} independent variable, x_i is the real value of the i^{th} independent variable, x_i* is the real value of the i^{th} independent variable at the centre point, and Δx_i is the step change value.

The independent variables and their levels are presented in Table 1, and the experimental design is shown in Table 2. The statistical analysis of the data was performed using Design-Expert software (version 6.0, Stat-Ease, Inc., Minneapolis, MN, USA), and results are shown in Tables 3 through 6. Growth of *A. terreus* and the production of cellulases were predicted according to Eq. (2),

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (2)$$

where Y is the predicted response factor, β_o is a constant, β_i represents the coefficient for each term, and X_1 and X_2 are the coded values of an independent variable.

Table 1. Actual Factor Levels Corresponding to Coded Factor Levels

Factor	Symbol	Actual factor at coded factor level of				
		-1.414	-1	0	+1	+1.414
Dissolve Oxygen Tension (DOT)	X_1	31	40	60	80	88
Initial pH	X_2	4.7	5	5.5	6	6.2

Table 2. Central Composite Design (CCD) of Factors in Coded Levels with Enzyme Activity as Response

Run no.	X_1	X_2	Y_1	Y_2	Y_3	Y_4
1	1.000	1.000	7.05	22.1	1.32	11.1
2	-1.414	0.000	11.3	37.3	1.44	13.42
3	0.000	-1.414	7.86	32.65	1.16	10.96
4	0.000	1.414	10.1	36.5	1.26	12.79
5	0.000	0.000	12.65	50	2.29	15.8
6	0.000	0.000	12.73	49.5	2.35	15.68
7	-1.000	1.000	11.5	45.6	1.95	14.66
8	0.000	0.000	12.7	51	2.11	15.83
9	-1.000	-1.000	10	41	1.47	11.95
10	0.000	0.000	12.69	47	2.26	15.78
11	1.000	-1.000	6.47	18.5	0.98	7.23
12	0.000	0.000	12.71	48.5	2.32	15.81
13	1.414	0.000	6.32	13.75	0.87	7.5

X_1 = DOT (%); X_2 = pH; Y_1 = Cell concentration (g/L); Y_2 = CMCase (U/mL); Y_3 = FPase(U/mL); and Y_4 = β-glucosidase (U/mL)

RESULTS AND DISCUSSION

Model Fitting and Statistical Analysis

The experimental responses of biomass concentration and cellulases activities with respect to the variation of two independent factors were evaluated to generate the best model equation for particular responses. Both linear and quadratic models were best fitted for biomass concentration, while only the quadratic model was fitted for cellulase activities. The quadratic models were chosen for all the responses, as it gave higher precision and are described in equations (3) to (6) with respect to coded factors. Therefore, the simplified second-order polynomial equation for cell concentration and cellulase production in terms of coded factors can be expressed as follows:

$$Y_1 = +12.70 - 1.88X_1 + 0.66X_2 - 1.98X_1^2 - 1.89X_2^2 - 0.23X_1X_2 \quad (3)$$

$$Y_2 = +49.20 - 9.91X_1 + 1.71X_2 - 11.40X_1^2 - 6.87X_2^2 - 0.25X_1X_2 \quad (4)$$

$$Y_3 = +2.27 - 0.24X_1 + 0.12X_2 - 0.49X_1^2 - 0.47X_2^2 - 0.03X_1X_2 \quad (5)$$

$$Y_4 = +15.78 - 2.08X_1 + 1.15X_2 - 2.64X_1^2 - 1.94X_2^2 + 0.29X_1X_2 \quad (6)$$

where X_1 and X_2 are DOT level and pH, respectively, Y_1 is cell concentration (g/L), Y_2 = CMCase (U/mL), Y_3 = FPase (U/mL), and Y_4 = β-glucosidase (U/mL).

All the models were examined for the goodness of fit. A number of indicators were used to assess the sufficiency of the particular fitted model, and the results are shown in Tables 3 to 6. The model significance (F and Prob>F values), determination coefficient (R^2), coefficient of variation (C.V.), adequate precision, and lack of fit criteria were used to judge the adequacy of the model. The large F values (cell concentration 363.47, CMCase 73.57, FPase 21.07, β-glucosidase 77.79) and very low prob>F values (cell concentration, CMCase, FPase, β-glucosidase <0.0001) suggested that the models were statistically reliable at more than 99% confidence level. High adjusted R^2 (cell concentration 0.99, CMCase 0.96, FPase 0.89, β-glucosidase 0.96) indicated that more than 94% of the variation was only due to the respective variables present in the models. Furthermore, adjusted R^2 values were very close with the predicted R^2 (cell concentration 0.97, CMCase 0.89, FPase 0.61, β-glucosidase 0.87). High R^2 (cell concentration 0.99, CMCase 0.98, FPase 0.93, β-glucosidase 0.98) indicated a good accord between predicted and experimental values. Low %C.V. for cell concentration (2.01), CMCase (6.02), FPase (10.76), and β-glucosidase (4.14) as well as very high adequate precision for cell concentration (46.97), CMCase (23.72), FPase (10.84), and β-glucosidase (22.57) indicated that the reliability and the precision of the experiments carried out were high.

Effect of Variables on Growth of *A. terreus* and Cellulase Production

The three-dimensional response curve plots were generated using the model equations (3) to (6) to assess the effect of DOT and initial pH on growth of *A. terreus* and the production of cellulase. The significance of each variables and its interaction were assessed by evaluating three-dimensional response curve plots (Fig. 1) and also the corresponding prob>F values (Tables 3 to 6). Both of the individual variables were found to be significant for growth of *A. terreus* and production of β-glucosidase. On the other hand, only the DOT level was found to be significant for the production of CMCase and FPase.

Very high DOT levels showed a greater negative effect than very low DOT levels for growth of *A. terreus* and the production of cellulase (Runs No. 11 and 2 in Table 2). Reduction and total inhibition of microbial growth due to high DOT levels in the bioreactor have also been reported (Onken and Liefke 1989). The formation of superoxide radicals (O_2^-) due to the presence of excess oxygen were destructive to cell metabolism and might inhibit the cell growth (Forage et al. 1985; Umikalsom et al. 1998). In contrast to DOT, a very low level of initial culture pH had a greater negative effect than very high level of initial pH for growth of *A. terreus* and the production of cellulase (Run No. 3 and 4 in Table 2). The use of highly acidic pH might have caused the inhibitory effect on sporulation and eventually reduced growth of *A. terreus* and the production of cellulase. The reduction of initial growth rate of *A. niger* due to acidic pH was also observed during the production of glucoamylase-green fluoroscent protein fusion protein (O'Donnell et al. 2001). Cellulase production by several *Aspergillus* spp. was enhanced at culture pH values ranging from 5 to 6 (D'Souza and Volfov 1982; El-Sersy et al. 2010; Juwaied et al. 2010). The combination of high DOT level and low initial culture pH and the combination of low DOT level and high initial culture pH have an extreme effect on growth of *A. terreus* and the production of cellulase. However, there was no interaction between these two variables for the growth of *A. terreus* and production of three main cellulase components, as indicated by the value of Prob (P)> F in the respective ANOVA (Tables 3 to 6).

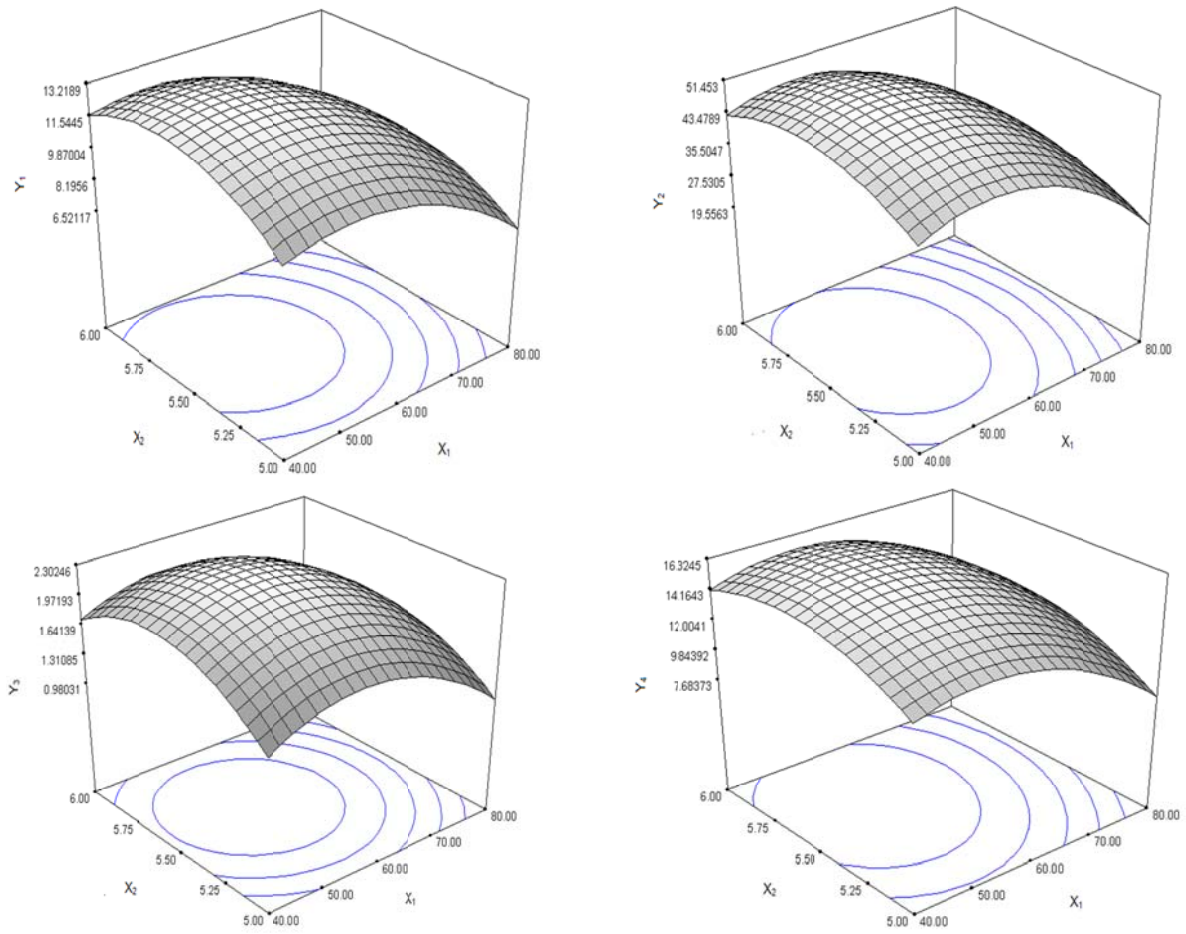

Figure 1. Contour and surface plots of the model equation fitted to the experimental data of a central composite design based on the influence of variation in initial culture pH and DOT on growth of *A. terreus.* Symbols: X_1 = Dissolved oxygen tension (%); X_2 = Initial pH; Y_1 = biomass conc. (g/L), Y_2 = CMCase (U/mL), Y_3 = FPase (U/mL), Y_4 = β-glucosidase (U/mL)

Table 3. Analysis of Variance (ANOVA) for Quadratic Model for Biomass Concentration from the Data of Central Composite Design Experiments

Source of variation	Sum of Squares	Degree of freedom	Mean Square	F Value	Prob (P)> F
Model	78.00	5	15.60	363.47	< 0.0001
X_1	28.21	1	28.21	657.28	< 0.0001
X_2	3.44	1	3.44	80.21	< 0.0001
X_1^2	27.22	1	27.22	634.14	< 0.0001
X_2^2	24.93	1	24.93	580.81	< 0.0001
X_1X_2	0.21	1	0.21	4.93	0.0618
Residual	0.30	7	0.043		
Cor Total	78.30	12			

Std. Dev.:0.21, R-Squared: 0.9962, Mean: 10.31, Adj R-Squared: 0.9934, C.V.: 2.01, Pred R-Squared: 0.9730, PRESS: 2.12, Adeq Precision: 46.976

Table 4. Analysis of Variance (ANOVA) for Quadratic Model for Carboxymethylcellulase (CMCase) Activity from the Data of Central Composite Design Experiments

Source of variation	Sum of Squares	Degree of freedom	Mean Square	F Value	Prob (P)> F
Model	1919.20	5	383.84	73.57	< 0.0001
X_1	786.16	1	786.16	150.69	< 0.0001
X_2	23.27	1	23.27	4.46	0.0726
X_1^2	904.07	1	904.07	173.29	< 0.0001
X_2^2	328.80	1	328.80	63.02	< 0.0001
X_1X_2	0.25	1	0.25	0.048	0.8330
Residual	36.52	7	5.22		
Cor Total	1955.72	12			

Std. Dev.: 2.28, R-Squared; 0.9813, Mean: 37.95, Adj R-Squared; 0.9680, C.V.: 6.02, Pred R-Squared: 0.8936, PRESS, 208.10, Adeq Precision: 23.728

Table 5. Analysis of Variance (ANOVA) for Quadratic Model for Filter-Paper-hydrolysis (FPase) Activity from the Data of Central Composite Design Experiments

Source of variation	Sum of Squares	Degree of freedom	Mean Square	F Value	Prob (P)> F
Model	3.42	5	0.68	21.07	0.0004
X_1	0.46	1	0.46	14.28	0.0069
X_2	0.12	1	0.12	3.56	0.1012
X_1^2	1.70	1	1.70	52.20	0.0002
X_2^2	1.51	1	1.51	46.55	0.0002
X_1X_2	4.900E-003	1	4.900E-003	0.15	0.7092
Residual	0.23	7	0.032		
Cor Total	3.65	12			

Std. Dev.; 0.18, R-Squared; 0.9377, Mean: 1.68, Adj R-Squared: 0.8932, C.V.: 10.76 Pred R-Squared: 0.6101, PRESS: 1.42, Adeq Precision: 10.846

Validation of the Models and the Potential Use of Isolated *A. terreus*

The optimal fermentation condition, as predicted by RSM, for enhancement of growth and the production of three main components of cellulase was DOT level set at 55% and initial culture pH set at 5.5. Batch fermentation in triplicates were performed at this optimal fermentation condition and the observed responses were in close agreement with the statistically predicted responses (Table 7).

Table 6. Analysis of Variance (ANOVA) for Quadratic Model for β-glucosidase Activity from the Data of Central Composite Design Experiments

Source of variation	Sum of Squares	Degree of freedom	Mean Square	F Value	Prob (P)> F
Model	112.01	5	22.40	77.79	< 0.0001
X_1	34.66	1	34.66	120.35	< 0.0001
X_2	10.51	1	10.51	36.48	0.0005
X_1^2	48.60	1	48.60	168.75	< 0.0001
X_2^2	26.06	1	26.06	90.50	< 0.0001
X_1X_2	0.34	1	0.34	1.17	0.3156
Residual	2.02	7	0.29		
Cor Total	114.03	12			

Std. Dev.:0.54, R-Squared: 0.9823, Mean: 12.96, Adj R-Squared: 0.9697, C.V.: 4.14 Pred R-Squared: 0.8750, PRESS: 14.26, Adeq Precision: 22.574

Table 7. Actual and Predicted Production of Biomass and Cellulases

Response		Actual value	Predicted value
Biomass concentration	(Y_1)	12.93 ± 0.03 g/L	13.10 g/L
CMCase	(Y_2)	50.33 ± 0.64 U/mL	51.07 U/mL
FPase	(Y_3)	2.29 ± 0.01 U/mL	2.30 U/mL
β-glucosidase	(Y_4)	15.98 ± 0.03 U/mL	16.24 U/mL

The time course of fermentation experiments run at different DOT levels (40, 55, 60, and 80%) and an initial culture pH was set at 5.5 are shown in Fig. 2. Although the rate of growth and cellulase production were different for different DOT levels, a similar trend was observed in all the conditions. Exponential growth was observed until 108 h of fermentation, and thereafter the stationary phase was maintained until the end of fermentation (240 h). CMCase and FPase activities were increased gradually with growth and became maximal when growth reached a stationary phase.

In contrast to CMCase and FPase production, β-glucosidase was still produced even during a stationary growth phase. Most β-glucosidase was intracellular when the culture was in the active stage of growth (Umikalsom et al. 1998). As the fermentation progressed, β-glucosidase might have secreted into the culture. It is possible that this enzyme is released by autolysis, i.e., when the culture is in the stationary phase (Berg and Von Hofsten 1976). However, it is often difficult to show whether an enzyme found in medium is secreted by growing cells or passively because of cellulolysis (Linger et al. 2010). From Fig. 2, it can be seen that the production of CMCase and FPase was growth-associated while β-glucosidase production can be considered as mixed growth system. In order to obtain high proportion of β-glucosidase in the culture filtrate, the fermentation should be extended after growth has reached a stationary phase. Enhanced growth of *A. terreus* and cellulase production at DOT level of 55% further validated the result of optimization.

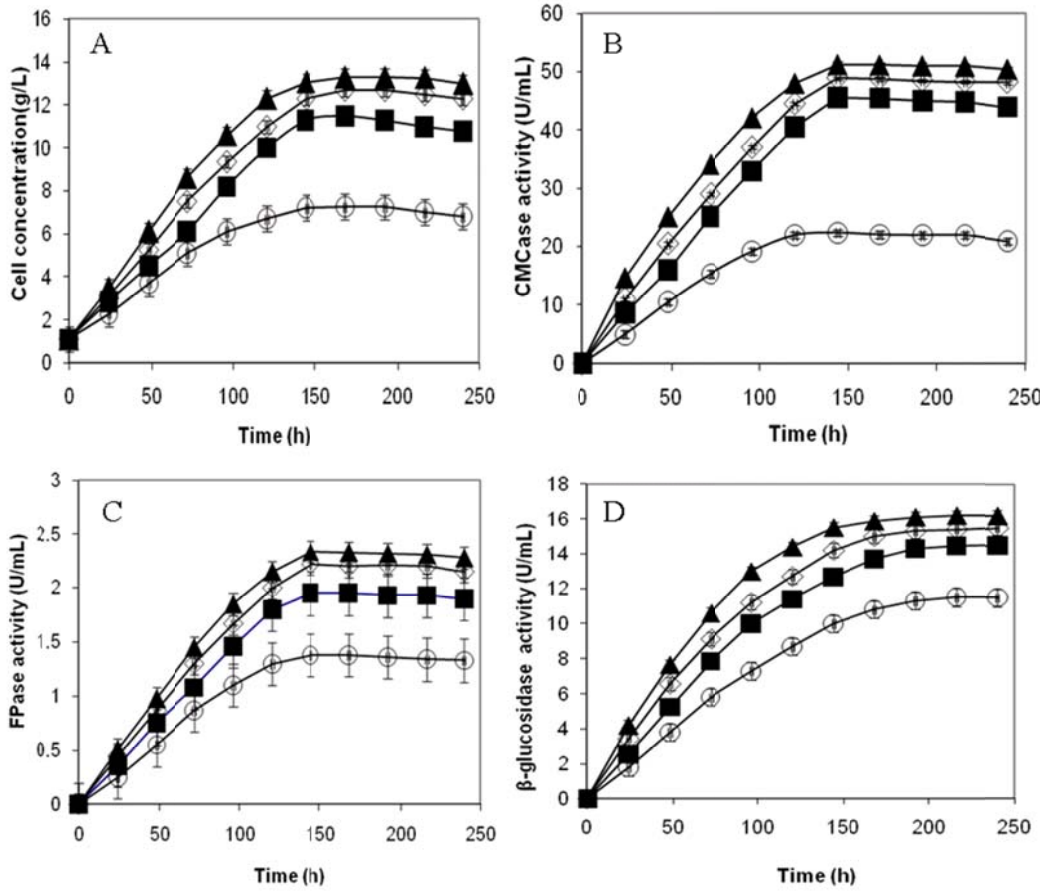

Figure 2. Comparison between different levels of dissolve oxygen tension for cellulase production by *Aspergillu terreus* in stirred tank bioreactor: (A) Cell concentration; (B) FPase activity; (C) CMCase activity; (D) β-glucosidase activity. Symbols: ■ 40%, ▲ 55%, ◇ 60%, ○ 80%. The initial pH was maintained at 5.5 for all the experiments.

Cellulase produced by *A. terreus* contained a high level of β-glucosidase activity. The ratio of β-glucosidase to FPase obtained in fermentation where the DOT level was controlled at 55% was about 8:1, and this was significantly higher than the reported ratios for cellulase from *Aspergillus niger* (Autam et al. 2010; Narasimha et al. 2006; Villena et al. 2007), *T. reesei* (Doppelbauer et al. 1987), *T. lignorum* (Baig 2005), *Fusarium oxyporum* (Ramanathan et al. 2010), *Gliocladium virens* (Gomes et al. 1989), and *Chaetomium globosum* (Umikalsom et al. 1998), which were in the range from 0.44:1 to 7:1. A high proportion of β-glucosidase would be beneficial for efficient hydrolysis of cellulosic materials, since β-glucosidase is required to reduce inhibition caused by the end product of cellobiose hydrolysis (Berlin et al. 2005).

CONCLUSIONS

RSM has been successfully used to evaluate the effect of DOT level and initial culture pH on growth of *A. terreus* and cellulase production. Although DOT level and initial culture pH contributed to growth of *A. terreus* and also production of three main components of cellulase, no significant interaction was observed between these factors on the responses. The optimum DOT level and initial culture pH for growth of

A. terreus and the production of three main components of cellulase were 55% and 5.5, respectively. In comparison to fermentation condition that gave the lowest production, the use of optimum conditions increased the production of CMCase, FPase, and β-glucosidase by about 3.66, 2.66, and 2.13 times, respectively. The proportion of β-glucosidase to FPase produced by *A. terreus* was higher than other cellulases reported in the literature. The use of delignified OPEFB as a main substrate in the fermentation would reduce the production cost of all three main components of cellulase.

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