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Utilization of Palm Kernel Cake for Production of β-Glucosidase by *Aspergillus niger* FTCC 5003 in Solid Substrate Fermentation Using an Aerated Column Bioreactor

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Abstract: The production of β-glucosidase by *Aspergillus niger* FTCC 5003 from palm kernel cake as a substrate was studied in solid substrate fermentation using a laboratory column bioreactor. The simultaneous effects of three independent variables, namely incubation temperature, initial moisture content of substrate and airflow rate on the production of β-glucosidase were evaluated using response surface methodology on the basis of a central composite face-centered design. A total of 18 experiments were carried out in which *Aspergillus niger* FTCC 5003 was cultivated on PKC in an aerated column bioreactor for 7 days under incubation temperature, moisture level and aeration rate determined. Experimental results showed that the highest activity of β-glucosidase (52.06 U g^{-1}) was obtained at an incubation temperature, an initial moisture level and an aeration rate of 32.5°C, 60% and 1.5 L min^{-1}, respectively. Statistical analysis revealed that the quadratic terms of incubation temperature and initial moisture content had highly significant effects on the production of β-glucosidase ($p<0.01$). The statistical results also showed that the interaction effect between initial moisture level and aeration rate significantly influenced the production of β-glucosidase ($p<0.05$). Optimum conditions suggested by the second-order polynomial regression model for attaining maximum β-glucosidase production were 31.6°C incubation temperature, 57.0% initial moisture and 0.5 L min^{-1} aeration rate with a predicted production value of 47.20 U g^{-1}.

Key words: Palm kernel cake, β-glucosidase, *Aspergillus niger*, solid substrate fermentation, column bioreactor

INTRODUCTION

Tropical agro-industrial residues have been found to be feasible substrates for the production of value-added products particularly microbial enzymes (Pandey *et al*., 2001). Palm kernel cake (PKC) is a tropical agro-industrial by-product which is obtained from the palm oil industry (Ong *et al*., 2004). Generally, PKC contains 12 to 18% crude fiber and 15 to 18 % crude protein (Awaludin, 2001). PKC components are also composed of 12% cellulose (Dusterhoft *et al*., 1992). PKC has already been used in the production of a variety of microbial enzymes such as alpha amylase, metalloprotease, tannase and mannanase (Ong *et al*., 2004; Ramachandran *et al*., 2004; Sabu *et al*., 2005; Sumantha *et al*., 2005).

β-glucosidase (EC 3.2.1.21) is one of the important components of cellulase enzyme complex which acts synergistically with endo-β-1,4-glucanase (EC 3.2.1.4) and exoglucanase (EC 3.2.1.91) to hydrolyze cellulosic substances into glucose. (Wood and Bhat, 1988). β-glucosidase have found various applications including the increase of bioaccessibility of isoflavones in human intestine and the enhancement of aromatic compounds of juices and wine in beverage industry (Bhatia *et al*., 2002). Solid Substrate Fermentation (SSF) has been employed in the biosynthesis of microbial biomass and industrial enzymes. SSF is characterized by the growth of microorganisms on water insoluble materials in absence or near the absence of free water (Mitchell and Lonsane, 1992). Environmental factors such as temperature, moisture content of substrate and airflow decisively affect SSF process, which in turn influences fermentation products (Pandey *et al*., 1999). SSF has widely been utilized in the production of microbial enzymes using agro-industrial residues. Pandey *et al*. (2001) have summarized different lignocellulosic substances, which have been used in the production of industrial enzymes through SSF technology.

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Although several attempts have been made to produce β-glucosidase using cellulosic compounds in SSF process, most of these studies have been focused on the production of enzyme in shake flask and much less work has been conducted to utilize bioreactor for the production of β-glucosidase under SSF process (Kang *et al*., 1999; Jäger *et al*., 2001; Leite *et al*., 2008; Sukumaran *et al*., 2009). Due to the existing complexity in scale-up processes, the expected productivity at large-scale methods is less than flask scale methods and hence the yields of microbial products obtained in a bioreactor are usually lower than those produced in a shake flask (Jin *et al*., 2004). Hence, it is necessary to develop SSF technology for the production of β-glucosidase in large-scale method using the bioreactor.

The current study reports on the bioprocess of PKC as a cheap and highly available agricultural by-product for the production of β-glucosidase by *Aspergillus niger* FTCC 5003 in SSF process using a laboratory bioreactor. This study also aimed to enhance β-glucosidase yield by the control of three independent variables including incubation temperature, initial moisture content of PKC and aeration rate in determined conditions. Statistical approach of Response Surface Methodology (RSM) was applied to study the combined effects of variables mentioned and to determine the optimum level of the variables tested for obtaining the maximum production of β-glucosidase (Box *et al*., 1978).

MATERIALS AND METHODS

This study began on 10 July 2006 and lasted till 18 July 2009. The preparation work of microorganism, substrate and medium was carried out in Malaysian Agriculture Research and Development Institute (MARDI). All experiments related to SSF process were also performed in MARDI. Analytical experiments and enzyme assay were conducted in Department of Microbiology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

Microorganism: *Aspergillus niger* FTCC 5003 was obtained from the culture collection of the MARDI. The stock culture was grown on a Potato Dextrose Agar (PDA) slant at 30°C for 7 days. Spore suspension was prepared by adding 50 mL of sterile Tween-80 (0.1%) to the PDA slant. The spore suspension obtained was kept at 4°C.

Medium of growth: Palm kernel cake was supplied by MARDI and used as a solid substrate. PKC was ground to a particle size of 2 mm and dried in an oven at 60°C for 48 h. Mandels' medium was used to moisten PKC (Sternberg, 1976). The composition of Mandels' medium was as follows (g L^{-1}): $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2$, 0.3; $MgSO_4.7H_2O$, 0.3; $MnSO_4.H_2O$, 0.0016; $FeSO_4.7H_2O$, 0.005; $ZnSO_4.7H_2O$, 0.0014; $CoCl_2$, 0.002; protease peptone, 0.75; urea, 0.3; and Tween 80, 1.0 . The initial pH of Mandels' medium was adjusted to 5.0 using 1.0 M HCl or 1.0 M NaOH.

Experimental design: A 2^3 full factorial central composite face-centered (CCF) design for three independent variables, each one at three levels with six star points and four replicates at central point was used to fit a second-order polynomial model in which a total of 18 experiments were required to be carried out (Haaland, 1989). The experimental variables selected, i.e., incubation temperature, initial moisture content and aeration rate were coded as X_1, X_2 and X_3 respectively. The behavior of the fitted model was explained by an empirical model equation as follows (Eq. 1):

$$Y = a_0+a_1 X_1+a_2 X_2+a_3 X_3 + a_{11}X_1^2 +a_{22} X_2^2+a_{33} X_3^2 + a_{12} X_1 X_2 +a_{13} X_1 X_3 +a_{23} X_2 X_3 \quad (1)$$

where, Y, is the measured response (β-glucosidase, U g^{-1}); a_o, represents the intercept; a_1, a_2 and a_3 are linear coefficients; a_{11}, a_{22} and a_{33} are squared coefficients and a_{12}, a_{13} and a_{23}, are interaction coefficients.

The test variables were coded at three levels of -1, 0 and +1 which represented the low, middle and high levels of the variables, respectively. The coded values and actual levels of the variables are given in Table 1. The design matrix of experiments is shown in Table 2. Design-Expert software (version 6.0.6 Stat-Ease, Inc.) was used for the regression analysis of data and the graphical design of response surface.

Solid substrate fermentation: A glass column bioreactor, which consisted of a jacketed vessel, was loaded with 100 g of PKC. Initial moisture of PKC was adjusted by addition of Mandels' medium. Bioreactor was sterilized at 121°C for 30 min. After cooling, PKC was inoculated with 1.0 mL of spore suspension containing 10^6 spores per gram of dry substrate (PKC) and then incubated for 7 days. Incubation temperature was controlled by circulating water through the jacket of bioreactor. Humid air was forced through the bed of culture to achieve both heat removal and oxygen supply. For each experiment, incubation temperature, initial moisture content and aeration rate were adjusted according to levels determined by the experimental design (Table 2).

Table 1: Independent variables and levels used in central composite face centered design

Variable	Symbol	Level					
		Actual range			Coded value		
		Low	Middle	High	Low	Middle	High
Incubation temperature (°C)	X_1	25	32.5	40.0	-1	0	+1
Initial moisture content (%)	X_2	40	60.0	80.0	-1	0	+1
Aeration rate (L min^{-1})	X_3	0.5	1.5	2.5	-1	0	+1

Table 2: Central composite face centered design and experimental results of β-glucosidase production by *Aspergillus niger* FTCC 5003 using palm kernel cake in SSF process for 7 days

Run	X_1[a]	X_2	X_3	β-glucosidase activity (U g^{-1})[b]	Specific activity (U mg^{-1})[c]
1	0	0	+1	35.35	0.56
2	0	0	0	44.26	0.54
3	+1	0	0	25.19	0.33
4	-1	+1	-1	3.45	0.16
5	-1	-1	+1	12.68	0.29
6	-1	-1	-1	13.97	0.35
7	-1	+1	+1	7.97	0.23
8	0	0	0	52.06	0.63
9	+1	+1	-1	1.96	0.08
10	0	0	0	49.45	0.63
11	0	0	0	48.84	0.64
12	0	-1	0	14.20	0.37
13	+1	-1	-1	7.64	0.19
14	+1	+1	+1	8.44	0.22
15	0	0	-1	49.38	0.63
16	+1	-1	+1	2.18	0.06
17	0	+1	0	9.73	0.21
18	-1	0	0	24.75	0.34

[a]X_1: Temperature (°C); X_2: Moisture level (%); X_3: Airflow rate (L min^{-1}), [b]Unit of enzyme activity per g of dry PKC, [c]Unit of enzyme activity per mg of protein released

Enzyme extraction: Enzyme was extracted from fermented PKC by adding 1000 mL of 0.05 M sodium citrate buffer (pH 5.0) to fermented PKC and shaking (170 rpm) for 24 h at 4°C. The suspended materials and the fungal biomass were then separated by filter paper (Whatman No. 1) at the temperature of 4°C. The clarified extract was used as the source of enzyme.

Analytical methods: β-glucosidase activity was measured by incubating 1.0 mL of an appropriately diluted culture supernatant with 10 mg salicin in 1.0 mL sodium citrate buffer (0.05 M, pH 4.8) at 50°C for 30 min. The reaction was terminated by addition of 3 mL of 3, 5-dinitrosalicylic acid (DNS) reagent. The tubes were placed in a boiling water bath for 5 min and then cooled to room temperature and the librated reducing sugar (as glucose) was determined spectrophotometrically by measuring the absorbance at the wavelength of 540 nm (Sadller *et al*., 1985). One unit (U) of enzyme activity was defined as the amount of enzyme required to liberate 1.0 μmol of glucose per minute under the assay conditions. The activity of β-glucosidase produced was expressed in unit per gram of dry PKC (U g^{-1}). The protein content of culture extract was measured using bovine serum albumin by the Lowry *et al*. method (1951).

RESULTS

Experimental results of β-glucosidase production according to CCF design are shown in Table 2. As observed, three independent variables (incubation temperature, initial moisture content and air flow rate) were controlled at levels determined by experimental design which were represented as -1, 0 and +1. Treatment 2, 8, 10 and 11 included center point in design which was repeated four times for estimation of experimental error. As can be seen from the results in Table 2, treatment 2, 8, 10, 11 and 15 showed high levels of β-glucosidase activity. As shown, the highest β-glucosidase production with an activity as high as 52.06 U g^{-1} was produced when *Aspergillus niger* (FTCC 5003) was grown at an incubation temperature of 32.5°C, an initial moisture of 60% and an aeration rate of 1.5 L min^{-1} (Run 8). As is evident, the peak level of specific activity (0.64 U mg^{-1}) was obtained in run 11. Experimental results also indicated that the lowest production of β-glucosidase (1.96 U g^{-1}) with a specific activity of 0.08 U mg^{-1} was obtained at 40°C temperature, 80% moisture level and 0.5 L min^{-1} aeration rate (Run 9). By applying multiple regression analysis the test results, a second-order polynomial equation (Eq. 2) was obtained to represent β-glucosidase production as a function of incubation temperature, initial moisture level and airflow rate:

$$Y = 6.67-0.34\,X_1-0.32\,X_2-7.408E\text{-}003\,X_3-1.37\,X_1^2-2.92\,X_2^2 +0.12\,X_3^2+0.34\,X_1X_2-0.071\,X_1X_3+0.49\,X_2X_3 \quad (2)$$

where, Y is β-glucosidase activity (U g^{-1}) and X_1, X_2 and X_3 are the coded values of incubation temperature (°C), initial moisture content (%) and aeration rate (L min^{-1}), respectively.

The statistical significance of the fitted model was evaluated using the Fisher's statistical test for Analysis of Variance (ANOVA), which is essential for determining patterns of interaction between experimental variables (Table 3). As observed, the calculated model's F value of 24.43 with a probability value (Prob >F) of less than 0.0001 revealed that the selected quadratic model was satisfactorily fitted to the experimental data ($p< 0.01$). The lack of fit F value (8.48) implied that the lack of fit was not significant and hence model was fit to the data.

Table 3: Analysis of variance for the quadratic model of β-glucosidase production by *Aspergillus niger* FTCC 5003 grown on PKC in SSF for 7 days

Source	Variable	Polynomial coefficients	Sum of squares	df	Mean square	F value	Prob>F
Model			68.65	9	7.63	24.43	<0.0001**
	Intercept	6.67		1			
	X_1	-0.34	1.15	1	1.15	3.68	0.0915
	X_2	-0.32	1.02	1	1.02	3.28	0.1076
	X_3	-7.408E-003	5.487E-004	1	5.487E-004	1.758E-003	0.9676
	X_1^2	-1.37	5.10	1	5.10	16.34	0.0037**
	X_2^2	-2.92	23.18	1	23.18	74.24	<0.0001**
	X_3^2	0.12	0.037	1	0.037	0.12	0.7384
	$X_1 X_2$	0.34	0.90	1	0.90	2.88	0.1283
	$X_1 X_3$	-0.071	0.041	1	0.041	0.13	0.7279
	$X_2 X_3$	0.49	1.94	1	1.94	6.20	0.0375*
Residual			2.50	8	0.31		
Lack of fit			2.33	5	0.47	8.48	0.0543
Pure error			0.17	3	0.055		

*Statistically significant at 95 % probability level. **Statistically significant at 99% of probability level. X_1: Temperature (°C); X_2: Moisture level (%); X_3: Airflow rate (L min^{-1}). X_1^2, X_2^2 and X_3^2 the quadratic terms; X_1X_2, X_1X_3 and X_2X_3 the interaction terms. $R^2 = 0.9649$; Adj $R^2 = 0.9254$

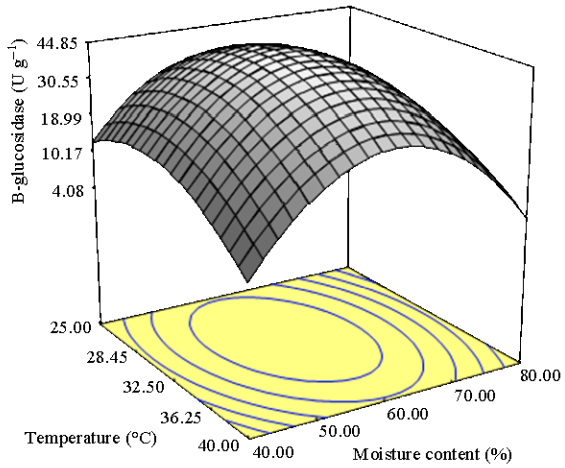

Fig. 1: Three-dimensional response surface graph of the combined effects of incubation temperature and moisture content on β-glucosidase production by *Aspergillus niger* FTCC 5003 using palm kernel cake in SSF process for 7 days

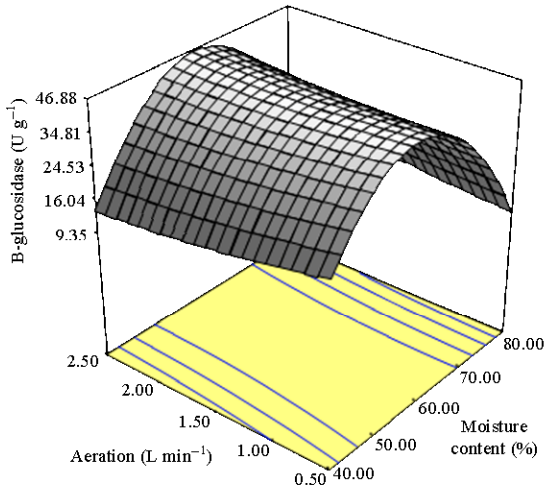

Fig. 2: Three-dimensional response surface graph of the combined effects of moisture content and aeration rate on β-glucosidase production by *Aspergillus niger* FTCC 5003 using palm kernel cake in SSF process for 7 days

Table 3 also shows the significance of linear, interaction and quadratic effects of variables based on their probability values. The values of Prob>F less than 0.05 indicate that the model terms are significant. As is evident, the quadratic terms of incubation temperature (X_1^2) and moisture content (X_2^2) was significant at 99% probability level ($p<0.01$). The high significance of X_1^2 and X_2^2 indicated that incubation temperature and initial moisture level acted as critical factors and even low changes in their values could affect β-glucosidase production to a remarkable level. The regression model also revealed that the interaction effect between moisture content and aeration rate (X_2X_3) on β-glucosidase yield were significant at 95% probability level ($p<0.05$). The multiple coefficient of determination (R^2) with an acceptable value of 0.9649 indicated that the model could explain 96.49% of the variability in the response. A high value of adjusted determination coefficient (Adj R^2 = 0.9254) also corroborated statistical significance of the fitted model. In order to describe the simultaneous effects of independent variables on the yield of β-glucosidase production, three dimensional response surface graphs were constructed on the basis of the regression model (Fig. 1-3). Figure 1 shows the combined effects of incubation temperature and initial moisture content of PKC on the production of β-glucosidase. As can be seen, an increase in β-glucosidase production occurred when temperature began to increase from 25°C to an optimum range of 31-33°C with the low level of moisture content. Following the rise in moisture content, the

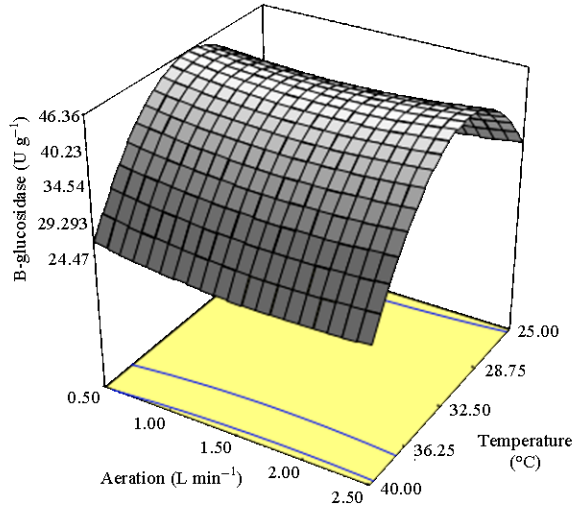

Fig. 3: Three-dimensional response surface graph of the combined effects of incubation temperature and aeration rate on β-glucosidase production by *Aspergillus niger* FTCC 5003 using palm kernel cake in SSF process for 7 days

level of β-glucosidase activity significantly increased. However, a decrease in response was observed when temperature increased higher than the optimum range. Similarly, β-glucosidase yield declined when the rise in initial moisture content was greater than the optimum range (57-60%). Figure 2 shows the influences of moisture level and aeration rate on β-glucosidase production. It is obvious that an increase in moisture content from 40% to the optimum range concomitantly caused the marked increase in β-glucosidase activity, while an aeration rise higher than 0.5 L min^{-1}showed a slight decrease in β-glucosidase activity. Similar trend between temperature and aeration rate is shown in Fig. 3 so that the quadratic rise in the yield of β-glucosidase was directly proportional with the increase of temperature up to optimal range, whereas a rise in aeration rate greater than 0.5 l min^{-1} had no significant effect in β-glucosidase production. Optimum conditions for attaining the highest production of β-glucosidase were determined by statistical model using Design-Expert software. The analysis results indicated that the optimal levels of incubation temperature, initial moisture of substrate and aeration rate were 31.6°C, 57.0% and 0.5 L min^{-1}, respectively. Statistical model predicted the activity value of 47.20 U g^{-1}could be achieved under optimal conditions.

DISCUSSION

Effect of incubation temperature: Environmental temperature is an important parameter, which dramatically influences the growth of microorganism and the production of cellulolytic enzymes (Mekala *et al*., 2008). Our work showed that increasing incubation temperature from 25°C to optimum level (31.6°C) raised the production of β-glucosidase up to the highest levels (Fig. 1, 3). An increase in the yield of β-glucosidase following a rise in incubation temperature can be attributed to the influence of temperature on the growth of microorganism, which consequently affects the amount of end-product (Lonsane *et al*., 1985; Patil and Dayanand, 2006). The results obtained in the present study was in agreement with that was reported by Prasertsan and Oi (1992), who showed that the highest production of β-glucosidase by *Aspergillus niger* on palm cake was measured when the growth temperature was set at 30°C. It was also noted that the peak level of β-glucosidase was produced when *Aspergillus niger* was cultivated on sugar cane bagasse pith in a shake flask at an incubation temperature of 29°C (Kirchner *et al*., 2005) contrary to what was found by Gao *et al*. (2008) who reported that the peak level of β-glucosidase produced by *Aspergillus terreus* on corn stover was obtained at 45°C. The diversity of optimal incubation temperature can partially be explained by the effect of other process parameters such as the moisture content of substrate, airflow and oxygen level on the environmental temperature, allowing the growth at various temperatures (Prior *et al*., 1992).

Effect of initial moisture content: The water content of solid substrate exists in different forms including a thin liquid film on the surface of particles and as a complex form within solid construction (Prior *et al*., 1992). Since SSF deals with the biological processes in which microorganism grows on solid materials with a limited moisture level, the moisture content of substrate is a crucial factor, which drastically influences fermentation process (Pandey *et al*., 2001). As shown in Fig. 1 and 2, the production of β-glucosidase was low at the moisture content of 40%. The low level of moisture content leads to the reductions of substrate swelling, nutrient diffusion and solubility of solid substances. These facts would result in insufficient nutrient supply for microorganism, which in turn causes the decrease of microbial growth and enzyme production (Prior *et al*., 1992; Venkateswarlu *et al*., 2000). The current study showed that the increase of moisture level from 40% to optimum level (57.0%) favored the production of β-glucosidase (Fig. 1, 2). This could be related to the increase of microorganism growth at high moisture content and a subsequent rise in the production of enzyme (Gao *et al*., 2008). However, higher levels of moisture content (70-80%) reduced the activity of β-glucosidase. The adverse effect of too high moisture level may be attributed

to the reduction of substrate porosity, low heat and mass transfer through the culture and the decrease of air exchange, which in turn result in the decrease of fungi growth and product formation (Venkateswarlu *et al*., 2000). Present findings are in contrast with the results obtained by Dogaris *et al*. (2009), who have shown that the growth of *Neurospora crassa* on the mixture of wheat bran and wheat straw with an initial moisture 70.5% leads to the highest yield of β-glucosidase in culture. The optimum moisture content for the production of β-glucosidase by *Fusarium oxysporum* on the mixture of brewer's spent grain and corn cobs was measured at the level of 74% in SSF process (Xiros *et al*., 2008). It was also noted that higher moisture level (80%) was necessary for the maximal production of β-glucosidase when *Aspergillus* niger was grown on corn stover under SSF process (Gao *et al*., 2008). The multiplicity in optimum moisture content could be due to the fact that optimum moisture greatly depends on the water-binding characteristics of substrate, temperature and selected microorganism (Prior *et al*., 1992).

Effect of aeration rate: Microbial activity of aerobic cultures is markedly affected by air supply to the system. The optimum rate of airflow is determined by the selection of microorganism, the particular amount of oxygen for product synthesis, the level of heat evolution to be removed, the quantity of carbon dioxide and other volatile metabolites, which would be dissipated, the thickness of substrate layer and the volume of pore space in the substrate (Lonsane *et al*., 1985). The consumption of oxygen by microorganisms in SSF process is carried out either directly from inert gas phase or from liquid phase as a thin film at the surface of substrate. On the other hand, the amount of oxygen consumption from a liquid film will depend on the interfacial surface area and the pressure of oxygen dissolved in the water of substrate particles. Hence, the use of forced aeration can favor oxygen uptake (Mitchell *et al*., 1992). The study of physical characteristics of PKC has elucidated that PKC particles quickly transfer heat evolved to air, which in turn reduces the requirement for a high rate of airflow during SSF process (Who *et al*., 2006). As shown in Fig. 2 and 3 an increase in airflow rate greater than 0.5 L min^{-1} slightly decreased β-glucosidase activity. The deleterious influence of the high aeration rates was possibly due to damaging effect of shear stress on filamentous fungi morphology and the reduction of moisture content of substrate which in turn could prevent microbial growth (Lonsane *et al*., 1985; Lu *et al*., 1997; Mitchell *et al*., 1999). In conclusion, this study investigates the potency of PKC in induction of β-glucosidase production by *Aspergillus niger* FTCC 5003. The findings of this research reveals that incubation temperature, moisture content of substrate and air flow affect the production of β-glucosidase. Further research is recommended to investigate the biodegradation of PKC in fed-batch system of SSF process to enhance the productivity of β-glucosidase further.

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REFERENCES

Awaludin, R., 2001. Feeding trials in Malaysia: palm kernel cake as a feedstuff for cattle. Palm. Oil. Dev., 34: 7-10.

Bhatia, Y., S. Mishra and V.S. Bisaria, 2002. Microbial β-glucosidases: cloning, properties and applications. Crit. Rev. Biotechnol., 22: 375-407.

Box, G.E.P., W.G. Hunter and J.S. Hunter, 1978. Statistic for Experimenters: An Introduction to Design, Data Analysis and Model Building. John Wiley and Sons Inc., New York, ISBN: 0-471-09315-7.

Dogaris, I., G. Vakontios, E. Kalogeris, D. Mamma and D. Kekos, 2009. Induction of cellulases and hemicellulases from *Neurospora crassa* under solid-state cultivation for bioconversion of sorghum bagasse into ethanol. Ind. Crops. Prod., 29: 404-411.

Dusterhoft, E.M., M.A. Posthumus and A.G.J. Voragen, 1992. Non-starch polysaccharides from sunflower (*Helianthus annuus*) meal and palm kernel (*Elaeis guineensis*) meal preparation of cell wall material and extraction of polysaccharide fractions. J. Sci. Food. Agric., 59: 151-160.

Gao, J., H. Weng, D. Zhu, M. Yuan, F. Guan and Y. Xi, 2008. Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. Bioresour. Technol., 99: 7623-7629.

Haaland, P.D., 1989. Experimental Design in Biotechnology. Marcel Dekker, New York, ISBN: 0-8247-7881-2.

Jäger, S., A. Brumbauer, E. Fehér, K. Réczey and L. Kiss, 2001. Production and characterization of ß-glucosidases from different *Aspergillus* strains. World. J. Microbiol. Biotechnol., 17: 455-461.

Jin, Z.H., J.P. Lin and P.L. Cen, 2004. Scale-up of rifamycin B fermentation with *Amycolatoposis mediterranei*. J. Zhejiang Univ. Sci. A, 5: 1590-1596.

Kang, S.W., E.H. Ko, J.S. Lee and S.W. Kim, 1999. Over-production of β-glucosidase by *Aspergillus niger* mutant from lignocellulosic biomass. Biotechnol. Lett., 21: 647-650.

Kirchner, O.G., M.S. Granados and P.R. Pascual, 2005. Effect of media composition and growth conditions on production of β-glucosidase by *Aspergillus niger* C-6. Applied Biochem. Biotechnol., 121: 347-359.

Leite, R.S.R., H.F. Alves-Prado, H. Cabral, F.C. Pagnocca, E. Gomes and R. Da-Silva, 2008. Production and characteristics comparison of crude β-glucosidases produced by microorganisms *Thermoascus aurantiacus* e *Aureobasidium pullulans* in agricultural wastes. Enzym. Microb. Technol., 43: 391-395.

Lonsane, B.K., N.P. Ghildyal, S. Budiatman and S.V. Ramakrishna, 1985. Engineering aspects of solid-state fermentation. Enzyme Microbiol. Technol., 7: 258-265.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Lu, M., J.D. Brooks and I.S. Maddox, 1997. Citric acid production by solid-state fermentation in a packed-bed reactor using *Aspergillus niger*. Enzym. Microb. Technol., 21: 392-397.

Mekala, N.K., R.R. Singhania, R.K. Sukumaran and A. Pandey, 2008. Cellulase production under solid-state fermentation by *Trichoderma reesei* RUT C30: Statistical optimization of process parameters. Applied Biochem. Biotechnol., 151: 122-131.

Mitchell, D.A. and B.K. Lonsane, 1992. Definition, Characteristics and Potential. In: Solid Substrate Cultivation, Doelle, H.W., D.A Mitchell and C.E. Rolz, (Eds.). Elsevier science publishers Ltd., England, ISBN: 1-85166-879-9, pp: 1-28.

Mitchell, D.A., B.K. Lonsane, A. Durand, R. Renaud and S. Almanza *et al*., 1992. General Principles of Reactor Design and Operation for SSC. In: Solid Substrate Cultivation, Doelle, H.W., D.A. Mitchell and C.E. Rolz, (Eds.). Elsevier Science Publishers Ltd., England, pp: 115-140.

Mitchell, D.A., A. Pandey, S. Penjit and N. Krieger, 1999. Scale-up strategies for packed-bed bioreactors for solid-state fermentation. Process. Biochem., 35: 167-178.

Ong, L.G., S. Abd-Aziz, S. Noraini, M.I. Karim and M.A. Hassan, 2004. Enzyme production and profile by *Aspergillus niger* during solid substrate fermentation using palm kernel cake as substrate. Applied Biochem. Biotechnol., 118: 73-79.

Pandey, A., P. Selvakumar, C.R. Soccol and P. Nigam, 1999. Solid-state fermentation for the production of industrial enzymes. Curr. Sci., 77: 149-152.

Pandey, A., C.R. Soccol, J.A. Rodriguez-Leon and P. Nigam, 2001. Solid–State Fermentation in Biotechnology. Asiatech Publishers Inc (API), New Delhi, ISBN: 81-87680-06-7.

Patil, S.R. and A. Dayanand, 2006. Optimization of process for the production of fungal pectinases from deseeded sunflower head in submerged and solid-state conditions. Bioresour. Technol., 97: 2340-2344.

Prasertsan, P. and S. Oi, 1992. Production of cellulytic enzymes from fungi and use in the saccharification of palm cake and palm fibre. World. J. Microbiol. Biotechnol., 8: 536-538.

Prior, B.A., J.C.D. Preez and P.W. Rein, 1992. Environmental Parameters. In: Solid Substrate Cultivation, Doelle, H.W., D.A. Mitchell and C.E. Rolz (Eds.). Elsevier Science Publishers Ltd., England, ISBN: 1-85166-879-9, pp: 65-85.

Ramachandran, S., A.K. Patel., K.M. Nampoothiri, S. Chandran, G. Szakacs, C.R. Soccol and A. Pandey, 2004. Alpha amylase from a fungal culture grown on oil cakes and its properties. Braz. Arch. Biol. Technol., 47: 309-317.

Sabu, A., A. Pandey, M.J. Daud and G. Szakacs, 2005. Tamarind seed powder and palm kernel cake: two novel agro residues for the production of tannase under solid state fermentation by Aspergillus niger ATCC 16620. Bioresour. Technol., 96: 1223-1228.

Sadller, J.N., C.M. Hogan and G. Louis-Seize, 1985. A comparison between the cellulase systems of *Trichoderma harzianum* E58 and Trichoderma reesei C30. Applied Microbiol. Biotechnol., 22: 139-145.

Sternberg, D., 1976. Production of cellulase by Trichoderma. Biotechnol. Bioeng. Symp., 6: 35-53.

Sukumaran, R.K., R.R. Singhania, G.M. Mathew and A. Pandey, 2009. Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. Renew. Energy., 34: 421-424.

Sumantha, A., C. Sandhya, G. Szakacs, C.R., Soccol and A. Pandey, 2005. Production and partial purification of a neutral metalloprotease by fungal mixed substrate fermentation. Food Technol. Biotechnol., 43: 313-319.

Venkateswarlu, G., P.S.M. Krishna, A. Pandey and L.V. Rao, 2000. Evaluation of Amycolatopsis mediterranei VA18 for production of rifamycin-B. Process. Biochem., 36: 305-309.

Who, F.C., A. Prabhakar, J. Janaun and K. Krishnaiah, 2006. Heat transfer studies in fluidized bed reactor using palm kernel cake as substrate. Proceedings of the 1st International Conference on Natural Resources Engineering and Technology, (ICNRET' 06), Putrajaya, Malaysia, pp: 656-663.

Wood, T.M. and K.M. Bhat, 1988. Methods for measuring cellulase activities. Methods Enzymol., 160: 87-112.

Xiros, C., E. Topakas, P. Katapodis and P. Christakopoulos, 2008. Evaluation of fusarium oxysporum as an enzyme factory for the hydrolysis of brewers spent grain with improved biodegradability for ethanol production. Ind. Crops. Prod., 28: 213-224.