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Cellulase production by solid state fermentation using bagasse with *Penicillium decumbens* L-06

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Abstract - The cellulase production by *Penicillium decumbens* L-06 in solid state fermentation (SSF) was investigated using bagasse as the substrate in this paper. The optimum conditions for cellulase production achieved by single factor testing were: the ratio of bagasse to wheat bran 1:1 (w/w), the ratio of water to material 3:1 (v/w), culture temperature 30 °C, initial pH 5.0, ammonium sulphate as nitrogen source with the concentration of 1%, 6 day's fermentation period. Box–Behnken factorial design (BBD) and response surface methodology (RSM) were further used to optimize conditions for cellulase (Filter paper activity) production. The maximal cellulase (Filter paper activity) production (3.89 FPU g^{-1}) was obtained under the optimized conditions (ratio of water to material 2.38:1, initial pH 5.28, cultivation time 150.5 h). It was well corresponded to the calculated results (3.97 FPU g^{-1}) by model prediction.

Key words: *Penicillium decumbens*; solid-state fermentation; cellulase, bagasse; Box-Behnken factorial design; response surface methodology.

INTRODUCTION

Cellulases are being widely used in industrial fields, for example, in starch processing, animal feed production, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry and textile industry (Ogel *et al*., 2001). Especially, cellulases have now becoming more and more important in hydrolyzing biomass into fermentable sugars which are then converted to bio-fuel like ethanol (Sun and Cheng, 2002; Sukumaran *et al.*, 2009) and hydrogen (Kumar and Das, 2001; Kapdan and Kargi, 2006) by microbial fermentation. Enzyme quantity is the major factor controlling the effectiveness and cost in the hydrolysis of cellulose, so it is necessary to improve the yields of the enzymes to make the process economically attractive (Romero *et al*., 1999).

Submerged fermentation (SmF) is generally used for industrial production of cellulase, but the cost of production and low yield of this enzyme are the major problems for industrial applications (Kang *et al*., 2004). In recent years, there has been a widespread resurgence of solid-state fermentation (SSF) process all over the word due to several advantages over systems mainly on engineering aspects (Pandey *et al*., 2000; Krishna, 2005). Producing cellulases by SSF is economical due to its lower capital investment, lower operating expenses (Panagiotou *et al*., 2003; Yang *et al*., 2004). The SSF process has a lower energy requirement, higher product yield with little risk of bacterial contamination, less wastewater generated and less environmental concerns regarding the disposal of solid waste (Lu *et al*., 1997). Another approach to reduce the cost of cellulase production is the use of lignocellulosic materials as substrates rather than expensive pure cellulose. In prior publications, abundant agricultural residue such as corn stover, wheat straw, rice straw, bagasse, etc. were used in cellulase production (Romero *et al*., 1999; Xia and Cen, 1999; Kang *et al*., 2004; Wen *et al*., 2005; Sukumaran *et al.*, 2009).

There are two ways by which the problem of fermentation parameters may be addressed: classical and statistical. The classical method is based on the "one-factor-at-a-time" method in which one independent variable is studied while maintaining all the other factors at a fixed level. This method may lead to unreliable results and inaccurate conclusions. Moreover, it does not guarantee the determination of optimal conditions, and is unable to detect the frequent interactions occurring between two or more factors. Response surface methodology (RSM), the statistical method, uses quantitative data from appropriate experiments to determine and simultaneously solve multivariate equations. It is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors, and analyzing optimum conditions of factors for desirable responses (Li *et al*., 2007).

In our previous work (Liu *et al*., 2008), the fungus *Penicillium decumbens* L-06 (GenBank accession number EU273880) with high cellulase activity was screened from rice straw compost. To

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our knowledge, the fungus *P. decumbens* to produce cellulase with bagasse as the substrate by solid state fermentation has not ever reported. In this study, we report the effects of different conditions in promoting the production of the cellulase enzyme by solid state fermentation and using statistical techniques like RSM to analyse the optimal parameter combination to maximize the filter paper activity (FPA).

MATERIALS AND METHODS

Organism and cultivation. The cellulase producing strain of *P. decumbens* L-06, was isolated and developed in this laboratory (Liu *et al*., 2008). It was maintained on potato dextrose agar (PDA) slants, stored at 4 °C and sub-cultured every two weeks. For inoculum preparation, the cultures were incubated on PDA at 30 °C for 16 or 18 h with 150 rpm agitation on rotary shaker and then transferred into solid-state fermentation medium according to ten percent inoculation quantity (10^6 spores/ml).

Substrate. The material used in this work was untreated bagasse and wheat bran. The bagasse was washed with water to remove all residual sugar, dried and milled to 40 mesh powder.

Solid-state fermentation medium. The substrate was mixed thoroughly with Mandel's solution (Mandels and Weber,. 1969; Sun *et al*., 2008). The Mandel's solution used had the following composition (we slightly modified): 10.0 g $(NH_4)_2SO_4$, 3.0 g KH_2PO_4, 1.0 g $MgSO_4{\cdot}7H_2O$, 0.5 g $CaCl_2$, 7.5 mg $FeSO_4{\cdot}7H_2O$, 2.5 mg $MnSO_4{\cdot}H_2O$, 2.0 mg $ZnSO_4{\cdot}7H_2O$, and 5.5 mg $CoCl_2{\cdot}6H_2O$ (pH 5.0) per 1000 ml distilled water. The contents were sterilized for 30 min at 1.1 kg cm^{-2} pressure.

Experimental design. Experiments were conducted in 250-ml Erlenmeyer flasks, each containing 5 g of crude sugarcane bagasse and wheat bran. Firstly, we conducted single-factor experiments to identify the variables with significant effects on cellulase production by *P. decumbens* L-06, and the tested factors included the ratio of bagasse to wheat bran, the ratio of water to solid material, initial pH, culture temperature, nitrogen source, ammonium sulphate concentration and fermentation time. The moisture content was kept at 70%. To remove the large amounts of heat produced in the substrate bed during growth of microorganisms, agitation was employed.

Then, based on the results of single-factor experiments, three major factors which affect FPA production were further studied. A three level, three variable Box-Behnken factorial design (BBD) (Minitab software 13.0) was applied to determine the best condition combination for cellulase production. Based on the investigations on single-factor experiment, the variables considered were the ratio of water to material, initial pH, fermentation time in the experimental design. The independent factors and the dependent variables used in this design were listed in Table 1. Table 2 listed the definition and coded levels that were carried out for developing the model. Each experiment was performed in triplicate and the average yield of cellulase was taken as the response, Y.

Regression analysis was performed, based on the experimental data, and was fitted into an empirical second-order polynomial model as shown in the following equation:

$$Y = \sum A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_i X_j \quad (1)$$

where Y was the response variable, A_0, A_i, A_{ii}, A_{ij} were the regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively, and X_i and X_j were independent variables (i„j).

TABLE 1 - Definition and coded levels for Box-Behnken design matrix

Factor	Levels		
	-1	0	1
Ratio of water to raw material (X1)/ml g^{-1}	1	3	5
pH (X2)	4.0	5.0	6.0
Fermentation time (X3)/d	4	6	8

TABLE 2 - Box-Behnken design matrix and the responses of the dependent variables of the FPA activity

Run	X1	X2	X3	FPA activity (IU/g)
1	-1	-1	0	1.9377
2	1	-1	0	1.6084
3	-1	1	0	1.9266
4	1	1	0	1.3155
5	-1	0	-1	0.5592
6	1	0	-1	1.4058
7	-1	0	1	3.5810
8	1	0	1	1.5240
9	0	-1	-1	1.2937
10	0	1	-1	1.8591
11	0	-1	1	1.0511
12	0	1	1	3.5793
13	0	0	0	3.7709
14	0	0	0	3.9696
15	0	0	0	3.8504

Experimental FPA activity was averages of triplicates.

The responses obtained from each set of experimental design (Table 2) were subjected to multiple nonlinear regression using the software Minitab, Version 13.0 to obtain the coefficients of the second polynomial model. The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2, and the statistical and regression coefficient significance were analyzed by Fisher's *F*-test.

Analytical methods. Fermented residues, 2 g, were suspended in 40 ml distilled water, incubated at 45 °C for 2 h and then centrifuged (8000 rpm, 10 min). The supernatant was used for the determination of enzyme activities. The mouldy bran dry weight was determined by drying 10g mouldy bran to constant weight at 105 °C. FPA and endo-β-1,4-glucanase activity (CMCase) were measured as described by Ghose (1987). One unit of enzyme activity was defined as the amount of enzyme required for liberating 1 μM of reducing sugar per minute and the cellulase activity was expressed as IU g^{-1} (units per gram dry mouldy bran).

RESULTS AND DISCUSSION

Effect of the ratio of bagasse to wheat bran on cellulase production

Here, bagasse was mainly used as the substrate and wheat bran was used as auxiliary material to produce cellulase. The designed experimental maximum ratio of the two materials was 1:1 (w/w) in this study. As indicated in Fig. 1, cellulase production by *P. decumbens* L-06 increased with the quantity of wheat bran. When the ratio reached 1:1, the FPA and CMCase activities were 3.4 and 41.2 IU g^{-1}at day 6, respectively. If the bagasse was used as the only fermentation material, the FPA and CMCase activities could not be examined, and similar results were also found by Xia and Chen (Xia and Cen, 1999). Therefore, it is reasonable to conclude that wheat bran was essential for cellulase production, and the ratio of 1:1 was chosen in this study. Actually, apart from bagasse (Ögel *et al*., 2001), other cellulosic materials could also be used as cellulose sources such as wood (Reczey *et al*., 1996), wheat straw (Romero *et al*., 1999), fruit pomace (Haddadin *et al*., 2001), dairy manure (Wen *et al*., 2005).

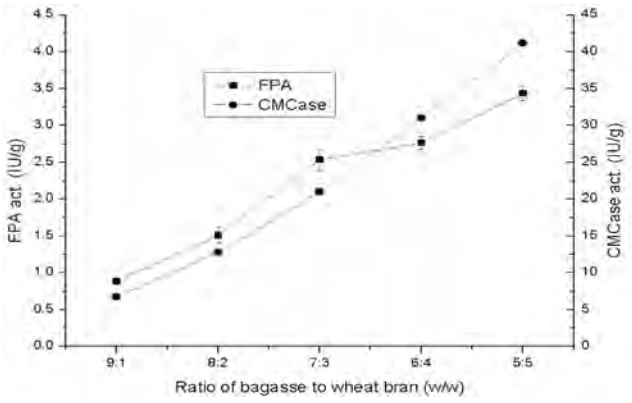

FIG. 1 - Effect of the ratio of bagasse to wheat bran on cellulase production. Cellulase production was conducted at 30 °C, the ratio of water to material was 3:1 (v/w), initial pH 5.0, 1% ammonium sulphate.

Effect of the ratio of water to material on cellulase production

In the solid state fermentation, the ratio of water to raw material posed significant effects on the cellulase production. As shown in Fig. 2, the maximum FPA and CMCase activity were achieved when the ratio was 3:1 (v/w), being 3.7 and 39.5 IU g^{-1} at day 6, respectively. Consequently, the ratio of 3:1 was chosen as the optimal water to raw material ratio for cellulase production. Xia and Chen (1999) also reported that the optimal water content in the solid substrate appears to be 75%.

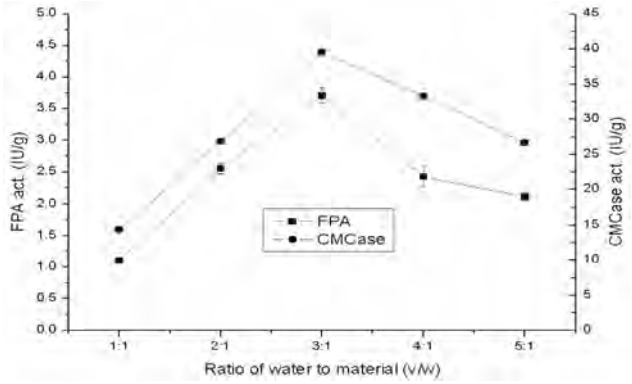

FIG. 2 - Effect of the ratio of water to material on cellulase production. Cellulase production was conducted at 30 °C, the ratio of bagasse to wheat bran was 1:1(w/w), initial pH 5.0, 1% ammonium sulphate.

Effect of culture temperature on cellulase production

Cellulase production at different culture temperature was shown in Fig. 3. At 25 °C, the least cellulase activities were determined, and the most favourable temperature was found to be 30 °C. At 30 °C, the FPA and CMCase activities were 3.9 and 42.3 IU g^{-1}, respectively. The responses of the FPA and CMCase activities to culture temperatures were very much consistent in this study.

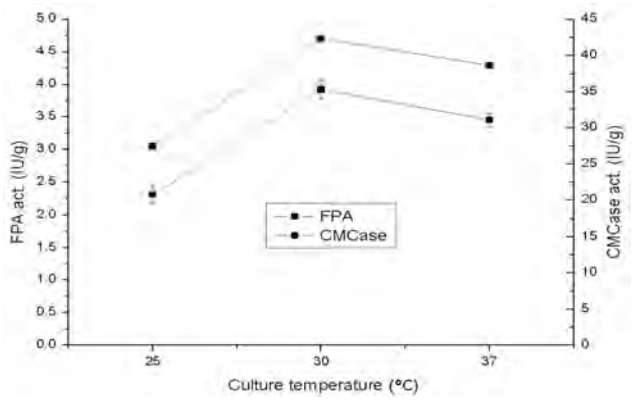

FIG. 3 - Effect of culture temperature on cellulase production. Cellulase production was conducted at the ratio of bagasse to wheat bran was 1:1 (w/w), the ratio of water to material was 3:1(v/w), initial pH 5.0, 1% ammonium sulphate.

Effect of initial pH on cellulase production

Cellulase can be produced over a large range of pH values. In this study, the initial culture pHs were set up from 3.5 to 6.5 with the gap of 0.5. As shown Fig. 4, the maximum values of both the FPA and CMCase activities was recorded at pH 5.0, being 3.4 and 41.8 IU g^{-1}, respectively. There were two turning points at pH 6.0 and 4.0 for FPA production, pH 5.5 and 4.0 for CMCase production. However, other results indicated that the FPA did not vary significantly within an initial pH range of 4-6 (Xia and Cen, 1999; Latifian *et al*., 2007), and possibly this phenomenon was favourable for cultivation in solid medium.

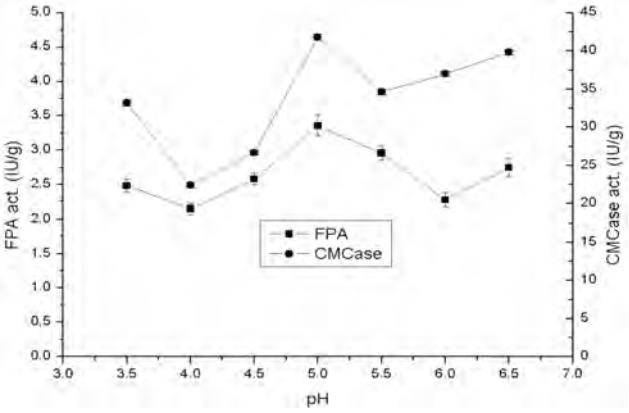

FIG. 4 - Effect of initial pH on cellulase production. Cellulase production was conducted at 30 °C, the ratio of bagasse to wheat bran was 1:1 (w/w), the ratio of water to material was 3:1 (v/w), 1% ammonium sulphate.

Effect of nitrogen source on cellulase production

Ammonium nitrate, tryptone, ammonium chloride, urea, ammonium sulphate were used as different nitrogen sources in this study, and the same nitrogen concentration was referred for each source. As indicated in Fig. 5, the order of capability to induce cellulase production was Ammonium sulphate > Ammonium Chloride > Ammonium nitrate > Urea > Tryptone. At the same time, it was found that only urea could accelerate the growth of *P. decumbens* L-06, and the colour of the solid state fermentation medium turned to greyish-green from white even at day 3. Actually, other researchers (Xia and Cen, 1999; Sun *et al*., 2008) ever added urea to promote fungi growth and result in the maximum cellulase production ahead of time.

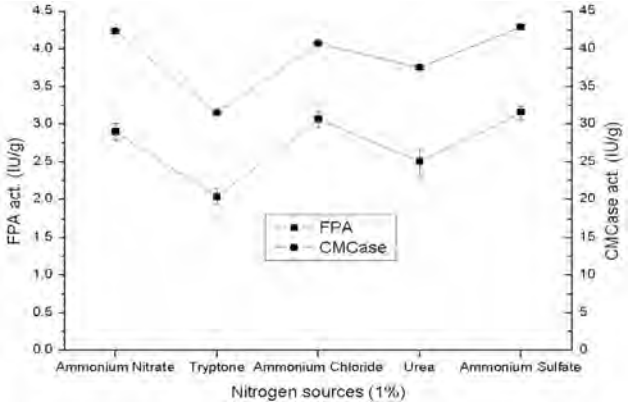

FIG. 5 - Effect of nitrogen source on cellulase production. Cellulase production was conducted at 30 °C, the ratio of bagasse to wheat bran was 1:1 (w/w), the ratio of water to material was 3:1 (v/w), initial pH 5.0.

Effect of fermentation time on cellulase production

Here, fermentation periods were set from day 1 to day 9, and the celluase activity was examined every 24 h. As indicated in Fig. 6, from the first day to the sixth day, the FPA kept increasing until reaching the maximum of 3.9 IU g^{-1} at day 6, and then decreased afterwards. However, the CMCase activity increased continuously from the first day until the maximum yield of 44.7 IU g^{-1} was achieved at day 8, and then decreased.

Box-Behnken design results and response surface analysis

The response surface methodology allowed the calculation of maximum yield based on the data from a few sets of experiments in which all the factors varied within chosen ranges. This method has been successfully applied in the optimization of medium compositions (Vasconcelos *et al*., 2000), conditions of enzymatic hydrolysis (Ma and Ooraikul, 1986) and enzyme production (Latifian *et al*., 2007; Li *et al*., 2007). In this study, the FPA was chosen as our research object. Therefore, the experiment design was shown in Table 1, the actual levels of the variables for each of the experiments in the design matrix were calculated and the results obtained were outlined in Table 2. Multiple regression analysis was performed on the experimental data, and the coefficients values of the coefficients were presented in Table 3. The analysis of variance (ANOVA) for the BBD was shown in Table 4. The mathematical model representing the FPA as a function of the independent variables within the region under investigation was expressed by the following equation:

$$(Y) = 3.86364 - 0.268846\ X_1 + 0.348707\ X_2 + 0.577212\ X_3 - 1.17245\ X_1^2 - 0.994132 X_2^2 - 0.923693\ X_3^2 - 0.0704324\ X_1X_2 - 0.725897\ X_1X_3 + 0.490697 X_2X_3 \quad (2)$$

where Y was the FPA, and X_1, X_2 and X_3 were the coded variables for the ratio of water to material, initial pH, fermentation time, respectively. Coefficient (R^2) of determination is defined as the ratio of the explained variation to the total variation,

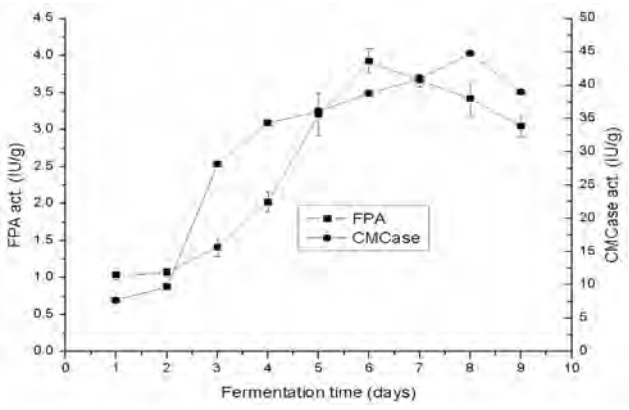

FIG. 6 - Effect of fermenattion time on cellulase production. Cellulase production was conducted at 30 °C, the ratio of bagasse to wheat bran was 1:1 (w/w), the ratio of water to material was 3:1 (v/w), initial pH 5.0, 1% ammonium sulphate.

TABLE 3 - Estimated Regression Coefficients for Y (FPA activity)

Term	Coefficients estimated	*t*-value	*P*-value
Constant	3.864	11.099	0.000
X1	- 0.269	- 1.261	0.263
X2	0.349	1.636	0.163
X3	0.577	2.708	0.042[a]
X1*X1	- 1.172	- 3.737	0.013[a]
X2*X2	- 0.994	- 3.168	0.025[a]
X3*X3	- 0.924	- 2.944	0.032[a]
X1*X2	- 0.070	- 0.234	0.825
X1*X3	- 0.726	- 2.408	0.061
X2*X3	0.491	1.628	0.165

[a] Significant at 5% level ($p < 0.05$).

TABLE 4 - Analysis of variance for Y (Filter paper activity)

Source	Degree of freedom	Adj SS	Adj MS	*F-value*	*P*-value
Regression	9	17.6250	1.95834	5.39	0.039
Linear	3	4.2164	1.40546	3.87	0.090
Square	3	10.3179	3.43932	9.46	0.017
Interaction	3	3.0907	1.03023	2.83	0.146
Residual Error	5	1.8177	0.36354		
Lack-of-Fit	3	1.7977	0.59922	59.88	0.016
Pure Error	2	0.0200	0.01001		
Total	14	19.4427			
	S = 0.6029	R-Sq = 90.7%	R-Sq(adj) = 73.8%		

and is a measurement of the degree of fitness (Nath and Chattopadhyay, 2007). A small value of R^2 indicates a poor relevance of the dependent variables in the model. The model can fit well with the actual data when R^2 approaches unity (Sin *et al*., 2006). As shown in Table 3 and 4, the R^2 value of this model was determined to be 0.907, which showed that the regression model defined well the true behaviour of the system. The *P-values* of the model were 0.039 ($P < 0.05$) (Table 4), which indicated that the model fitness was significant. According to the *P-values* (Table 3), if the value was less than 0.05 which indicated significance level, X_1 and X_2 were therefore not significant, but X_3 was significant; X_1^2, X_2^2 and X_3^3 were significant; X_1 and X_2, X_1 and X_3, X_2 and X_3 interactions were not significant. Other researches (Sin *et al*., 2006; Sun *et al*., 2008) indicated that the FPA did not vary significantly within an initial pH range of 4-6. From the results X_2 (initial pH) of *P-valus* in this study, initial pH was not significant in affecting the FPA.

To determine optimal levels of the variables for the FPA, three dimension surface plots were constructed. The effects of the ratio of water to material, initial pH, fermentation time, as well as their interactions, were shown in Fig. 7. There was an optimal value for the ratio of water to material to obtain the highest FPA, and lower or higher this value would lead to decrease in the FPA. This optimal value for the ratio of water to material could vary with different initial pH and fermentation time employed (Fig. 7A, 7B, 7C and 7D). Initial pH had a similar effect on the FPA as the ratio of water to material (Fig. 7C and 7E). The FPA reached a maximum at a fixed pH, but it would decrease when exceeding the optimal value (Fig. 7E and 7F). The interaction of the ratio of water to material and fermentation time posed a similar effect on the FPA as the interaction of the ratio of water to material and pH (Fig. 7C and 7E).

To further validate the optimal culture condition, the optimal values of the variables affecting the yield of the FPA given by the software were calculated in the equation, which gave the following results: $X_1 = 2.377$; $X_2 = 5.281$; $X_3 = 6.273$. Therefore, the optimal combination of the variables was the following: the ratio of water to material was 2.38 (v/w), initial pH was 5.28, fermentation time was 150.5 h. Under these conditions, the predicted value of Y (FPA) from the model was 3.97 IU g^{-1}. When *P. decumbens* L-06 was cultivated under the optimal combination achieved by response surface analysis (RSA) method, the FPA was found to be 3.89 IU g^{-1}, and this value was very close to the theoretical predicted one, indicating the validity of RSA method, and the effectiveness of the experimental design matrix for the cellulase yield, the FPA in this study.

Acknowledgments

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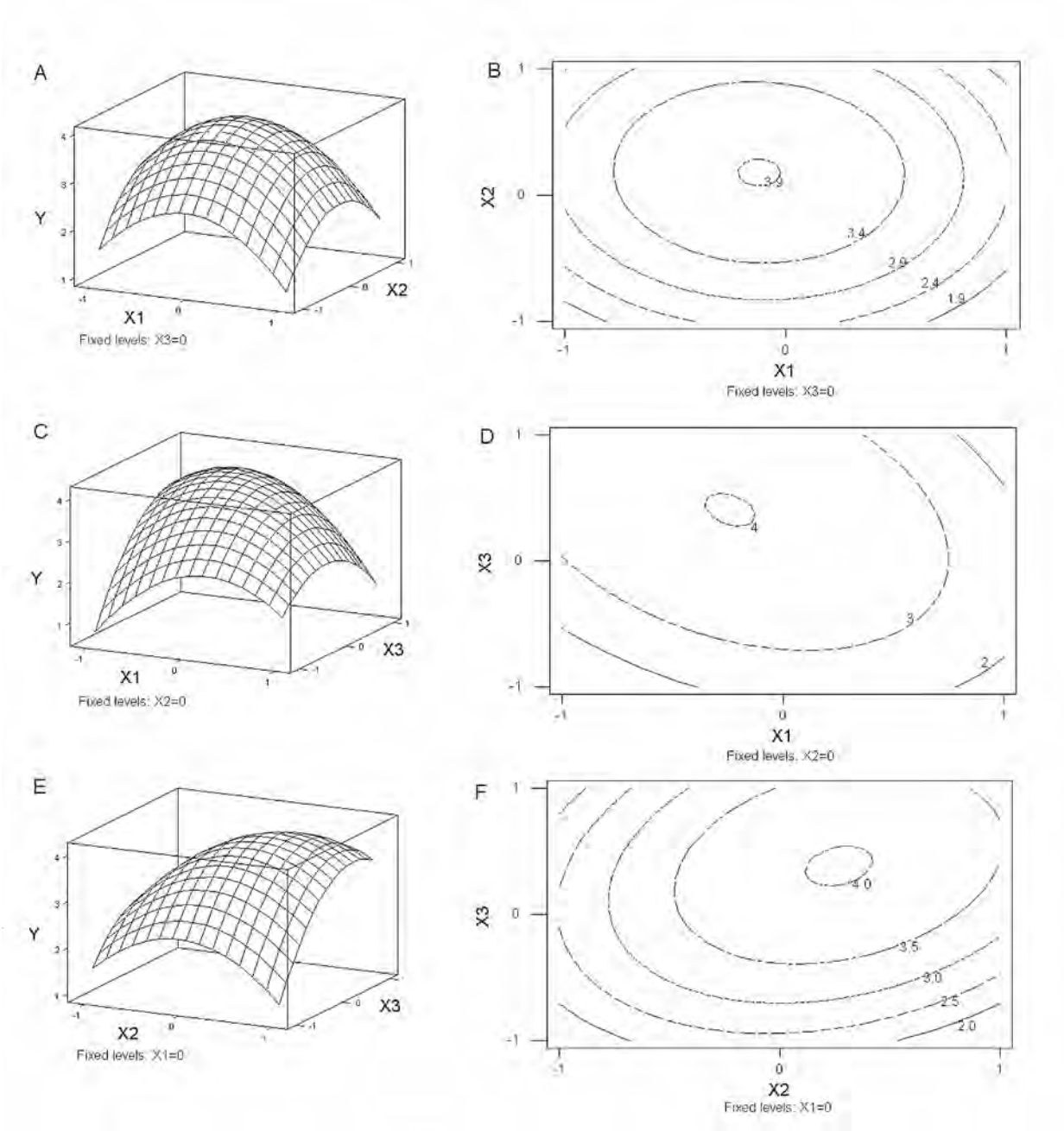

FIG. 7 - Response surface (3D) and contour plots showing interaction effects added on the response Y (Filter paper activity). A and B was the effects of the ratio of water to raw material (X1) and pH (X2); C and D was the effects of the ratio of water to raw material (X1) and fermentation time (X3); E and F was the effects of pH (X2) and fermentation time (X3).

REFERENCES

Ghose T.K. (1987). Measurement of cellulase activities. Pure Appl. Chem., 59: 257-268.

Haddadin M.S.Y., Abu-Reesh I.M., Haddadin F.A.S., Robinson R.K. (2001). Utilisation of tomato pomace as a substrate for the production of vitamin B_{12} - a preliminary appraisal. Bioresour. Technol., 78: 225-230.

Kang S.W., Park Y.S., Lee J.S., Hong S.I., Kim S.W. (2004). Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresour. Technol., 91, 153–156.

Kapdan I.K., Kargi F. (2006). Bio-hydrogen production from waste materials. Enzyme Microb. Technol., 38 (5): 569-582.

Krishna C. (2005). Solid-state fermentation systems: An overview. Crit. Rev. Biotechnol., 25: 1-30.

Kumar N., Das D. (2001). Continuous hydrogen production by immobilized *Enterobacter cloacae* IIT-BT 08 using lignocellulosic materials as solid matrices. Enzyme Microb. Technol., 29 (4-5): 280-287.

Latifian M., Hamidi-Esfahani Z., Barzegar M. (2007). Evaluation of culture conditions for cellulase production by two *Trichoderma reesei* mutants under solid-state fermentation conditions. Bioresour. Technol., 98: 3634-3637.

Li Y., Cui F.J., Liu Z.Q., Xu Y.Y., Zhao H. (2007). Improvement of xylanase production by *Penicillium oxalicum* ZH-30 using response surface methodology. Enzyme Microb. Technol., 40: 1381-1388.

Liu Y.T., Xuan S.X., Long C.N., Long M.N., Hu Z. (2008). Screening, identifying of cellulose-decomposing strain L-06 and its enzyme-producing conditions. Chinese J. Biotech., 24 (6): 1112-1116.

Lu M.Y., Brooks J.D., Maddox I.S. (1997). Citric acid production by solid state fermentation In a packed bed reactor using *Aspergillus niger*. Enzyme Microb. Technol., 21 (6): 392-397.

Ma A.Y.M., Ooraikul B. (1986). Optimization of enzymatic hydrolysis of canla meal with response surface methology. J. Food Process Pres., 10: 99-113.

Mandels M., Weber J. (1969). The production of cellulases. Adv. Chem. Se., 95: 394-414.

Nath A., Chattopadhyay P.K. (2007). Optimization of oven toasting for improving crispness and other quality attributes of ready to eat potato-soy snack using response surface methodology. J. Food Eng., 80: 1282-1292.

Ögel Z.B., Yarangümeli K., Dündar H., Ifrij I. (2001). Submerged cultivation of *Scytalidium thermophilum* on complex lignocellulosic biomass for endoglucanase production. Enzyme Microb. Technol., 28 (7-8): 689-695.

Panagiotou G., Kekos D., Macris B.J., Christakopoulos P. (2003). Production of cellulytic and xylanolytic enzymes by *Fusarium oxysporum* grown on corn stover in solid state fermentation. Ind. Crops Products, 18: 37-45.

Pandey A., Soccol C.R., Mitchell D. (2000). New developments in solid state fermentation: I-Bioprocesses and products. Process Biochem., 35: 1153-1169.

Reczey K., Szengyel Z., Eklund R., Zacchi G.. (1996). Cellulase production by *T. reesei*. Bioresour. Technol., 57: 25-30.

Romero M.D., Aguado J., González L., Ladero M. (1999). Cellulase production by *Neurospora crassa* on wheat straw. Enzyme Microb. Technol., 25: 244-250.

Sin H.N., Yusof S., Hamid N.S.A., Rahman RA. (2006). Optimization of enzymatic clarification of sapodilla juice using response surface methodology. J. Food Eng., 73: 313-319.

Sukumaran R.K., Singhania R.R., Mathew G.M., Pandey A. (2009). Cellulase production using biomass feed stock and its application in ligno-cellulose saccharification for bio-ethanol production. Renew. Energ., 34 (2): 421-424.

Sun X.Y., Liu Z.Y., Zheng K., Song X., Qu Y.B. (2008). The composition of basal and induced cellulase systems in *Penicillium decumbens* under induction or repression conditions. Enzyme Microb. Technol., 42: 560-567.

Sun Y., Cheng J.Y. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour. Technol., 83 (1): 1-11.

Vasconcelos A.F.D., Barbosa A.M., Dekker R.F.H., Scarminio I.S., Rezende M.I. (2000). Optimization of laccase production by *Botryosphaeria* sp. In the presence of veratryl alcohol by the response-surface method. Process Biochem., 35: 1131-1138.

Wen Z.Y., Liao W., Chen S.L. (2005). Production of cellulase by Trichoderma reesei from dairy manure. Bioresour. Technol., 96: 491-499.

Xia L.M., Cen P.L. (1999). Cellulase production by solid state fermentation on lignocellulosic waste from the xylose industry. Process Biochem., 34(9): 909-912.

Yang Y.H., Wang B.C., Wang Q.H., Xiang L.J., Duan C.R. (2004). Research on solid state fermentation on rice chaff with a microbial consortium. Colloid Surf., 34: 1-6.