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Application of Response Surface Methodology (RSM) for Optimization of Physico-chemical Parameters for the Production of Endoglucanase by *Trichoderma ressei* Rut C-30 using Agro-residues

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ABSTRACT

Response surface methodology (RSM) involving central composite design (CCD) was employed to optimize the physico-chemical parameters for the production of endoglucanase by *Trichoderma ressei* Rut C-30 under solid state fermentation using a novel mixture of waste paper and wheat bran. Most effective variables for the endoglucanase production in screening experiments were incubation day, substrate ratio, solid: liquid ratio and pH of the medium. A quadratic model was developed through RSM in terms of related independent variables to maximize the endoglucanase production as the response. Incubation day and solid: liquid ratio were found to be the most significant factors. The predicted optimal parameters were tested in the laboratory and the final endoglucanase activity obtained was very close to the predicted value (22.93 IU/g, predicted; 25.43 IU/g, tested). After optimization, endoglucanase activity increased by ~1.77-fold. Our result shows that optimization of enzyme production is the most useful way to obtain concentrated enzyme extracts from solid state cultivation and that *T. ressei* Rut C-30 using cheap agro-residues can be an attractive source for endoglucanase production.

Keywords: agro-residues, endoglucanase, response surface methodology, solid-state fermentation, *Trichoderma reesei* Rut C-30
Abbreviations: CCD, central composite design; **CMC**, carboxymethyl cellulose; **RRL**, Regional Research Laboratory; **RSM**, response surface methodology; **SSF**, solid state fermentation; **SmF**, submerged fermentation

INTRODUCTION

Lignocellulose is a potentially valuable source of energy. It is composed of holocellulose bound to lignin. Different plant materials contain various ratios of cellulose, hemicellulose and lignin (Sánchez 2009). Lignocellulolytic microorganisms produce cellulases and hemicellulases that hydrolyze plant lignocelluloses (Esterbauer *et al*. 1991; Nieves *et al.* 1998; Jørgensen *et al.* 2003). These microorganisms and their enzymes are being used for valorisation of plant residues together with many other industrial and environmental applications (Bhat 2000). Currently the most important applications of cellulases are the production of dissolving pulp, treatment of wastewater, deinking of recycled waste paper in the paper and pulp industry and in the enzymatic saccharification of lignocellulosics present in agricultural residues (Ko *et al*. 2009; Sukumaran *et al*. 2009).

Cellulolytic enzyme systems can be produced by a number of different microorganisms, such as aerobic and anaerobic bacteria (Gilkes *et al.* 1991), white rot fungi (Uzcategui *et al.* 1991), soft rot fungi (Wood *et al.* 1988) and anaerobic fungi (Barichievic and Calza 1990). *T. reesei* Rut C-30 culture is the most widely utilized soft rot fungus that has been employed for the production of various cellulases using various agro-residues (Chahal *et al*. 1982; Singhania *et al*. 2006).

High cost of production and low yield of the enzyme are the major impediments for their industrial application. Therefore, investigations on the ability of the cellulose hydrolyzing microbial strains to utilize inexpensive substrate have been under taken. A little work has been directed for improvement of the fermentation processes (Esterbauer *et al*. 1991; Haltrich *et al*. 1996). Solid state fermentation (SSF) offers advantages over submerged fermentation (SmF), such as in the nature of the substrates, low media cost, higher productivity, less effort in downstream processing, reduced energy and cost requirements. Aeration also tends to require lower pressure than that needed for liquid cultivation (Smits *et al*. 1996).

Although there have been many studies regarding endoglucanase (EC 3.2.1.4) production using *Trichoderma* strains, a complete optimization of operational conditions for the production of the enzyme has received little attention in the literature (Reczey *et al*. 1996; Gerber *et al*. 1997). The effect of particle size, aeration rate and harvest time for the production of cellulase by *T. reesei* QM 9414 was evaluated through RSM by Rocky-Salimi and Hamidi-Esfahani (2010). Optimization of such processes with RSM may provide great benefits in the production of endoglucanase.

RSM is a combination of mathematical and statistical techniques that is useful in analyzing the effects of several independent variables on the system response without the need of a predetermined relationship between the objective function and the variables (Draper and John 1988; Draper and Lin 1990; Myers and Montgomery 2002; Tanyildizi 2005). The present study was performed to investigate and optimize the conditions for endoglucanase production employing agro-residues (waste paper and wheat bran) through RSM for batch runs designed with composite central design (CCD). Four factors, viz., incubation day, substrate ratio, solid: liquid ratio and medium pH were selected as process (independent) parameters while endoglucanase activity was

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selected as a response (dependent) parameter. Four factors in a broad treatment interval were studied simultaneously for the first time in literature to optimize the response while using a novel combination of agro-residues.

MATERIALS AND METHODS

Microorganism and material

A hyperactive strain of *Trichderma reesei* Rut C-30 was procured from the Regional Research Laboratory (RRL), Trivandrum and was maintained on 2% (w/v) malt extract - agar slants at around 28°C and sub-cultured once in two weeks. Wheat bran (WB) and waste paper (WP), locally available from the market, were used as a substrate for enzyme production. All chemicals were procured from Merck, India and Sigma Co., USA.

Inoculum development for endoglucanase production

10 ml of sterile distilled water was added to 6-day culture slants of *T. reesei* Rut C-30. Spores were scraped off with inoculating loop, aseptically. The spore suspension was taken and agitated in a vortex-cyclomixer for 5 min in order to disperse the spores evenly. Spore count was determined by haemocytometer and spore (conidia) suspension containing ~3.6×10^6 spores/ml was used as inoculum for subsequent fermentation.

Production medium

The primary culture medium (Das *et al*. 2008) used for endoglucanase enzyme production through solid-state fermentation by growing *T. reesei* Rut C-30. pH adjustment was made using either 0.1N HCl or liquid ammonia. The fermentation medium was sterilized at 121°C or 15 psi for 20 min.

Substrate and solid-state fermentation

WB and WP were utilized as substrate for SSF. 10 ml of modified Mandel's medium (Das *et al*. 2008) contained KH_2PO_4 (2 g/l), $(NH_4)_2SO_4$ (1.4 g/l), $MgSO_4{\cdot}7H_2O$ (0.3 g/l), $CO(NH_2)_2$ (0.2 g/l), peptone (0.2 g/l), yeast extract (0.2 g/l), vitamin (nicotinic acid, 15 mg/l), hormone (alpha- napthalene acetic acid, 7 mg/l), metal ion (ferric chloride, 5 mg/l) and surfactant (Tween-80, 15 ml/l) along with lactose (0.1%) and $NaNO_3$ (0.1%) was added to 5 g of well mixed autoclaved substrate in the 250 ml Erlenmeyer flasks. pH adjustment was made using either 0.1N HCl or liquid ammonia. The fermentation medium was sterilized at 121°C or 15 psi for 20 min. After sterilization, 0.2 ml spore suspension, described above was aseptically added to the substrate and mixed thoroughly. In preliminary experiments various process parameters influencing the enzyme production during SSF were studied including type of substrate, substrate ratio, incubation day, moisture content, solid: liquid ratio and pH. Among these factors the most promising ones, incubation day (X_1) (4, 5 and 6 days), substrate ratio (X_2) (waste paper: wheat bran) (1:4, 1:5 and 1:6), solid: liquid ratio (X_3) (1:0.5, 1:1 and 1:1.5) and medium pH (X_4) (4.5, 5.0 and 5.5) were intensively studied (**Table 1**).

Enzyme extraction and assay

The fermented biomass of *T. reesei* Rut C-30 was soaked in 10 ml of 5% glycerol-water solution for 2 h at room temperature for leaching out the extracellular enzyme. The biomass was then passed through folds of cheese cloth under pressure, and filtrate was centrifuged at 10,000 rpm at 4°C for 10 min in cold centrifuge (Remi-24) to remove the spores and other insolubles. The clarified supernatant was used as the source of the enzyme. The endoglucanase activity was determined according to Mandels *et al*. (1976), using 1% Na-carboxymethyl cellulose (CMC). The activity was expressed in International Units (IU). IU was calculated as number of µm of product (glucose) equivalents released per milliliter per minute.

Table 1 Independent variables and their levels in the experimental design.

Independent variables	Symbols	Code levels				
		-2	-1	0	+1	+2
Incubation day	(X_1)	3	4	5	6	7
Substrate ratio	(X_2)	3:1	4:1	5:1	6:1	7:1
Solid : Liquid ratio	(X_3)	1:0	1:0.5	1:1	1:1.5	1:2
pH	(X_4)	4.0	4.5	5.0	5.5	6.0

Experimental design

In order to ascribe the effect of factors on response surface in the region of investigation, a central composite design (CCD) with four factors at five levels was performed with three replicates using RSM (**Table 1**). In order to obtain ratio for factor level of substrates the values of one substrate (waste paper) and in solid: liquid ratio, solid substrates were made constant and other variable factors (wheat bran and liquid medium) were entered in design expert software. Enzyme activity (IU/g) of endoglucanase (Y) was taken as a response from the 30 sets analyzed (**Table 2**).

Response surface methodology

Response surface methodology may be summarized as a collection of statistical tools and techniques for constructing and exploring an approximate functional relationship between a response variable and a set of design variables (Venter 1998). The response variable was fitted by a second order model in order to correlate the response variable to the independent variables. The general form of the second degree polynomial equation is:

$$Y_i = \beta_o + \sum \beta_i \chi_i + \beta_{ii} \chi \sum_i^2 + \sum \beta_{ij\chi_i\chi_j}$$

where Y_i is the predicted response, X_iX_j are input variables which influence the response variables Y; β_o is the offset term; β_i is the i^{th} linear coefficient; β_{ii} the i^{th} quadratic coefficient and β_{ij} is the ij^{th} interaction coefficient.

The second order polynomial coefficients were calculated using the statistical software Design-Expert 8.0 (Stat-Ease, Inc., Minneapolis, USA). The data obtained from RSM on endoglucanase production were subjected to the analysis of variance (ANOVA). Statistical significance of the model equation was determined by Fisher's test value, and the proportion of variance explained by the model was given by the multiple coefficient of determination, R squared (R^2) value. It also includes the Student's *t*-value for the estimated coefficients and the associated probabilities *p*(*t*). For each variable, the quadratic models were represented as contour plots (2D).

RESULTS AND DISCUSSION

On the basis of initial results of endoglucanase production the boundary limits of each variable were determined (**Table 1**). Data from the 30 sets were analyzed to yield regression equation and regression coefficient (R^2). The responses Y was fitted with second order polynomial equation (see equation at base of this page).

The statistical significance of the model equation was evaluated by the F-test for analysis of variance (ANOVA). The ANOVA statistics for the Y response is shown in **Table 3**. The results of this quadratic models indicated that this could be used to navigate the design space. **Table 3** evinces that the prob > *F*-values for endoglucanase production is lower than 0.05 indicating that quadratic model was significant. The coefficient of determination (R^2) that was found

$$Y(\text{endoglucanase activity}) = 23.01 - 2.44X_1 - 2.93X_2 + 0.89X_3 - 2.49X_4 - 3.43X_1^2 - 3.03X_2^2 - 4.22X_3^2 - 2.02X_4^2 + 0.74X_1X_2 - 0.38X_1X_3 + 1.78X_1X_4 - 0.85X_2X_3 + 3.80X_2X_4 - 0.96X_3X_4$$

Table 2 Experimental design and results of the central composite design.

Run	Variables				Response (Y) endoglucanase activity	
	X_1 incubation days	X_2 substrate ratio	X_3 solid:liquid ratio	X_4 pH	Actual value (IU/g)	Predicted value (IU/g)
1	5	1:5	1:1	5.0	24.11	24.52
2	5	1:5	1:1	5.0	24.01	24.52
3	4	1:6	1:1.5	5.5	8.82	9.58
4	4	1:6	1:0.5	5.5	12.97	10.64
5	4	1:6	1:1.5	4.5	14.79	12.44
6	4	1:6	1:0.5	4.5	3.22	9.69
7	6	1:6	1:0.5	5.5	3.97	11.57
8	6	1:6	1:0.5	4.5	7.97	3.49
9	6	1:4	1:1.5	5.5	8.02	7.48
10	6	1:4	1:0.5	5.5	11.42	6.67
11	6	1:4	1:1.5	4.5	23.17	18.40
12	6	1:6.	1:1.5	4.5	1.87	4.74
13	6	1:6	1:1.5	5.5	6.15	9.00
14	4	1:4	1:1.5	4.5	30.75	29.07
15	5	1:5	1:1	5.0	22.14	24.52
16	6	1:4	1:0.5	4.5	8.60	13.77
17	5	1:5	1:1	5.0	23.13	24.52
18	4	1:4	1:1.5	5.5	13.64	11.02
19	4	1:4	1:0.5	4.5	32.88	22.93
20	4	1:4	1:0.5	5.5	5.65	8.70
21	5	1:5	1:2	5.0	4.24	6.39
22	5	1:3	1:1	5.0	7.80	15.25
23	7	1:5	1:1	5.0	5.44	2.89
24	5	1:5	1:0	5.0	3.80	2.82
25	5	1:5	1:1	4.0	14.63	18.40
26	5	1:7	1:1	5.0	9.80	3.52
27	5	1:5	1:1	5.0	24.54	21.49
28	5	1:5	1:1	5.0	23.14	21.49
29	3	1:5	1:1	5.0	8.90	12.63
30	5	1:5	1:1	6.0	11.03	8.43

Table 3 ANOVA analysis for response Y (endoglucanase activity IU/g).

Source	Sum of squares	DF	Mean square	F-value	Prob > F
For Y					
Model	1689.97	14	120.71	3.38	< 0.0148
Residual	499.71	14	35.69		
Lack of fit	496.21	10	49.62	56.62	0.0007
Pure error	3.51	4	0.88		
$R^2 = 0.7718$					
Adeq. precision = 6.017					

to be close to 1 (0.77 for Y) also advocated a high correlation between observed and predicted values. The "lack of fit tests" compares the residual error to the "Pure Error" from replicated experimental design points. The *P*-values, less than 0.05, for the responses indicate that lack of fit for the model was significant. Adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable. The adequate precision for Y was 6.02. These high values of adequate precision demonstrated that model is significant for the process.

Usually it is essential to ensure that the selected model provides an adequate approximation to the real system. Model adequacy can be assessed by plotting predicted versus actual value. The correlation coefficient between actual and predicted values for Y was 0.77. This R^2 values illustrate good agreement between the calculated and observed results within the range of experiment.

Perturbation plot (**Fig. 1**) shows the comparative effects of all the physico-chemical components on endoglucanase activity. In **Fig. 1**, a steep curvature in incubation days and solid: liquid ratio shows that the response of endoglucanase activity was very sensitive to these factors. However, with respect to substrate ratio and pH the responses appeared relatively less sensitive.

Experimental run 19 shows (**Table 2**) the highest level of endoglucanase activity (32.88 IU/g) which suggests that the endoglucanase activity by *T. reesei* Rut C-30 was increased at moderate cultivation time (4 days), substrate ratio (1 WP: 4 WB), solid: liquid ratio (1:0.5) and pH (4.5). The highest enzymatic activity experimentally obtained according to the CCD was 1.77-fold higher than that obtained under conditions previously employed in our laboratory (18.6 IU/g at 5 days, 1:4 substrate ratio, 1:1 solid: liquid ratio and pH 4.8). The endoglucanase activity rapidly increased between days 1-3, and reached a plateau on day 4 and 5. Since endoglucanase is the key enzyme in the degradation of cellulose, a process necessary for cell survival, the trend in its production by *T. reesei* Rut C-30 increased in the early logarithmic phase of the cell growth. This result was similar to Thygesen *et al*. (2003) in which maximum endoglucanase activity (0.19 U/ml) was achieved after 106 h under SmF and also to Reczey *et al*. (1996) who reported maximum cellulase synthesis between 3-5 days by *T. reesei*. In the present study the endoglucanase activity was found at the highest level from the cultivation of mixed substrates of waste paper: wheat bran in 1:4 ratio. Jecu (2000) also reported the importance of mixed substrate for endoglucanase production employing a mixture of wheat bran and wheat straw as a substrate for *Aspergillus niger*. The observed endoglucanase activity (32.88 IU/g) in *T. reesei* Rut C-30 was higher as compared to endoglucanase activities reported for *A. niger* (66 nkat/g of dry substrate), *Schizophyllum commune* (420 nkat/g of dry substrate) and *Trichoderma reesei* (19 nkat/g of dry substrate) under SSF using wheat straw as substrate (Thygesen *et al*. 2003). Moisov (2010) also used wheat straw as a substrate in SSF medium for cellulase production by *T. viride* and reported a maximum cellulase activity of 2.2 U/ml. The favorable solid: liquid

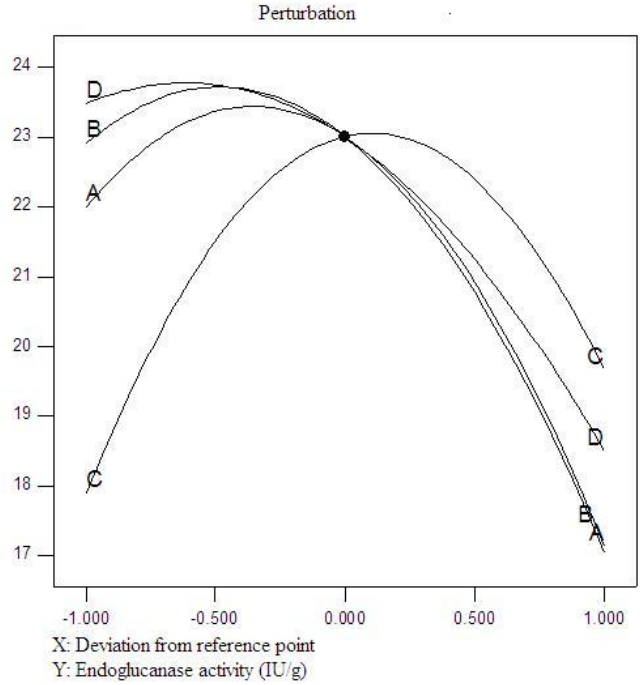

Fig. 1 Perturbation plots. Plots for endoglucanase activity by *Trichoderma ressei* Rut C-30; (A) Incubation day, (B) Substrate ratio, (C) Solid: Liquid ratio and (D) pH.

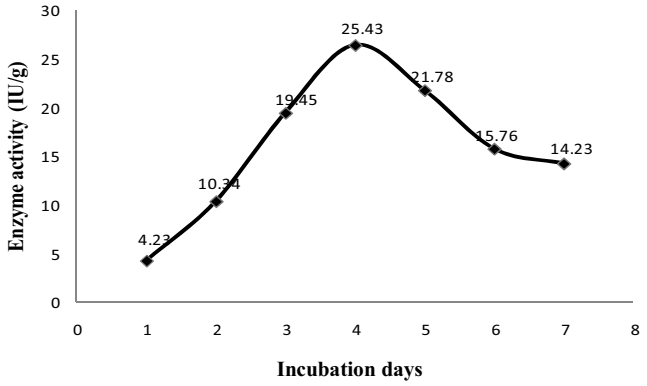

Fig. 2 Endoglucanase (♦) activity profile under the optimal conditions suggested by the model. Substrate ratio = 1:4, solid: liquid ratio = 1:0.5 and pH = 4.5.

ratio for maximum endoglucanase activity is mainly at the higher levels (1:0.5) of solid: liquid ratio. Satyanarayana (1994), Babu and Satyanarayana (1995) and Raimbault and Alazard (1980) also investigated relation between several solid substrates to moistening agent ratios. In the present study, pH 4.5 was found to be most suitable for the higher endoglucanase activity by *T. reesei* Rut C-30. This result is similar to the report of Vlaev *et al*. (1997) in which 4.5 pH favored the growth of *Trichoderma* sp. strain 414 and enhanced the activity of cellulase. The results varied from the report of Sami *et al*. (2008) who found that the CMCase was more active on substrate at pH 5.8. The results indicated that an optimum pH for fungal cellulases varies from species to species; though in most cases the optimum pH ranges from 3.0 to 6.0 (Rodriguez *et al*. 2005; Niranjane *et al*. 2007).

A validation of the model is given in **Fig. 2**, shows that cultivation of *T. reesei* Rut C-30 for the maximum endoglucanase production under optimal conditions was at substrate ratio (1:4), solid: liquid ratio (1:0.5) and pH (4.5). The maximum endoglucanase activity obtained was 25.43 IU/g in 4 days. In this case, the model predicted endoglucanase activity of 22.92 IU/g in 4 days. The experimental value was found to be 9.87% higher than the predicted value, confirming the closeness of the model to the experimental result.

Fig. 3 shows that the shapes of contour curves of variables are independent of each other. The contour plot of the interaction effect of solid: liquid ratio and pH on endoglucanase activity of *T. reesei* Rut C-30 in **Fig. 3A**. It evinces that endoglucanase activity tends to be maximum within the range of pH (4.5-4.8) and solid: liquid ratio (1:0.9-1:1.2). **Fig. 3B** shows contour plot of substrate ratio against pH. The endoglucanase activity tends to increase with low substrate ratio and pH. **Fig. 3C** illustrated the contour plot as function of substrate ratio versus solid: liquid ratio. It shows that increase in the initial substrate ratio and solid: liquid ratio within the range tested improved endoglucanase activity and that maximum endoglucanase activity is achieved at an initial substrate ratio in the range of 1:4.5-1:1.5 and a solid: liquid ratio in the range of 1:0.9-1:1.2. Our results were found be highly correlated with Iqbal *et al*. (2010) and Ahmed *et al*. (2010), who were reported maximum CMCase activity at 40% moisture level. **Fig. 3D** presents variation in endoglucanase activity owing to simultaneous change in incubation day and pH. Relative to **Fig. 3A**, the highest endoglucanase activity occurred at low pH. High endoglucanase activity was obtained at a short length of incubation that declined with lengthening of incubation time. Such a variation appears to be connected with the accumulation of hydrolysis products such as glucose and cellobiose, which are inhibitors for cellulase-system activity and could adversely affect the rate of endoglucanase production (Castellanos *et al*. 1995; Xia and Cen 1999). **Fig. 3E** shows the effect of incubation day and solid: liquid ratio on endoglucanase activity at fixed pH (5.0) and substrate ratio (1 WP: 5 WB). Increasing the incubation day and initial solid: liquid ratio within the range tested improved endoglucanase activity this was achieved at an incubation day in the range of 4-5 and solid: liquid ratio in the range of 1:0.9-1:1.2. **Fig. 3F** indicates the interaction effect of incubation day and substrate ratio on endoglucanase activity where reduction in incubation day and substrate ratio resulted in an increase in endoglucanase activity.

CONCLUSION

In this work, evidences have been found to determine the optimal conditions for the simultaneous production of endoglucanase by *T. reesei* Rut C-30 in SSF employing experimental factorial design and response surface methodology. The maximal activity of endoglucanase produced was 25.43 IU/g, when optimized conditions were employed. The enzyme activity predicted by the model at optimal conditions agreed fittingly with experimental data, thus confirming the model validity. The results obtained in this study are expected to facilitate further work on the purification of the endoglucanase produced by *T. reesei* Rut C-30.

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REFERENCES

Ahmed I, Zia MA, Iqbal HMN (2010) Bioprocessing of proximally analyzed wheat straw for enhanced cellulase production through process optimization with *Trichoderma viride* under SSF. *International Journal of Biological and Life Sciences* **6**, 164-170

Bhat MK (2000) Cellulases and related enzymes in biotechnology. *Biotechnology Advances* **18**, 355-383

Babu KR, Satyanarayana T (1995) Amylase production by thermophilic *Bacillus coagulans* in solid state fermentation. *Process Biochemistry* **30**, 305-309

Barichievic EB, Calza RE (1990) Supernatant protein and cellulase activities of the anaerobic ruminal fungus *Neocallimastix frontalis* EB 188. *Applied and Environmental Microbiology* **56**, 43-48

Castellanos OF, Sinitsyn AP, Vlasenko EY (1995) Comparative evaluation of hydrolytic efficiency toward microcrystalline cellulose of *Penicillium* and

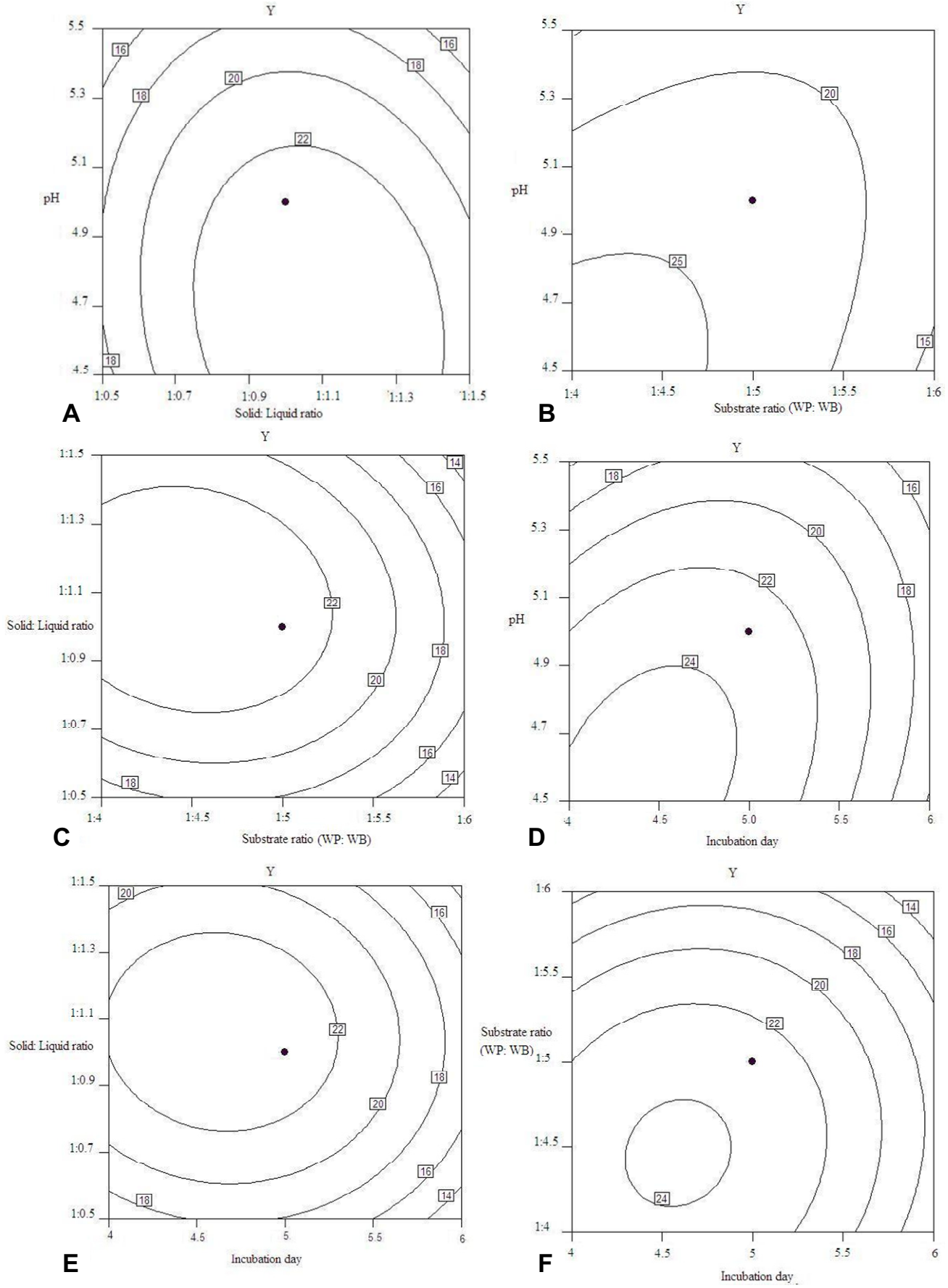

Fig. 3 Contour plots. Plots determined the effect of (**A**) pH × Solid: liquid ratio, (B) pH × Substrate ratio, (C) Solid: liquid ratio × Substrate ratio, (D) pH × Incubation day, (E) Solid: liquid ratio × Incubation day, (F) Substrate ratio × Incubation day on endoglucanase activity. Not plotted variables are fixed at zero level in all of the six graphs.

Trichoderma cellulases. *Bioresource Technology* **52**, 119-124

Chahal DS, McGuire S, Pikor H, Noble G (1982) Production of cellulase complex by *Trichoderma reesei* Rut C-30 on lignocellulose and its hydrolytic potential. *Biomass* **2**, 127-137

Draper NR, Lin DKJ (1990) Small response-surface designs. *Technometrics* **32**, 187-194

Draper NR, John JA (1988) Response-surface design for quantitative and qualitative variables. *Technometrics* **30**, 423-428

Das M, Banerjee R, Bal S (2008) Multivariable parameter optimization for endoglucanase production by *Trichoderma reesei* Rut C-30 from *Ocimum gratissimum* seed. *Brazilian Archives of Biology and Technology* **51**, 35-41

Esterbauer H, Steiner J, Labudova I, Hermann A, Hayn M (1991) Production of *Trichoderma* cellulase in laboratory and pilot scale. *Bioresource Technology* **36**, 51-65

Gerber PJ, Heitmann JA, Joyce TW (1997) Purification and characterization of xylanases form *Trichderma*. *Bioresource Technology* **61**, 127-140

Gilkes NR, Kilburn DG, Miller Jr RC, Warren RAJ (1991) Bacterial cellulases. *Bioresource Technology* **36**, 21-35

Haltrich D, Nidetzky B, Kulbe KD, Steiner W, Zupancic S (1996) Production of fungal xylanases. *Bioresource Technology* **58**, 137-161

Iqbal HMN, Asgher M, Ahmed I, Hussain S (2010) Media optimization for hyper-production of carboxymethyl cellulase using proximally analyzed agro-industrial residue with *Trichoderma harzianum* under SSF. *International Journal for Agro Veterinary and Medical Sciences* **4**, 47-55

Jecu L (2000) Solid state fermentation of agricultural wastes for endoglucanase production. *Industrial Crops and Products* **11**, 1-5

Jorgensen H, Eriksson T, Borjesson J, Tjerneld F, Olsson L (2003) Purification and characterization of five cellulases and xylanase from *Penicillium brasilianum* IBI 20888. *Enzyme and Microbial Technology* **32**, 851-861

Ko KJ, Bak JS, Jung MW, Lee HJ, Choi IG, Kim TH, Kim KH (2009) Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. *Bioresource Technology* **100**, 4374-4380

Mandels M, Andreotti R, Roche C (1976) Measurement of saccharifying cellulose. *Biotechnology and Bioengineering Symposium* **6**, 21-33

Moisov K (2010) Application of solid-state fermentation for cellulose enzyme production using *Trichoderma viride*. *Perspectives of Innovations, Economics and Business* **5**, 108-110

Myers RH, Montgomery DC (2002) *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, John Wiley and Sons Inc., USA, 43 pp

Nieves RA, Ehrman CI, Adney WS, Elander RT, Himmel ME (1998) Survey and analysis of commercial cellulase preparations suitable for biomass conversion to ethanol. *World Journal of Microbiology and Biotechnology* **14**, 301-304

Niranjane AP, Madhou P, Stevenson TW (2007) The effect of carbohydrate carbon sources on the production of cellulase by *Phlebia gigantean*. *Enzyme and Microbial Technology* **40**, 1464-1468

Raimbault M, Alazard D (1980) Culture method to study fungal growth in solid fermentation. *Applied Microbiology and Biotechnology* **9**, 199-209

Reczey K, Szengyel Zs, Eklund R, Zacchi G (1996) Cellulase production by *T. reesei*. *Bioresource Technology* **57**, 25-30

Rocky-Salimi K, Hamidi-Esfahani Z (2010) Evaluation of the effect of particle size, aeration rate and harvest time on the production of cellulase by *Trichoderma reesei* QM9414 using response surface methodology. *Food and Bioproducts Processing* **88**, 61-66

Rodriguez IA, Escobedo CP, Paramo MGZ, Romero EL, Camacho HC (2005) Degradation of cellulose by the bean-pathogenic fungus *Colletotrichum lindemuthianum*. Produciton of extracellular cellulolytic enzymes by cellulose induction. *Antonie van Leeuwenhoeck* **87**, 301-310

Sami AJ, Awais M, Shakorri AR (2008) Preliminary studies on the production of endo-1,4-β-D-glucanases activity produced by *Enterobacter cloacae*. *African Journal of Biotechnology* **7**, 1318-1322

Sánchez C (2009) Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnology Advances* **27**, 185-194

Satyanarayana T (1994) Production of bacterial extracellular enzymes by solid state fermentation. In: Pandey A (Ed) *Solid State Fermentation*, Wiley Eastern Ltd., New Delhi, India, pp 122-129

Singhania RR, Sukumaran RK, Pillai A, Prema P, Szakacs G, Pandey A (2006) Solid-state fermentation of lignocellulosic substrates for cellulase production by *Trichoderma reesei* NRRL 11460. *Indian Journal of Biotechnology* **5**, 332-336

Smits JP, Rinzema A, Tramper J, Van Sonsbeek HM, Knol W (1996) Solid-state fermentation of wheat bran by *Trichoderma reesei* QM9414: substrate composition changes, C balance, enzyme production, growth and kinetics. *Applied Microbiology and Biotechnology* **46**, 489-496

Sukumaran RK, Singhania RR, Mathew GM, Pandey A (2009) Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. *Renewable Energy* **34**, 421-424

Tanyildizi MS, Ozer D, Elibol M (2005) Optimization of α-amylase production by *Bacillus* sp. using response surface methodology. *Process Biochemistry* **40**, 2291-2296

Thygesen A, Thomsen AB, Schmidt AS, Jorgensen H, Ahring BK, Olsson L (2003) Production of cellulose and hemicelluloses degrading enzymes by filamentous fungi cultivated on wet oxidized wheat straw. *Enzyme and Microbial Technology* **32**, 606-615

Uzcategui E, Ruiz A, Montesino R, Johansson G, Pettersson LG (1991) The 1,4-β-D-glucan cellobiohydrolases from *Phanerochaete chrysosporium.* I. A system of synergistically acting enzymes homologous to *Trichoderma reesei*. *Journal of Biotechnology* **19**, 271-286

Venter G (1998) Non-dimensional response surfaces for structural optimization with uncertainty. PhD thesis, University of Florida, USA

Vlaev SD, Djejeva G, Raykovska V, Schugerl K (1997) Cellulase production by *Trichoderma* sp. grown on corn fibre substrate. *Process Biochemistry* **32**, 561-565

Wood TM, McCrae SI, Wilson CA, Bhat KM, Gow LM (1988) Aerobic and anaerobic fungal cellulases, with special reference to their mode of attack on crystalline cellulose. *FEMS Symposium No. 43, Biochemistry and Genetics of Cellulose Degradation* **43**, 31-52

Xia L, Cen P (1999) Cellulase production by solid state fermentation on lingocellulosic waste from the xylose industry. *Process Biochemistry* **34**, 909-912