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RESEARCH ARTICLE

ENHANCED PRODUCTION OF CELLULASE FROM TAPIOCA STEM USING RESPONSE SURFACE METHODOLOGY

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Abstract

Medium composition for production of cellulase by *Cellulomonas fimi NCIM-5015* from tapioca stem as a substrate in submerged fermentation was optimized using a stepwise strategy. Plackett-Burman design was employed for screening, the most significant nutrient components which influence the cellulose production from 12 nutrient components. Results revealed that peptone, yeast extract, KH_2PO_4 and $CaCl_2.2H_2O$, have significant effects on cellulase production, with confidence levels above 95%. Central composite design (CCD) was used to determine the optimal concentrations of these four components and the experimental results were fitted with a second-order polynomial model at 95% level ($P < 0.05$). The optimum conditions are peptone (0.957 g/L), yeast extract (2.5 g/L), KH_2PO_4 (3.09 g/L), and $CaCl_2{\cdot}2H_2O$ (0.409 g/L). Under these conditions, the production of cellulase was found to be 0.58 IU/ml.

Keywords: *cellulase, Plackett-Burman Design, tapioca stem, response surface methodology*

Introduction

Lignocellulosic biomass like agricultural, forestry residues and municipal solid waste etc., is considered to be an attractive alternative energy source because it is renewable, abundant, production of such resources does not compete with food crops for land, which offer economic, environmental and strategic advantages (Perez *et al.*, 2002; Wyman, 1996).

Lignocellulose mainly consists of cellulose, hemicellulose and lignin (Sanchez, 2009; Juha´sz *et al.*, 2005; Muthuvelayudham and Viruthagiri, 2010). Where cellulose consists of linear chains of hundreds or thousands of glucose molecules, while the most abundant hemicelluloses are comprised of xylans, they frequently consist of heterogeneous mixtures of pentoses (xylose, arbinose), hexoses (mannose, glucose, galactose) and sugar acids (Saha, 2003).

Lignocellulosic materials could be naturally degraded to monomeric sugars by enzymatic hydrolysis done by cellulase and xylanase, which are widely spread among bacterial and fungal species (Xia and Sheng, 2004).

The enzymatic hydrolysates of lignocellulosic materials containing reducing sugars can be further used for the production of biofuels (e.g. hydrogen, ethanol) and other high-value chemicals (Amouri and Gargouri, 2006).The term cellulase is a group of enzymes consists of three types of enzymes: β-1,4-D-glucan cellobiohydrolase (EC 3.2.1.91), which cleaves cellobiosyl units from the ends of cellulose chains: endo-β-1,4-D-glucanase (EC 3.2.1.4),which cleaves internal glucosidic bonds; β-1,4-glucosidase (EC 3.2.1.21), which cleaves glucose units from cellooligosaccharides (Zhang, 2006).

The cost of enzymes plays an important role in determining the economics of an enzymatic

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hydrolysis and it can be reduced by finding optimum conditions for their production (Lynd *et al.*, 2002). Reducing the costs of enzyme production by optimizing the fermentation medium is the basic research for industrial application.

The composition of fermentation media including carbon sources, nitrogen sources, mineral salts, trace elements, vitamins and other growth factors) is a very important factor that determines the nutrimental and chemical environment for the whole cell biocatalysts in the reactor and thus may influence the production of cellulase (Lynd *et al.*, 2002).

The use of different statistical designs for medium optimization has been recently employed for cellulase and xylanase production by fungal cultures (Senthilkumar *et al.*, 2005; Hao *et al.*, 2006; Francis *et al.*, 2003; Dey *et al.*, 2001). These statistical methods, as compared to the common ”one-factor-at-a-time” method proved to be powerful and useful tools, since optimization by one-factor-at-a-time method involves changing one variable while fixing the others at certain levels, which is laborious and time consuming, especially for large number of variables (Xu *et al.*, 2003).

However, statistical method offers several advantages one-factor-at-a-time method being quick and reliable, screens significant nutrients, helps understanding the interactions among the nutrients at various concentrations and reduces the total number of experiments resulting in reducing chemicals, glass wares, experimental time, and manpower (Srinivas *et al.*, 1994; Carvalho *et al.*, 1997). Statistical methods such as Plackett–Burman design, the Box–Behnken design and central composite design are most widely used techniques (Rafaat *et al.*, 2012; Soni *et al.*, 2010).

Placket–Burman design is mainly used as statistical technique for screening and selection of significant variables followed by central composite design (CCD) of response surface methodology (RSM) which provides important information regarding the optimum level of each variable, their interaction with other variables and effect on the product yield. It involves minimum number of experiments for large number of factors, by which improvement in enzyme production has been successfully observed (Rodrigues *et al.*, 2003; Majumder, and Goyal, 2008; Ghanem *et al.*, 2000).

In the present study the production of cellulase by *Cellulomonas fimi NCIM-5015* in batch process, using tapioca stem as substrate was enhanced by medium optimization. The medium optimization of the bioprocess was carried out by three steps (1) screening the most significant medium components which enhance enzymes production by using Plackett-Burman design. (2) Optimization of the most significant medium components by application of central composite design (CCD) of response surface methodology (RSM), and (3) verification of the model.

Materials and methods

Microorganism and culture media

Cellulomonas fimi NCIM-5015 used in this study was purchased from the National Chemical Laboratory, Pune, India. Stock cultures were maintained on nutrient agar slants which contain 1 g of beef extract, 0.5 g of NaCl, 1 g of peptone, 2 g of agar, in 100 ml of distilled water. pH 7.0 to 7.5, at 4^{o}C. The sub culturing was performed every 15 days to assure its viability. All the chemicals used here are analytical grade procured from Himedia, India.

Substrate preparation

Pretreatment decreases the crystallinity of tapioca stem while removing lignin and other inhibitors thereby enabling its enzymatic hydrolysis. 100 g of the washed ground tapioca stem was treated separately with 2000 mL of 2% NaOH solution and autoclaved at 121°C for 30 minutes.

Then it was filtered, washed with distilled water and excess alkali present was neutralized with 1N phosphoric acid. Again it was filtered and the residue material was dried at 65°C in a hot air oven to constant weight. To the cellulosic material obtained, the same volume of distilled water was added and heated at 121°C for 30 minutes. The suspension was filtered and the solid material was dried at 65°C to constant weight in hot air oven. The dried tapioca stem powder was used as a carbon source.

Cultivation of Cellulomonas fimi NCIM-5015

Alkali pretreated tapioca stem powder was used as substrate for cellulase production. Fermentation was carried out in Erlenmeyer flasks (250 mL) with 10 g of alkali pretreated tapioca stem powder, supplemented with nutrients concentrations defined by the experimental design, pH of the medium was adjusted to 7.0 with 1 N NaOH or 1 N HCl. Each flask was covered with hydrophobic cotton and autoclaved at 121°C for 20 min. After cooling, each flask was inoculated with 10% v/v of inoculum incubated at 28°C on a rotary shaker (150 rpm) (Orbital shaking incubator-Remi instruments Ltd, Mumbai, India) (Emtiazi and Nahvi, 2000). During the preliminary screening process, the experiments were carried out for 96 hours, and it was found that at the 72 h, the maximum enzyme production occurs. Hence, experiments were carried out for 72 h. All the experiments were carried out in triplicate and the average values are reported.

Cellulase enzyme assay

In culture filtrate, 20 – 90% ammonium sulfate [$(NH_4)_2SO_4$] was added and precipitated. Precipitates were separated by centrifugation and redissolved in citrate buffer (0.05 M) and centrifuged. Filter paper activity (FPA) was determined according to the method of the International Union of Pure and Applied Chemistry (IUPAC) and expressed as international units (IU) (Ghose, 1987). A rolled Whatman No. 1 filter paper strip of dimension 1.0 x 6 cm (50 mg) was saturated with 1 mL of sodium citrate buffer (0.05 mol/L, pH 4.8) in a tube, and 1 mL of an appropriately diluted enzyme was added to the tube and incubated at 50°C for 30 min. Thereafter, tubes were removed from the water bath, and the reaction was stopped by addition of 3 mL of 3,5-dinitrosalicylic acid reagent per tube. The tubes were incubated for 5 min in a boiling water bath for color development and were cooled rapidly. The reaction mixture was diluted appropriately and was measured against a reagent blank at 540 nm in an UV-VIS spectrophotometer (Elico-India).

One international unit of cellulose activity is the amount of enzyme that forms 1 µmol glucose (reducing sugars as glucose) per minute during the hydrolysis reaction. Reducing sugar was determined by the dinitrosalicylic acid (DNS) method (Miller, 1959).

Plackett–Burman design

For screening purpose, various medium components were evaluated. Based on Plackett–Burman factorial design, each factor was examined in two levels, -1 for low level and +1 for high level (Plackett and Burman, 1946).

Table 1 shows the factors under investigation as well as levels of each factor used in the experimental design, whereas Table 2 represents the design matrix.

Table 1. *Nutrient Screening Using A Plackett-Burman Design*

Nutrient Code	Nutrient (g/L)	Low (-1)	High (+1)
A	Peptone	0.25	1.25
B	Yeast extract	0.5	3.5
C	$MnSO_4.7H_2O$	0.0010	0.002
D	KH_2PO_4	2.0	4.0
E	K_2HPO_4	1.0	5.0
F	$NaNO_3$	0.5	1.0
G	$CoCl_2.6H_2O$	0.02	2.0
H	$MgSO_4.7H_2O$	0.3	0.5
I	$CaCl_2.2H_2O$	0.3	0.5
J	$(NH_4)_2SO_4$	0.06	0.1
K	Corn steep liquor	0.4	0.8
L	$FeSO_4.7H_2O$	0.005	0.1

Table 2. *Plackett-Burman experimental design matrix for screening of important variables for cellulase*

RunOrder	A	B	C	D	E	F	G	H	I	J	K	L	FPA IU/mL
1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	0.14
2	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	0.14
3	-1	1	1	1	1	-1	-1	1	1	-1	1	1	0.33
4	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	0.15
5	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	0.29
6	1	1	1	1	-1	-1	1	1	-1	1	1	-1	0.23
7	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	0.3
8	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	0.13
9	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	0.2
10	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	0.24
11	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0.06
12	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	0.26
13	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	0.18
14	1	-1	1	-1	1	1	1	1	-1	-1	1	1	0.12
15	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	0.18
16	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	0.15
17	1	-1	1	1	1	1	-1	-1	1	1	-1	1	0.32
18	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	0.31
19	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	0.12
20	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	0.19

In the present work, 12 assigned variables were screened in 20 experimental designs. All experiments were carried out in triplicate and the average of the cellulase activity was taken as responses (Table 2).

From the regression analysis the variables, which were significant at 95% level ($P < 0.05$) were considered to have greater impact on cellulase production. Significant nutrient components for cellulase production such as nitrogen source, and inorganic salts were screened and identified by the Plackett-Burman design using statistical software package MINITAB (Release 15.1, PA, USA).

Central composite design

Response surface methodology (RSM) was used to optimize the selected four significant nutrient components, namely, peptone, yeast extract, KH_2PO_4 and $CaCl_2{\cdot}2H_2O$ which enhances the cellulase.

The four independent variables were studied at five different levels (Table 3), and sets of 30 experiments were carried out (Table 4).

It consists of a complete 2^k factorial design, where k is the number of the test variables and is equal to 4, six replications of the center points to estimate the experimental error and have a satisfactory orthogonality for the coefficients estimation (all factors at level 0), six star points (2 axis points on the axis of each variable at a distance of α ($= 2^{k/4}$, = 2 for $k = 4$), whereas the other two factors are at level 0.

Hence, the total number of design points is $N = 2^k + 2k + n_0 = 30$ experiments, where n_0 the number of replicate runs at center point of the variables.

Table 3. *Ranges of the independent variables used in RSM*

	Variable	Code	Levels (g/L)				
			-2	**-1**	**0**	**1**	**2**
1.	Peptone	X_1	0.25	0.5	0.75	1.0	1.25
2.	Yeast extract	X_2	0.5	1.25	2.0	2.75	3.5
3.	KH_2PO_4	X_3	2.0	2.5	3.0	3.5	4.0
4.	$CaCl_2.2H_2O$	X_4	0.3	0.35	0.4	0.45	0.5

Table 4. *Central composite design (CCD) of factors in coded levels with enzymes activities as responses*

Run	X1	X2	X3	X4	FPA IU/mL	
					Observed	**Predicted**
1	1	-1	-1	-1	0.27	0.28
2	0	0	0	0	0.52	0.52
3	0	0	0	0	0.52	0.52
4	0	0	0	0	0.52	0.52
5	0	0	0	-2	0.28	0.27
6	-1	-1	1	1	0.24	0.23
7	-2	0	0	0	0.16	0.22
8	-1	-1	1	-1	0.17	0.15
9	-1	-1	-1	1	0.17	0.14
10	1	-1	1	1	0.41	0.41
11	1	1	1	1	0.51	0.52
12	0	0	0	0	0.52	0.52
13	0	-2	0	0	0.16	0.16
14	1	1	-1	1	0.43	0.44
15	0	0	-2	0	0.27	0.25
16	0	2	0	0	0.47	0.46
17	-1	1	1	1	0.39	0.37
18	0	0	0	2	0.36	0.36
19	-1	1	-1	-1	0.35	0.34
20	-1	1	1	-1	0.32	0.29
21	0	0	0	0	0.51	0.52
22	1	-1	1	-1	0.29	0.3
23	-1	1	-1	1	0.32	0.32
24	1	1	1	-1	0.4	0.42
25	2	0	0	0	0.55	0.49
26	1	-1	-1	1	0.25	0.29
27	0	0	0	0	0.52	0.52
28	-1	-1	-1	-1	0.16	0.16
29	1	1	-1	-1	0.41	0.43
30	0	0	2	0	0.31	0.32

In this study, the Central composite design (CCD), using statistical software package “Design Expert 7.1.5” (Stat Ease Inc., Minneapolis, USA) was employed .which was used to obtain a quadratic model, consisting of factorial trials and star points (α=2) to estimate quadratic effects and central

points to estimate the pure process variability with cellulase production as response.

The statistical software package "Design Expert 7.1.5" was used to analyze the experimental data. All variables were taken at a central coded value of zero. The minimum and maximum ranges of variables investigated are listed in Table 3. Upon the completion of experiments, the average maximum cellulase was taken as the response (Y_1).

A multiple regression analysis of the data was carried out for obtaining an empirical model that relates the response measured to the independent variables. A second-order polynomial equation is

$$Y = \beta 0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad (1)$$

where Y is the measured response, β_0 is the intercept term, β_i are linear coefficients, β_{ii} are quadratic coefficient, β_{ij} are interaction coefficient, and X_i and X_j are coded independent variables. The following equation was used for coding the actual experimental values of the factors in the range of (−1 to +1):

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (2)$$

$$\Delta X_i, \quad i = 1, 2, 3, \ldots, k, \quad (3)$$

where x_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_0 is the value of X_i at the center point, and ΔX_i is the step change. Statistical analysis of the data was performed by design package Design Expert 7.1.5 to evaluate the analysis of variance (ANOVA) to determine the significance of each term in the equations fitted and to estimate the goodness of fit in each case. The fitted polynomial equation was then expressed in the form of three-dimensional response surface plots to illustrate the main and interactive effects of the independent variables on the dependent ones. The combination of different optimized variables, which yielded the maximum response, was determined to verify the validity of the model. In order to verify the accuracy of the predicted model an experiment was conducted with initial and optimized media. The optimal concentrations of the critical variables were obtained by analyzing 3D plots. The statistical analysis of the model was represented in the form of analysis of variance (ANOVA).

Results and Discussion

Plackett-Burman experiments (Table 2) showed a wide variation in cellulase activity. This variation reflected the importance of optimization to attain higher productivity.

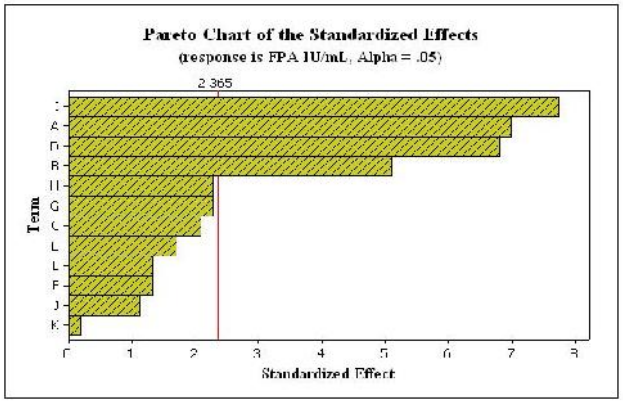

Figure 1. *Pareto chart showing the effect of media components on cellulase activity*

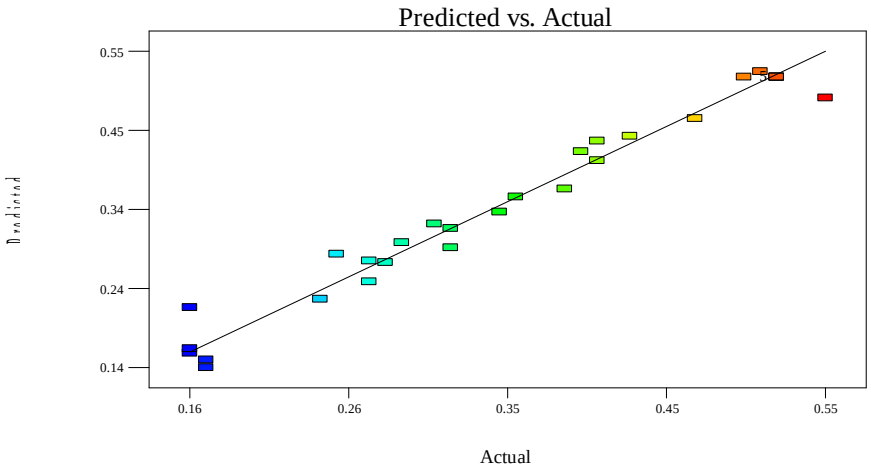

***Figure 2**. Predicted response Vs Actual value for FPA*

From the Pareto charts (Figure 1,2) the variables, namely, Peptone, Yeast extract, $KH_2PO_4$and $CaCl_2.2H_2O$ were selected for further optimization to attain a maximum production of cellulase. The levels of factors Peptone, Yeast extract, $KH_2PO_4$and $CaCl_2{\cdot}2H_2O$ and the effect of their interactions on cellulase production was determined by central composite design of RSM.

Thirty experiments were performed at different combinations of the factors shown in Table 3.

The observed responses along with design matrix are presented in Table 4, and the results were analyzed by ANOVA.

The second-order regression equations provided the levels of cellulase as the function of peptone, yeast extract, $KH_2PO_4$and $CaCl_2{\cdot}2H_2O$ which can be presented in terms of coded factors as in the following equations:

$$Y_1 = 0.52 + 0.068X_1 + 0.075X_2 + 0.019X_3 + 0.021X_4 - 6.875 \times 10^{-3}X_1X_2 + 8.125\,10^{-3}X_1X_3 + 6.875\,10^{-3}X_1X_4 - 9.375 \times 10^{-3}X_2X_3 - 6.25 \times 10^{-3}X_2X_4 + 0.024X_3X_4 - 0.041X_1^2 - 0.051X_2^2 - 0.057X_3^2 - 0.049X_4^2$$

where Y_1 is the cellulase activity (IU/mL) X_1, X_2, X_3 and X_4 are peptone, Yeast extract, KH_2PO_4 and $CaCl_2.2H_2O$, respectively. ANOVA for the response surface is shown in Table 5.

Table 5: Analysis of Variance (ANOVA) for FPA

Source	Coefficient factor	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	0.52	0.46929	14	0.03352	37.7345	< 0.0001
X_1	0.068	0.1107	1	0.1107	124.62	< 0.0001
X_2	0.075	0.1335	1	0.1335	150.286	< 0.0001
X_3	0.019	0.00844	1	0.00844	9.49812	0.0076
X_4	0.021	0.01084	1	0.01084	12.1998	0.0033
X_1 X_2	-6.875E-03	0.00076	1	0.00076	0.85131	0.3708
X_1 X_3	8.125E-03	0.00106	1	0.00106	1.18902	0.2927
X_1 X_4	6.875E-03	0.00076	1	0.00076	0.85131	0.3708

X_2 X_3	-9.375E-03	0.00141	1	0.00141	1.58302		0.2276
X_2 X_4	-6.25E-04	6.25E-06	1	6.25E-06	0.00704		0.9343
X_3 X_4	0.024	0.00951	1	0.00951	10.7012		0.0052
X_1^2	-0.041	0.0455	1	0.0455	51.2199	< 0.0001	
X_2^2	-0.051	0.07059	1	0.07059	79.4589	< 0.0001	
X_3^2	-0.057	0.08905	1	0.08905	100.244	< 0.0001	
X_4^2	-0.049	0.06715	1	0.06715	75.5913	< 0.0001	
Residual		0.01333	15	0.00089			
Lack of Fit		0.01299	10	0.0013	19.4875		0.0022
Pure Error		0.00033	5	6.67E-05			
Cor Total		0.48262	29				

Std. Dev. 0.030, R-Squared 0.9724, Mean 0.36 , Adj R-Squared 0.9466, C.V. %8.32 , Pred R-Squared 0.8440, PRESS 0.075, Adeq Precision 18.209

The Model F-value of 37.73 for cellulase implies the model is significant. There is only 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > *F*" less than 0.05 indicate that model terms are significant. Values greater than 0.1 indicate that the model terms are not significant.

In the present work, the linear effects of X_1 , X_2 ,X_3, X_4,the interactive effects of X_3 X_4 and square effects of X_1^2, X_2^2, X_3^2, X_4^2 are significant model terms for cellulase production. To test the fit of the model equation, the regression - based determination coefficient R^2 was evaluated. The nearer the values of R^2 to 1, the model would explain better for variability of experimental values to the predicted values. The coefficient of determination (R^2) for cellulase activity was calculated as (R^2=0.9724 for cellulase) which are nearly equal to 1) 97.24% of the responses. The predicted R^2 value of cellulase activity was 84.40% was in reasonable agreement with the adjusted R^2 value of 94.66%. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Adeq Precision of 18.209 indicates an adequate signal. This model can be used to navigate the design space. The above models can be used to predict the cellulase production within the limits of the experimental factors. Figure 2 shows that the actual response values agree well with the predicted response values of cellulase. The interaction effects of variables on cellulase was studied by plotting 3D surface curves against any two independent variables, while keeping another variable at its central (0) level. The plotting 3D surface curves of the calculated responses (cellulase activity) from the interactions between the variables are shown in Figures 3(a)-3(f). Figures 3(a), 3(b), 3(c) show the dependency of cellulase on Peptone. The cellulase activity increases with increase in peptone about 0.957 g/L and thereafter cellulase decreases with further increase in peptone. The same trend was observed in Figures 3(a), 3(d), 3(e) which shows the dependency of cellulase on yeast extract. The cellulase activity increases with increase in yeast extract about 2.5g/L and thereafter cellulase activity decreases with further increase in yeast extract. The Figures 3(b), 3(d), 3(f) shows the dependency of cellulase on KH_2PO_4. The cellulase activity increases with increase in KH_2PO_4 about 3.09g/L and thereafter cellulase activity decreases with further increase in KH_2PO_4. The dependency of cellulase on $CaCl_2.2H_2O$ shows from the Figures 3(c), 3(e), 3(f). The cellulase increases with increase in $CaCl_2.2H_2O$ about 0.409g/L and thereafter cellulase activity decreases with further increase in $CaCl_2.2H_2O$.

The optimum conditions for the maximum production of cellulase was determined by response surface analysis and also estimated by optimizer tool using statistical software package "Design Expert 7.1.5". The optimum conditions are Peptone (0.957g/L), Yeast extract (2.5g/L), KH_2PO_4 (3.09g/L), and $CaCl_2.2H_2O$ (0.409g/L). The predicted results are shown in Table 4.

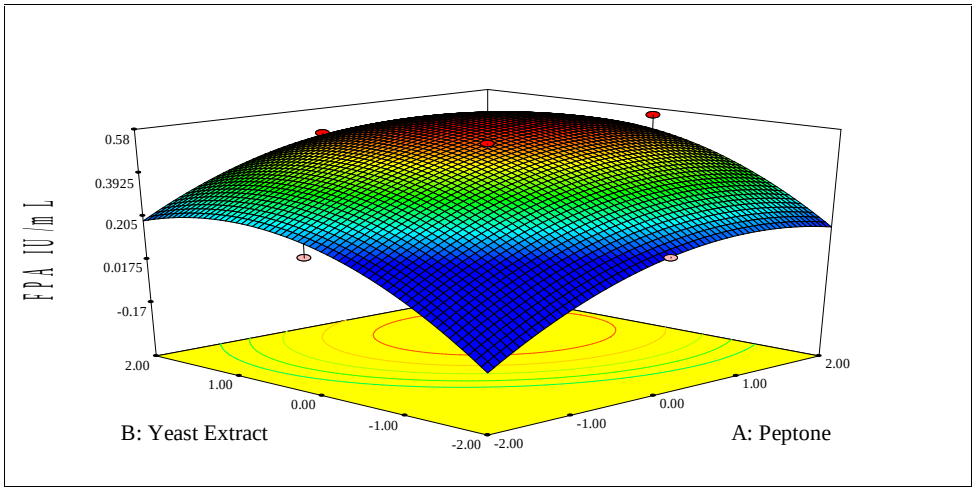

Figure 3(a). *Three-dimensional response surface plot for cellulase production showing the interactive effects of peptone and yeast extract*

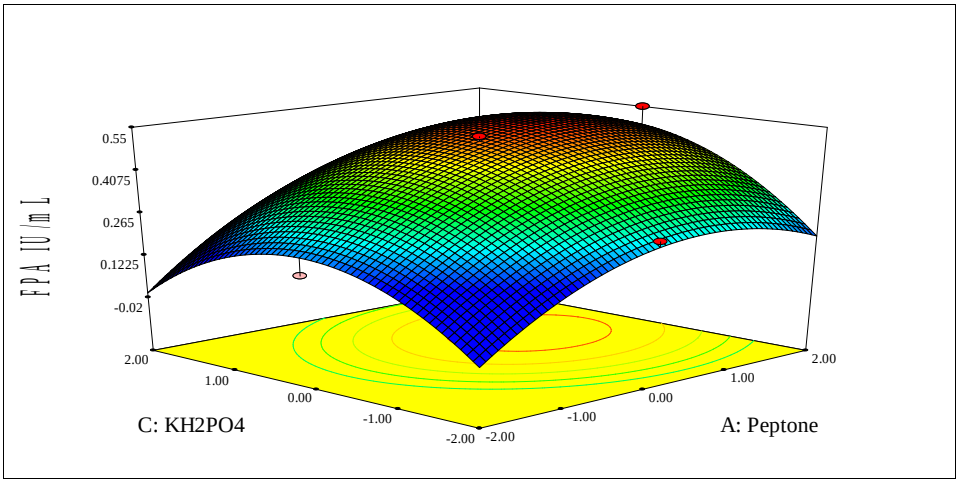

Figure 3(b). *Three-dimensional response surface plot for cellulase production showing the interactive effects of peptone and KH_2PO_4*

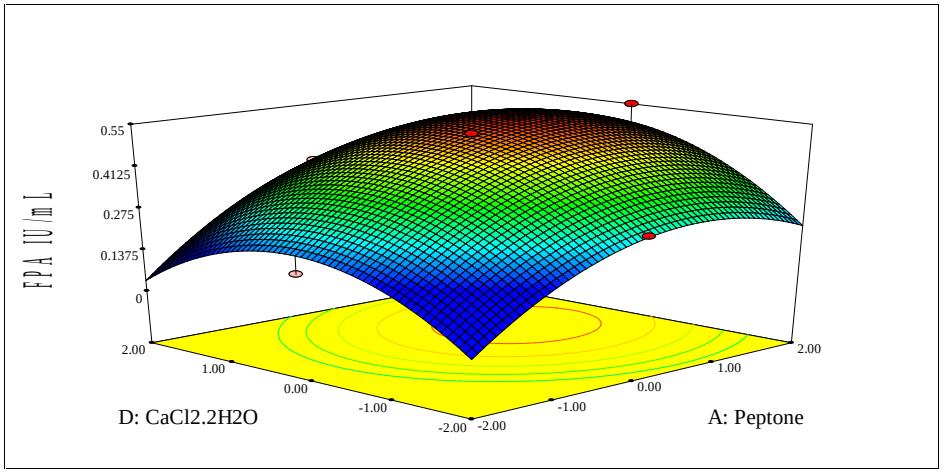

Figure 3(c). *Three-dimensional response surface plot for cellulase production showing the interactive effects of peptone and $CaCl_2{\cdot}2H_2O$*

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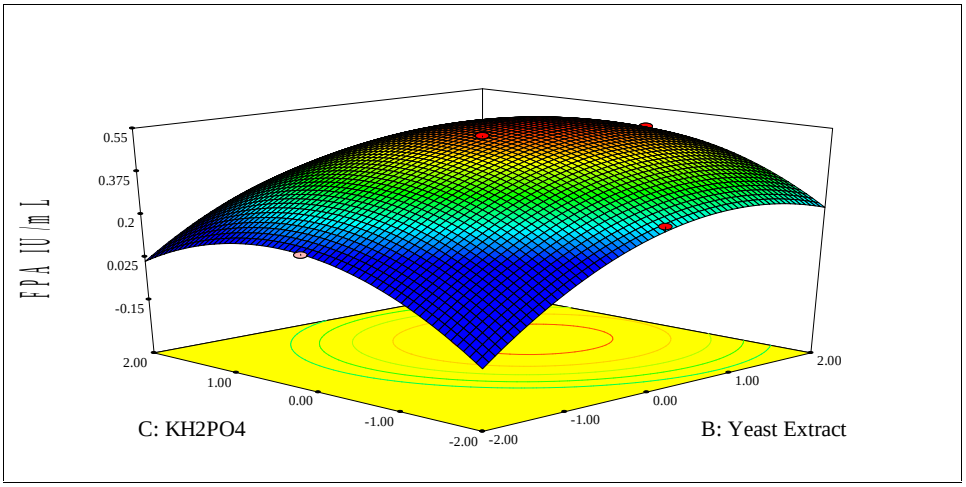

Figure 3(d). *Three-dimensional response surface plot for cellulase production showing the interactive effects of yeast extract and KH_2PO_4*

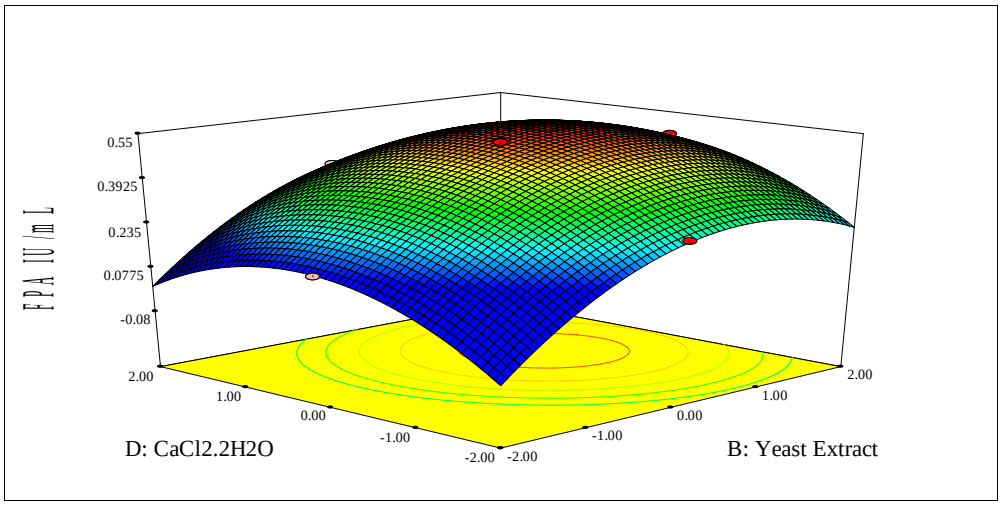

Figure 3(e). *Three-dimensional response surface plot for cellulase production showing the interactive effects of yeast extract and $CaCl_2.2H_2O$*

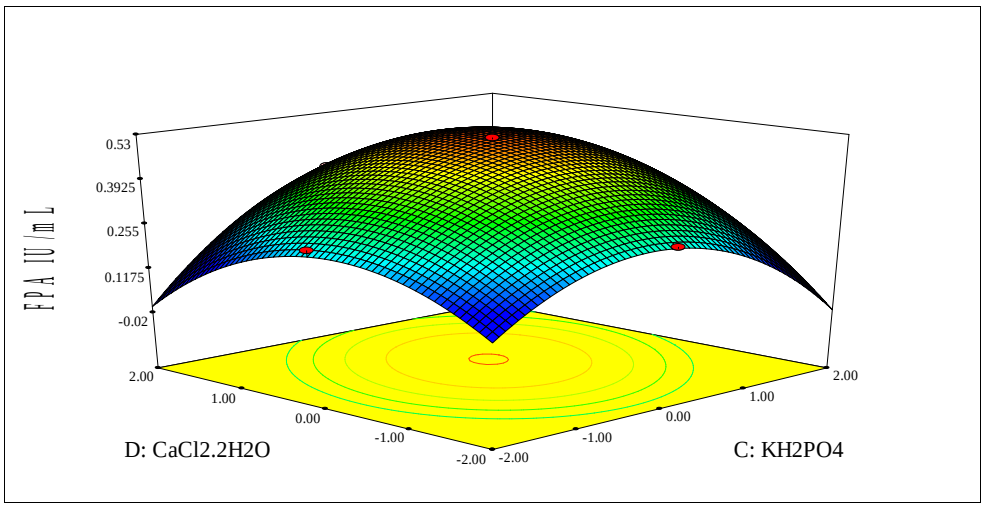

Figure 3(f). *Three-dimensional response surface plot for cellulase production showing the interactive effects of KH_2PO_4 and $CaCl_2{\cdot}2H_2O$*

Validation of the experimental

Validation of the experimental model was tested by carrying out the batch experiment under optimal operation conditions. Three repeated experiments were performed, and the results are compared. The cellulase activity obtained from experiments was very close to the actual response predicted by the regression model, which proved the validity of the model. At these optimized conditions, the maximum cellulase activity was found to be 0.58 IU/ml.

Conclusions

In this work medium components for higher cellulase activities from *Cellulomonas fimi NCIM-5015* were optimized by RSM. Using Placket-Burman design peptone, yeast extract, $KH_2PO_4$and $CaCl_2{\cdot}2H_2O$ was found to be the most significant variables, which significantly enhanced cellulase activity.

Central composite design was employed to optimize these selected nutrients. The optimal levels of components were obtained as peptone (0.957g/L), yeast extract (2.5g/L), KH_2PO_4 (3.09g/L), and $CaCl_2{\cdot}2H_2O$ (0.409g/L).This study showed that the tapioca stem constitutes a good carbon source for the production of cellulase. Using the optimized conditions, the produced enzyme activity of cellulase reaches 0.58 IU/ml. The results show a close agreement between the expected and obtained activity level.

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