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Research Article

Enhanced production of cellulasefromagro-industrial residuesby optimization of mediumcomponents using central composite design

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Abstract

In this present work the production of cellulase by *CellulomonasfimiNCIM-5015* in submerged fermentation using wheat straw as a sole carbon source, was enhanced by medium optimization. A Plackett-Burman based statistical screening procedure was used to identifythe most significant nutrient components which influence the cellulase production.From the experiments,twelve nutrient components were screened and results revealed thatpeptone, yeast extract, $KH_2PO_4$and $MgSO_4.7H_2O$ werethe most significant nutrient components. The screened nutrients components were further optimized by using central composite rotary designand response surface methodology,the combined effects of these nutrients on production of cellulasewas studied.The optimum conditions are Peptone-(0.846g/L), yeast extract-(2.14g/L), KH_2PO_4-(3.05g/L)and $MgSO_4.7H_2O$-(0.405g/L). Under these conditions, the production of cellulasewas found to be 0.74 IU/ml which represented a 6.73 fold increase in production compared tounoptimized conditions.

Keywords:*Cellulomonasfimi*,fermentation, agricultural wastes,Plackett-BurmanDesign,wheat straw,RSM, India.

Introduction

Cellulaseis anindustriallyimportant enzyme in bio-energy and various other biotechnological processes, such aspaper and pulp, food, textile, agriculture and environment [1,2].Biodegradation or bioconversion of cellulose to monomeric sugars is conducted through the synergetic actions of enzymes, including cellulolytic enzymes[3].Cellulase is a group of enzymeswhich includes Endo-1, 4-β-glucanase, or carboxymethylcellulase (CMCase), Exo-1, 4-β-glucanase or cellobiohydrolase (CBH) and β-glucosidase or cellobiase[4], whichchange cellulose into glucose through hydrolysis.Cellulases area hydrolytic enzyme, synthesized by a large diversity of microorganisms including both fungi and bacteria during their growth on cellulosic material[5]. These microorganisms can be aerobic, anaerobic,mesophilic or thermophilic. Among them, the genera of *Clostridium, Cellulomonas, Thermomonospora, Trichoderma* and *Aspergillus*are the most extensively studied cellulase producers [6, 7, 8, 9].

However, the production cost of enzymes for these biotechnological processes is very high andaccounts for about 40–60% of the total production cost [10,11].Reducing the costs of enzyme production by optimizing (i)utilizing the most abundant renewable lignocellulosic biomass, especially agricultural waste residues, agro-industrial waste and their by-products as substrates like wheat straw,wheat bran can help to reduce the cost of enzymes and another reason isagricultural residues are not only inexpensive, but also abundant and easily available, supplying the microorganism better nutrition [12].(ii)optimizing the fermentation medium is the basic research for industrial application.Development of economical mediumrequires selection of carbon, nitrogen, phosphorous and trace element sources.

In general, culture condition optimization by the traditional ‘one-factor-at-a-time’ techniqueis used. This method is not only time consuming but also often leads to an incomplete understanding of the systembehaviour, resulting in a bafflement and failure of predictive response. To overcome these limitations, statistically-based experimental designs such as Placket-Burman design and response surface methodology (RSM) can be effectively used to study the effects of factors and searching optimum levels of parameters for desired responses and performing a minimum number of experiments [13].

In the present study the production of cellulase by*Cellulomonasfimi NCIM-5015* in batch process, using wheat straw as substrate was enhanced by medium optimization. The medium optimization of the bioprocess was carried out by three steps (1) screening the most significant nutrients which affects enzyme production by using Plackett-Burman design. (2) Optimization of the most significant nutrients by application of central composite design, and (3) verification of the model by monitoring the experimental production pattern[1].According to earlier studies andour best knowledge this is the papers have dealt with thedescribed 3-step procedure in order to optimize cellulaseproduction by applied*CellulomonasfimiNCIM-5015*strain.

Materials and Methods

Microorganism and culture media

*CellulomonasfimiNCIM-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 room temperature.The sub-culturing was performed every 15 daysto assure its viability.The inoculum was prepared by adding 5.0mL of steriledistilled water to the agar slants and shaking vigorously. The spore suspension that obtained was adjusted to 1 X10^7 spores per mL and used as the inoculums.

Substrate preparation

100 g of the washed ground wheat straw 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 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 a hot air oven[14].The dried wheat straw powder was used as a carbon source.

Cultivation of Cellulomonasfimi NCIM-5015

Alkali pretreated wheat straw powder was used as substrate for cellulase production. Fermentation was carried out in Erlenmeyer flasks (250 mL) with 10 g of alkali pretreated wheat straw powder in 100 ml of enzymeproduction medium.The compositionof media varied according to the experimental design described in thiswork.pH of the medium was adjusted to 7.0 with 1 molNaOH or 1 molHCl. Each flask was covered with hydrophobic cotton and autoclaved at 121^{o}C for 20 min.After cooling, each flask was inoculated with 10% v/v of inoculum incubated at 28^{o}C on a rotary shaker (150 rpm)[15]. After incubation, the samples were withdrawn at regular time intervals and the sampleswere filtered through GD-120 glassfibre filter disks (Whatman)to remove the residual insoluble substrate. Then, the liquid contentobtained after filtration was centrifuged at 10,000×g for 10 minat 4◦Cto separate the cells.The cell-freesupernatant was analyzed for enzyme activity.

During the preliminary screening process, the experiments were carried out for 96 hours and it was found that at the 72 hrs,the maximum enzyme production occurs. Hence, experiments were carried out for 72 hrs. 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 re-dissolved 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)[17]. 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 [18].

Screening of medium components byPlackett-Burman(PB) experimental design

The main application of Plackett-Burman experimental design was to identify which ingredients of the medium have most significant effect on the cellulase producing capability of *Cellulomonasfimi NCIM-5015*.When this kind of statistical experimental design is employed it is assumed that no interactions between different factors occur in the range of variables under consideration [19].A total of twelve variables were considered for screening (Table 1) with each factor examined in two levels: −1 for low level and +1 for high level of each factor are listed in (Table 1).

The PB design is a fractional factorial design and the main effect (the contrast coefficient) of such a design may be simply calculated as the difference between the average of measurements made at the high level (+1) of the factor and the average of measurements made at the low level (-1). Contrast coefficients allow the determination of the effect of each constituent. A large contrast coefficient either positive or negative indicates that a factor has a large impact on titre; while a coefficient close to zero means that a factor has little or no effect.

The P-value is the probability that the magnitude of a contrast coefficient is due to random process variability. A low P-value indicates a ‘‘real’’ or significant effect. The significance of each variable was determined by applying the Student’s t-test.

Table 1.Nutrient screening using 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_2 PO_4	2.0	4.0
E	K_2H PO_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

All the 20 experiments were carried out in duplicate and the averages of thecellulase activitywas taken as the response(Table 2). From the regression analysis the variables, which were significant at or above 95% level ($P < .05$), were considered to have greater impact on cellulase activity and were further optimized by central composite design.

Table 2.Plackett-Burman experimental design matrix for screening of important variables for cellulase and xylanase production.

Run Order	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	0.31
2	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	0.19
3	-1	1	1	1	1	-1	-1	1	1	-1	1	1	0.34
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	0.32
6	1	1	1	1	-1	-1	1	1	-1	1	1	-1	0.43
7	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	0.23
8	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	0.15
9	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	0.38
10	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	0.36
11	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0.046
12	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	0.27
13	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	0.41
14	1	-1	1	-1	1	1	1	1	-1	-1	1	1	0.34
15	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	0.29
16	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	0.051
17	1	-1	1	1	1	1	-1	-1	1	1	-1	1	0.28
18	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	0.38
19	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	0.31
20	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	0.12

Central Composite Design (CCD) and statistical analysis

Once 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).The screened nutrients were further optimized by Response surface methodology (RSM) based central composite design(CCD).

It consists of a complete 2^k factorialdesign, where k is the number of the test variables and isequal to 4, five replications of the center points to estimatethe experimental error and have a satisfactory orthogonality for the coefficients estimation (all factors at level 0), six starpoints (2 axis points on the axis of each variable at a distanceof α (= $2^{k/4}$, = 2 for $k = 4$), whereas the other two factorsare at level 0. Hence, the total numberof design points is $N = 2^k + 2k + n_0 = 30$experimentswhere n_0

the number of replicate runs at centre point of the variables. The central composite design along with the experimentaland predicted values of cellulase production is shownin Table 3. The relationships and interrelationships of the variables were determined by fitting the second-order polynomial equation to data obtained from 30 experiments.The response values (Y) used in each trial was theaverage of the duplicates.

A second-order polynomial equation is:

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

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):

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

ΔX_i, $i = 1, 2, 3, \ldots, k$, (3)

wherex_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 $\Delta X_i$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).

The minimum and maximum ranges of variables investigated are listed in Table 4. Upon the completion of experiments, the average maximum cellulasewas taken as the response (Y). A multiple regression analysis of the data was carried out for obtaining an empirical model that relates the response measured to the independent variables.

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. Table 3 shows the analysis of regression coefficients, the effect, standard error,*t*-value and P values of 12 ingredients. Generally, a large *t*-value associated with a low *P*-value of a variable indicates a high significance of the corresponding model term. $CaCl_2.2H_2O$, $(NH_4)_2SO_4$ and Corn steep liquordisplayed a negative effect for enzyme production, whereas all other nutrients had a positive effect on enzyme activity (Table 3). The components were screened at the confidence level of 95% on the basis of their effects. The variables with confidence levels greater than 95% were considered as significant. Peptone, yeast extract, $KH_2PO_4$and $MgSO_4.7H_2O$ showed confidence level above 95% and these nutrients were considered to be significant. The rest of the components showed confidence level below 95% and were considered to be insignificant .None of the components had significant negative effect. Neglecting the variables which were insignificant.

Table3. Calculated t-value, probability and confidence level as per Plackett–Burman design for cellulase production.

Term	Effect coef	Coef	SE	T	P	Confidence%
Constant	0.26785	0.008	33.48	0		100
A	0.1303	0.06515	0.008	8.14	0	100
B	0.1103	0.05515	0.008	6.89	0	100
C	0.0123	0.00615	0.008	0.77	0.467	53.3
D	0.0743	0.03715	0.008	4.64	0.002	99.8
E	0.0145	0.00725	0.008	0.91	0.395	60.5
F	0.0123	0.00615	0.008	0.77	0.467	53.3
G	0.0305	0.01525	0.008	1.91	0.098	90.2
H	0.0943	0.04715	0.008	5.89	0.001	99.9
I	-0.0355	-0.0178	0.008	-2.22	0.062	93.8
J	-0.0075	-0.0038	0.008	-0.47	0.653	34.7
K	-0.0295	-0.0148	0.008	-1.84	0.108	89.2
L	0.0345	0.01725	0.008	2.16	0.068	93.2

From the Pareto chart also represented (Figure 1) that the variables, namely, peptone, yeast extract, $KH_2PO_4$and $MgSO_4.7H_2O$wassignificant. These nutrientswere selected for further optimization to attain a maximum production of cellulase.

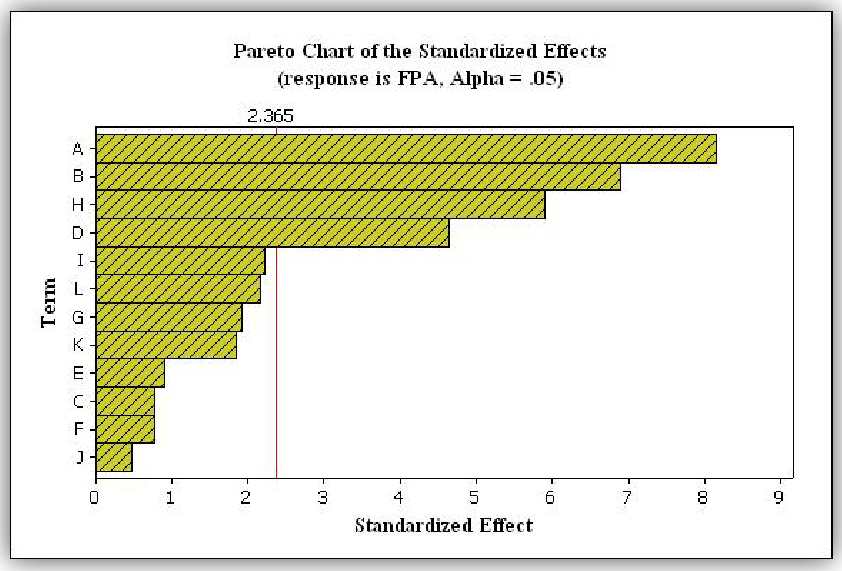

Figure 1.Pareto chart showing the effect of media components on cellulase activity.

The central composite design (CCD) of RSMwas employed to optimize the selected four significant nutrient components, namely, peptone, Yeast extract, $KH_2PO_4$and $MgSO_4.7H_2O$ which enhances the cellulase production. The four independent variables were studied at five different levels (Table 4), and sets of 30 experimentswere carried out (Table 5).and the results were analyzed by ANOVA(Table 6).

Table 4.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. $KH_2 PO_4$	X_3	2.0	2.5	3.0	3.5	4.0
4. $MgSO_4.7H_2O$	X_4	0.3	0.35	0.4	0.45	0.5

Table 5.Central composite design (CCD) of factors in coded levels with enzyme activities as responses.

Run	X_1	X_2	X_3	X_4	FPA IU/mL	
					Observed	Predicted
1	0	0	2	0	0.62	0.59
2	0	0	0	0	0.71	0.7
3	1	1	1	1	0.66	0.67
4	1	-1	1	1	0.61	0.61
5	0	-2	0	0	0.54	0.53
6	-1	1	-1	-1	0.58	0.58
7	0	0	0	0	0.71	0.7
8	-1	1	1	-1	0.51	0.52
9	-1	1	1	1	0.54	0.54
10	0	0	0	0	0.69	0.7
11	-1	-1	1	-1	0.51	0.51
12	0	0	0	0	0.71	0.7
13	1	-1	-1	1	0.57	0.57
14	0	0	0	-2	0.61	0.59
15	0	0	-2	0	0.6	0.61
16	-1	1	-1	1	0.55	0.55
17	-1	-1	1	1	0.49	0.51
18	1	1	-1	-1	0.7	0.68
19	-2	0	0	0	0.45	0.44
20	1	-1	1	-1	0.59	0.6
21	0	0	0	0	0.71	0.7
22	1	-1	-1	-1	0.63	0.62
23	0	0	0	2	0.58	0.57
24	2	0	0	0	0.67	0.66
25	0	0	0	0	0.71	0.7
26	-1	-1	-1	1	0.47	0.46
27	0	2	0	0	0.66	0.64
28	-1	-1	-1	-1	0.53	0.53
29	1	1	-1	1	0.69	0.68
30	1	1	1	-1	0.62	0.6 3

The second-order regression equation provided the levels of cellulase as the function of peptone, yeast extract, $KH_2PO_4$and $MgSO_4.7H_2O$ which can be presented in terms of coded factors as in the following equation (3):

$$\begin{aligned} Y_{1=}\ 0.61 &+ 0.065417X_1 + 0.042917X_2 + 0.000417X_3 + 0.019583X_4 - 0.00938X_1 X_2 \\ &- 0.01563X_1 X_3 + 0.013125X_1 X_4 + 0.006875X_2 X_3 - 0.00438X_2 X_4 \\ &- 0.01063X_3 X_4 - 0.09427X_1^2 - 0.07552X_2^2 - 0.06052X_3^2 \\ &- 0.06052X_4^2 \end{aligned}$$

where Y is the cellulase activity (IU/mL),whereas X_1, X_2, X_3 and $X_4$are peptone, yeast extract, $KH_2PO_4$and $MgSO_4.7H_2O$, respectively.

The independent variables were fitted to the second order model equation and examined for the goodness of fit. Several indicators were used to evaluate the adequacy of the fitted model and the results are shown in Table 6. The determination coefficient R^2 value,correlation coefficient R^2value, coefficients of variation (CV) and model significance (F-value) wereused to judge the adequacy of the model. R^2, or coefficient of determination, is the proportion ofvariation in the response attributed to the model rather than to random error. The F-value is the ratio of the mean square due to regression to the mean square due to error and indicates the influence (significance) of each controlled factor on the tested model.

The Model F-value of 62.43for cellulase implies the modelsare 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.

The coefficient of variation (CV) is the ratio of the standard error of estimate to the mean value of the observed response, expressed as a percentage. A model can be considered reasonably reproducible if the CV is not greater than 10%. Usually, the higher the value of CV, the lower is the reliability of experiment.Here, a lower value of CV (2.36) for cellulase indicated a greater reliability of the experiments performed [19].

To test the fit of the model equation, the regression-based determination coefficient R^2 was evaluated. The closer the values of R^2 to 1, the better the model would explain the variability between the experimental and the model predicted values [20].The coefficient of determination (R^2) for cellulase activitywas calculated as (R^2=0.9831 which are nearly equal to 1) 98.31% variability of the response, and only about 1.69% of the total variation cannot be explained by the model.The predicted R^2 value of cellulaseactivity 91.06% was in reasonable agreement with the adjusted R^2 value of cellulase activity 96.74%.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable.Adeq Precision of 26.227 for cellulase indicates an adequate signal. These models can be used to navigate the design space.

The statistical significance of (equ.3) was checked by *F* test, the results of ANOVA are shown in Table 6. The results demonstrated that the model is highly significant and is evident from Fischer's *F*-test with a low probability value (*P* model >*F* less than 0.05) (Table 6). Model coefficients estimated by regression analysis for each variable is shown in Table 6. The significance of each coefficient was determined by *t*-values and *P*-values. The larger the magnitude of*t*-test value and smaller the *P*-value indicates the high significance of the corresponding coefficient [21].

Table 6.Analysis of Variance (ANOVA)for FPA.

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value Prob> F
Model	0.180095	14	0.012864	62.41259	< 0.0001
X_1	0.073704	1	0.073704	357.5943	< 0.0001
X_2	0.019838	1	0.019838	96.24663	< 0.0001
X_3	0.000938	1	0.000938	4.548518	0.0499
X_4	0.000938	1	0.000938	4.548518	0.0499
$X_1 X_2$	0.000506	1	0.000506	2.456199	0.1379
$X_1 X_3$	5.63E-05	1	5.63E-05	0.272911	0.6090
$X_1 X_4$	0.000306	1	0.000306	1.485849	0.2417
$X_2 X_3$	0.002256	1	0.002256	10.94677	0.0048
$X_2 X_4$	0.001406	1	0.001406	6.822776	0.0196
$X_3 X_4$	0.003306	1	0.003306	16.04111	0.0011
X_1^2	0.041407	1	0.041407	200.8986	< 0.0001
X_2^2	0.022836	1	0.022836	110.7947	< 0.0001
X_3^2	0.01905	1	0.01905	92.42732	< 0.0001
X_4^2	0.024857	1	0.024857	120.6021	< 0.0001
Residual	0.003092	15	0.000206		
Lack of Fit	0.002758	10	0.000276	4.1375	0.0652
Pure Error	0.000333	5	6.67E-05		
Cor Total	0.183187	29			

Std. Dev. 0.014, R-Squared 0.9831, Mean 0.61 , Adj R-Squared 0.9674,
C.V. %2.36 , Pred R-Squared0.9106, PRESS 0.016, Adeq Precision 26.227

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.

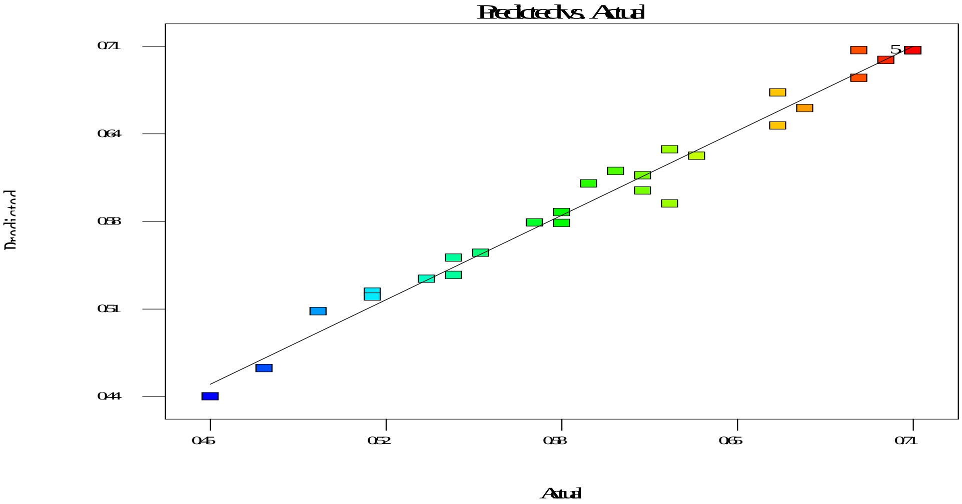

Figure 2.Predicted response vsactual value for FPA.

According to Table 6, values of“Prob>F” less than 0.05,the linear effects of X_1 , X_2 , X_3,X_4, the interactive effects of X_2 X_3, X_2 X_4 , 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 [22].

This interaction was investigated by plotting the 3D response surfaces with the vertical (Z)axis representing Enzyme activity(response) yield and two horizontal axes representing the coded levels of two explanatory factors, while maintaining other variables at their median levels, shown in Figures 5(a)–5(f).The contour plots might be circular,saddle,elliptical, or rising ridges[23].The response surfaces having circular contour plot indicate no interaction, whereas, an elliptical or saddle nature of the contour plot indicates significant interaction between the corresponding variables.

From Figures 5(a), 5(b), 5(c) contour plot shows nearly circular plots indicate no interaction, whereas Figures 5(d), 5(e), 5(f) contour plots are in elliptical shape indicates there is significant interaction between X_2 X_3, X_2 $X_4$and X_3 X_4.

Figures 5(a), 5(b), 5(c) show the dependency of cellulase on Peptone. The cellulase activityincreases with increase in Peptone up to 0.846g/L and thereafter cellulase activity decreases with further increase in Peptone. The same trend was observed in Figures 5(a), 5(d), 5(e) which shows the dependency of cellulase on yeast extract. The cellulase and activityincreases with increase in yeast extract up to 2.14 g/L and thereafter cellulase activity decreases with further increase in yeast extract. From Figures 5(b), 5(d) and 5(f) can be seen the effect of KH_2PO_4on cellulaseactivity. Thecellulase activity increases with increase in KH_2PO_4up to 3.05g/L and thereafter cellulase activity decreases with further increase in KH_2PO_4. The influenceof $MgSO_4.7H_2O$on cellulaseactivity shown from the Figures 5(c), 5(e) and 5(f). The cellulase activity increases with increase in $MgSO_4.7H_2O$up to 0.405g/L and thereafter cellulase activity decreases with further increase in $MgSO_4.7H_2O$.

The optimum conditions for the maximum production of cellulasewere determined by response surface analysis using statistical software package “Design Expert 7.1.5”. The optimum conditions are Peptone-(0.845g/L), Yeast extract (2.14g/L), KH_2PO_4-(3.05g/L)and $MgSO_4.7H_2O$-(0.405g/L),The predicted results are shown in Table 5.

Validation of the experimental model

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 activityobtained 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 were found to be 0.74 IU/ml which was nearly 6.73 fold increase with respect to the control basal medium which showsonly 0.11 U/mL enzyme activity.

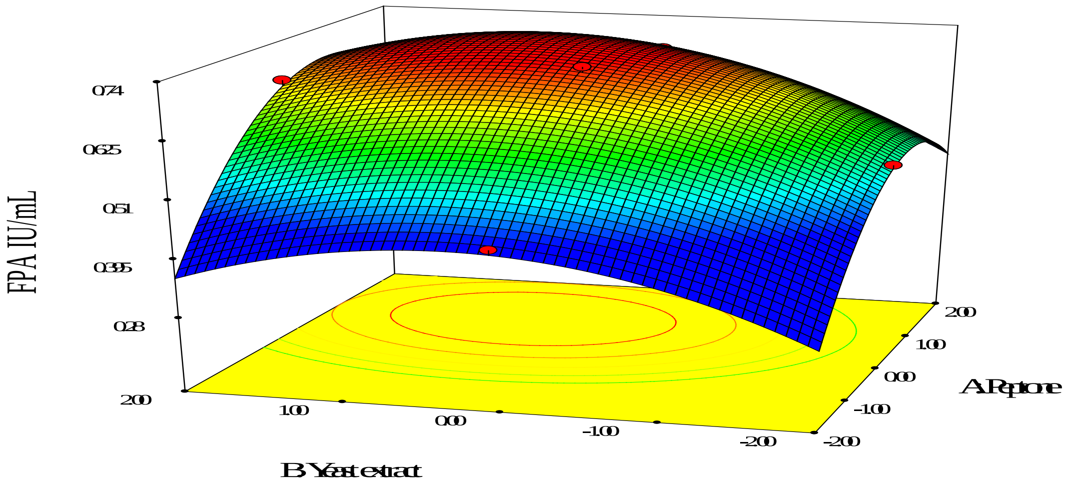

Figure 5(a). Three-dimensional response surface plot for cellulase production showing the interactive effects of medium components in peptone and yeast extract.

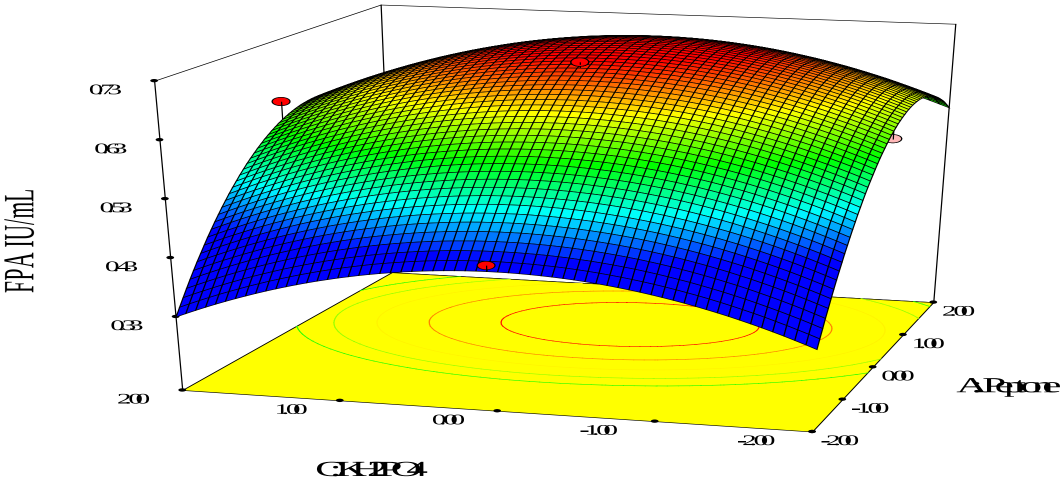

Figure 5(b). Three-dimensional response surface plot for cellulase production showing the interactive effects of medium components in peptone andKH_2PO_4.

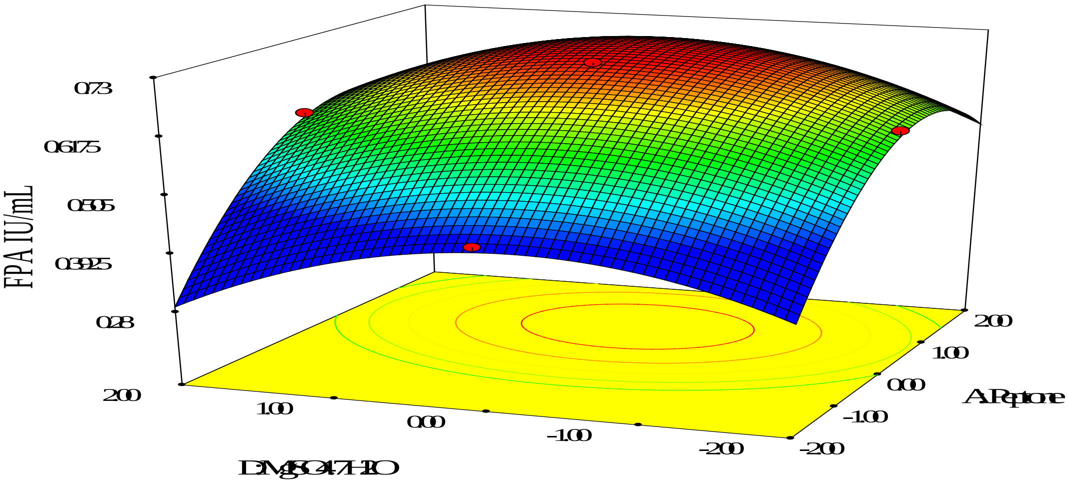

Figure 5(c). Three-dimensional response surface plot for cellulase production showing the interactive effects of medium components in peptone and$MgSO_4.7H_2O$.

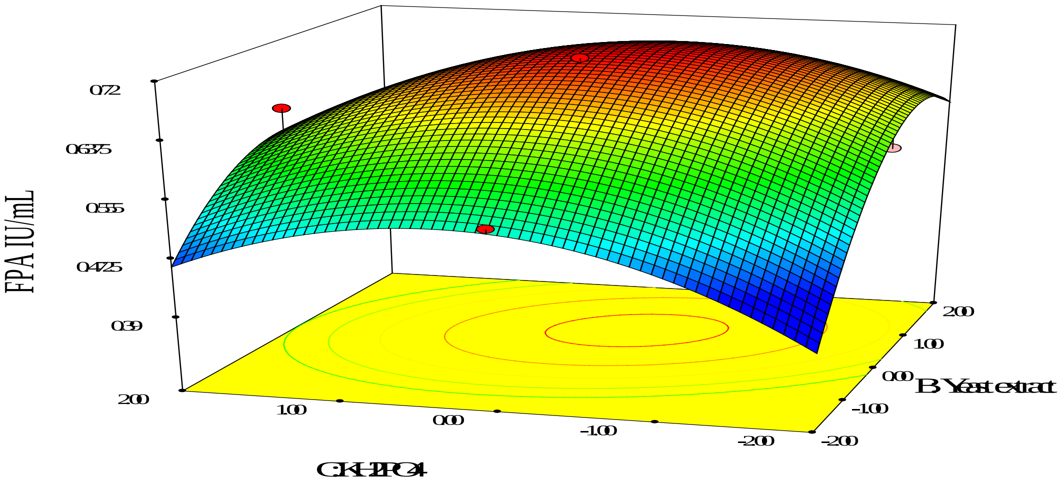

Figure 5(d). Three-dimensional response surface plot for cellulase production showing the interactive effects of medium components in yeast extract and KH_2PO_4.

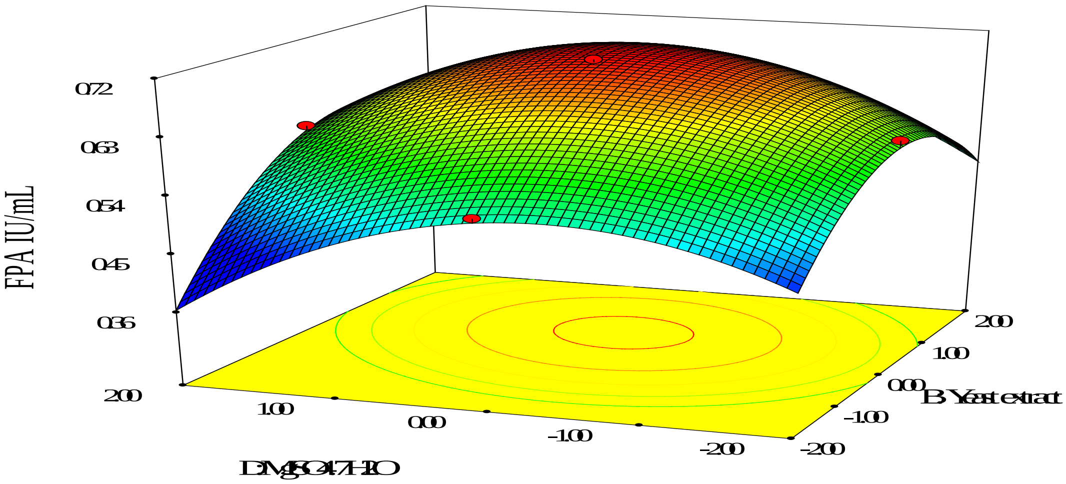

Figure 5(e). Three-dimensional response surface plot for cellulase production showing the interactive effects of medium components in yeast extractand $MgSO_4.7H_2O$.

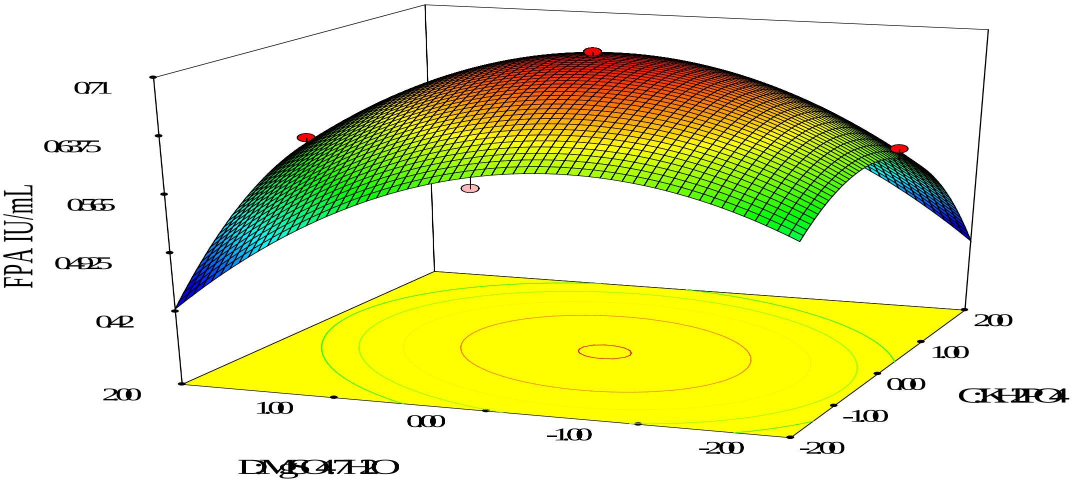

Figure 5(f). Three-dimensional response surface plot for cellulase production showing the interactive effects of medium components in $KH_2PO_4$and $MgSO_4.7H_2O$.

Conclusions

In this work medium components for higher cellulaseactivityfrom *Cellulomonasfimi NCIM-5015* were optimized by RSM. Using Placket-Burman design peptone, yeast extract, $KH_2PO_4$and $MgSO_4.7H_2O$ were found to be the most significant variables, which significantly enhanced cellulase activity. Central composite design was applied to study the combined effects of these nutrients. The optimal levels of components were obtained as peptone(0.846g/L), yeast extract (2.14g/L), KH_2PO_4-(3.05g/L)and $FeSO_4.7H_2O$(0.405g/L). This study showed that wheat straw constitutes a good carbon source for the production of cellulase. Using the optimized conditions, the produced enzyme activity of cellulase reaches 0.74 IU/ml,which was a 6.73 fold increase in production than un-optimized conditions.These results show a close agreement between the expected and obtained activity level.

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