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Research Article

CENTRAL COMPOSITE DESIGN (CCD) FOR CELLULASE PRODUCTION USING *ASPERGILLUSNIGER* ISOLATED FROM KATTALAGARKOVIL, TAMILNADU

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ABSTRACT

In the present study, the leaf litter soil samples were collected from ten different locations of Kattalagarkovil (Tamilnadu, India). The soil samples were serially diluted and plated on Sabouraud's Dextrose Agar (SDA). The fungal strains were identified based on the morphology using Lacto phenol cotton blue mount and cellulase producing fungi were confirmed by Congo red method. The potential cellulase producing fungal strain was confirmed as *Aspergillus* sp. by colony morphology on Sabouraud's Dextrose agar plate and Lacto Phenol Cotton Blue Staining. The potential cellulase producing fungi was identified as *Aspergillus niger* by 18S rRNA sequencing. The fungal gene sequence was submitted in NCBI with the accession Number MF621961.In this study optimum parameters for cellulase production by *Aspergillus niger* using central composite design (CCD) model was analyzed. The optimal level of the key variables (pH, temperature, peptone and KCl) was used to determine the effect of their interactions on cellulase production using the statistical tool (CCD). At these optimized conditions the maximum cellulase production was found to be 0.977 IU/ml.

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INTRODUCTION

Cellulose is most abundant compound of plant formed by stoking of glucose units obtained through photosynthesis. The major components of plant cell walls are cellulose, hemicellulose and lignin, with cellulose being the most abundant component (Anderson *et al*., 2007).

Enzymatic hydrolysis of cellulose involves the sequential action of a group of enzymes known as cellulase, which belongs to the super family of glycosylhydrolases, they catalyze the hydrolysis of the glycosidic bond between two or more carbohydrates. The enzyme activity was defined as the amount of an enzyme that, at optimum conditions of temperature, pH and ionic strength are able to transform into their respective products a micromole of substrate in one minute, and is known as IU unit international and specific enzymatic activity refers to mg protein (Solis *et al*., 2016).

Cellulases are the inducible bioactive compounds produced by microorganisms during their growth on Cellulosic matters. Cellulases are the inducible bioactive compounds produced by microorganisms during their growth on Cellulosic matters. Cellulolytic activity is a multicomplex enzyme system and consists of three major components; endo-β-glucanase (EC 3.2.1.4), exo- β -glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) (Shankar, 2012).

RSM is now considered as a standard statistical approach for designing experiments, building models, evaluating the effects of many factors and finding the optimal conditions for desirable responses and reducing the number of required experiments. In biological processes, especially in the production of enzyme, RSM has been adopted to optimize the growth of microorganisms and the production of enzyme (Shankar and Isaiarasu, 2012).

In this study, RSM was adopted to determine the optimal conditions for the production of cellulase from *Aspergillus niger* and the interactions amongthe factors that influence the response of the cellulase production.

MATERIALS AND METHODS

Microorganism and cultural conditions

Cellulose hydrolyzing fungi *Aspergillus niger* isolated from the leaf litter soil sample of Kattalagarkovil, Tamilnadu, was used in this study. The culture was maintained on CMC (Carboxy

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Methyl Cellulose) agar slants and stored in the refrigerator (4°C) for further analysis.

Cellulase production medium

Strains were screened for cellulase production in liquid culture which contained ($NaNo_3$-3.0g/L; K_2HPO_4-1.0g/L; $MgSo_4.7H_2O$-0.5g/L; KCl-0.5g/L; $FeSo_4.7H_2O$-0.01g/L and CMC-1.0g/L at pH 7.0) in Distilled water 1000ml (Shankar, 2012).

Cellulase Assay

The culture medium was centrifuged at 5000 rpm for 20 min at 4°C. The supernatant was used as crude enzyme source for cellulase assay. Endo glucanase activity was assayed as per the method of Ghose, (1987) using 0.5ml of 1% CMC as the substrate in 0.2M citrate phosphate buffer (pH-7) and incubated at 45°C for 30 min. The reaction was terminated by addition of 2ml of DNS reagent and tubes were kept at boiling water bath for 5 min. After cooling the tubes at room temperature, 7ml of distilled water was added in each tube. The intensity of the color was read at 540nm in UV-VIS spectrophotometer (Systronics, 119). Standard curve was performed with glucose solution. One unit of enzyme activity was defined as the amount of enzyme required for release 1µ mol of glucose per minute under assay condition. Enzyme activity was expressed in units. Cellulase activity was calculated using this formula:
IU/ml = concentration of glucose / 0.5×30×0.180 One micromole of glucose equals 0.180 mg.

Statistical Optimization

CCD and RSM analysis

From the optimized nutrient composition for *Aspergillus niger* growth rate, the effect of the pH range, temperature, nitrogen sources (Peptone), Metal ions (KCl) level were studied using Central Composite Design (CCD) (Shankar *et al*., 2014).

A Central Composite Design consists of:

1. A complete 2^K factorial design, where the factor levels are coded to the usual -1, +1 value. This is called the factorial portion of the design.
2. No center points (no >_ 1)
3. Two axial points on the axis of the design variable at a distance of ± *a* from the design center. This is called the axial portion of the design.

The total number of design points is thus equal to $\alpha = [2^k]^{1/4}$.
For this investigation, pH range (X1), temperature (X2), nitrogen source (Peptone) (X3), Metal ions (KCl) (X4) are the independent variables in a series of cellulase production experiment.

Thus K = 4 $\alpha = 2 \times ^{4/4} \alpha = 2$
A CCD with six star points (*a* = 2) and six replicates at the center point (no 6) with a total number of experiments (N) N = 30.

The experiments was conducted by five different level o were employed simultaneously covering the spectrum of variables for the production of cellulase in the Central Composite Design. Table.1indicates the range and levels of the independent variables selected for the production of cellulase. To understand the effects of the parameters pH range, temperature, nitrogen sources (Peptone), Metal ions (KCl)their interactions on the production of cellulase process, statistically designed experiments were used.

Statistical analysis software

Experimental designs and the polynomial coefficients were calculated and analyzed using a trial version of Design-Expert software (version 7.1.6., Stat-Ease Inc., Minneapolis, USA). Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA).

Table 1 Range and levels of the independent variables selected for the production of cellulose

Variables	-α	Low value	Coded variable	High value	+α
pH	3	4	5	6	7
Temperature (°C)	20	30	40	50	60
Nitrogen source (Peptone) (%)	0.3	0.4	0.5	0.6	0.7
Metal ions (KCl) (%)	0.01	0.015	0.02	0.025	0.03

Table 2 Central composite design for cellulase production by *Aspergillus niger*

Std	Run	pH	Temperature	Peptone%	KCl%	Response:1
22	1	5.00	40.00	0.70	0.02	
30	2	5.00	40.00	0.50	0.02	
27	3	5.00	40.00	0.50	0.02	
1	4	4.00	30.00	0.40	0.01	
8	5	6.00	50.00	0.60	0.01	
18	6	7.00	40.00	0.50	0.02	
24	7	5.00	40.00	0.50	0.03	
9	8	4.00	30.00	0.40	0.03	
11	9	4.00	50.00	0.40	0.03	
15	10	4.00	50.00	0.60	0.03	
13	11	4.00	30.00	0.60	0.03	
17	12	3.00	40.00	0.50	0.02	
20	13	5.00	60.00	0.50	0.02	
23	14	5.00	40.00*	0.50	0.01	
4	15	6.00	50.00	0.40	0.01	
6	16	6.00	30.00	0.60	0.01	
14	17	6.00	30.00	0.60	0.03	
7	18	4.00	50.00	0.60	0.01	
28	19	4.00	50.00	0.60	0.01	
26	20	5.00	40.00	0.50	0.02	
3	21	4.00	50.00	0.40	0.01	
16	22	6.00	50.00	0.60	0.03	
5	23	4.00	30.00	0.60	0.01	
12	24	6.00	50.00	0.40	0.03	
21	25	5.00	40.00	0.30	0.02	
29	26	5.00	40.00	0.50	0.02	
2	27	6.00	30.00	0.40	0.01	
10	28	6.00	30.00	0.40	0.03	
19	29	5.00	20.00	0.50	0.02	
25	30	87	40.00	0.50	0.02	

Mass scale production

In order to increase the amount of cellulase production in various pH, temperature, incubation time, carbon source, nitrogen source and agricultural residues; optimization process was carried out using *Aspergillus niger*. Among the isolates maximum yield of cellulase was produced by *Aspergillus niger*. Fermentation was carried out in a 3L fermentor (Lark, Chennai) in optimized production media (Sudha *et al.,* 2018).

RESULTS

Central Composite Design

Response surface methodology was used to optimize the levels of the significant variables identified by the 2-level fractional factorial design. A CCD matrix was developed depending on

the number of factors considered for optimization. Based on the identification of variables by the 2-level fractional factorial, a central composite design was developed for variables significantly to enhancecellulase production. All the non-significant factors were maintained at central points ('0' coded level) of the levels used in the 2-level fractional factorial design.

Table.1. Shows the five levels of variables chosen for trials in CCD. Response surface methodology (RSM) was used to optimize cultivation conditions for cellulase production, 30 experimental runs with different combinations of four factors and five levels were carried out (Table.2). The variables used for the factorial analysis were named as X1, X2, X3 and X4 in this design respectively. The effects of four independent variables on cellulase production and the experimental response along with the predicted response in Table.3.

Table 3 Observed and predicted responses for cellulase production performed using CCD design

Std	Run	pH	Temperature	Peptone %	KCl%	X Actual value	Y Predicted value
22	1	5	40	0.70	0.02	0.712	0.712
30	2	5	40	0.50	0.02	0.977	0.977
27	3	5	40	0.50	0.02	0.977	0.977
1	4	4	30	0.40	0.01	0.600	0.595
8	5	6	50	0.60	0.01	0.400	0.394
18	6	7	40	0.50	0.02	0.401	0.406
24	7	5	40	0.50	0.03	0.500	0.501
9	8	4	30	0.40	0.03	0.300	0.303
11	9	4	50	0.40	0.03	0.278	0.278
15	10	4	50	0.60	0.03	0.390	0.390
13	11	4	30	0.60	0.03	0.604	0.605
17	12	3	40	0.50	0.02	0.260	0.259
20	13	5	60	0.50	0.02	0.420	0.426
23	14	5	40	0.50	0.01	0.670	0.599
4	15	6	50	0.40	0.01	0.600	0.599
6	16	6	30	0.60	0.01	0.602	0.602
14	17	6	30	0.60	0.03	0.678	0.674
7	18	4	50	0.60	0.01	0.502	0.500
28	19	4	50	0.60	0.01	0.977	0.977
26	20	5	40	0.50	0.02	0.977	0.977
3	21	4	50	0.40	0.01	0.520	0.521
16	22	6	50	0.60	0.03	0.510	0.515
5	23	4	30	0.60	0.01	0.762	0.768
12	24	6	50	0.40	0.03	0.600	0.591
21	25	5	40	0.30	0.02	0.612	0.615
29	26	5	40	0.50	0.02	0.977	0.977
2	27	6	30	0.40	0.01	0.612	0.613
10	28	6	30	0.40	0.03	0.555	0.556
19	29	5	20	0.50	0.02	0.662	0.613
25	30	5	40	0.50	0.02	0.977	0.977

There was a considerable variation in the cellulase production depending on the four chosen variables. The maximum cellulase production (0.977 IU/ml) was achieved. This adequately indicated that choosing appropriate cultivation conditions could evidently enhance the yield of cellulase. By applying multiple regression analysis on the experimental data, the following second order polynomial equation was found to explain the cellulase production by only considering the significant terms and was shown as below:

Final equation in terms of coded factors

Cellulase = + 0.98 + 0.037 * A - 0.058 * B + 0.024 * C - 0.043 * D + 0.015 * A * B - 0.046 * A * C + 0.059 * A * D - 0.048 * B * C + 0.012 * B * D + 0.032 * C * D - 0.16 * A^2 - 0.11* B^2 - 0.078 * C^2 - 0.098 * D^2

Final equation in terms of actual factors

Cellulase = - 8.01971 + 1.58429 * pH + 0.092929 *Temperature + 11.03042 * nitrogen + 46.87500 * KCl + 1.49375E-003 * pH * Temperature - 0.46062 * pH * nitrogen + 11.76250 * pH * KCl - 0.048437 * Temperature * nitrogen + 0.24375 * Temperature * KCl + 64.37500 * nitrogen * KCl - 0.16122* pH^2 - 1.08594E-003 * $Temperature^2$ - 7.83437 * $nitrogen^2$ - 3903.75000 * KCl^2

The independent variables were fitted to the second order model equation and examined for the goodness of fit. Several indiicators were used to evaluate the adequacy of the fitted model and the results are shown in Table.4. The determination coefficient R^2 value, correlation coefficient R value, coefficient of variation (CV) and model significance (F- value) were used to judge the adequacy of the model. R^2 should be at error, Suggested for a good fit of a model, R^2 should be atleast 80%. The closer value of R (correlation coefficient) to 1, the better is the correlation between the experimental and predicted values. Here the value of R (0.9978) for Eq. (3.2) being close to 1 indicated a close agreement between the experimental results and the theoretical values predicted by the model equation. The coeficient 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 value of CV, the lower is the reliability of experiment. Here, a lower value of CV (0.91) indicated a greater reliability of the experiments performed.

The model significance (F-value) indicates the level of confidence that the selected model cannot be due to experimental error. Linear and quadratic terms were significant at the 1% level. Therefore, the quadratic model was selected in this optimization study. The Student T distribution and the corresponding P value, along with the parameter estimate, are given in the Table.4.

The P-values are used as a tool to check the significance of each of the coefficients which, in turn, are necessary to understand the pattern of the mutual interactions between the best variables. The parameter estimates and the corresponding P-values showed that among the independent variables, X1 (pH), X2 (temperature), X3 (Nitrogen), X4 (KCl) had a significant effect on cellulase production. So, compared with the traditional 'one – variable at a time' approach which is unable to detect the frequent interactions occuring between two or more factors although they often do occur, RSM has immeasurable effects and tremendous advantages.

The significant factors identified by manual optimization design were considered for the next stage in the medium optimization using response surface optimization technique for further study. The analysis of variance (ANOVA) was employed (shown in the Table.4) for the determination of significant parameters. ANOVA consists of classifying statistical results and testing whether the means of a specified classification differ significantly.

Table 4 Analysis of Variance (ANOVA) for cellulase production

Source	Sum of squares	Df	Mean square	F Value	p-value Prob> F
Model	1.40	14	0.100	4439.68	< 0.0001[c]
A-pH	0.032	1	0.032	1447.08	< 0.0001[c]
B-Temperature	0.081	1	0.081	3622.14	< 0.0001[c]
C-Nitrogen	0.014	1	0.014	630.83	< 0.0001[c]
D-KCl	0.044	1	0.044	1942.33	< 0.0001[c]
AB	3.570E-003	1	3.570E-003	159.02	< 0.0001[c]
AC	0.034	1	0.034	1512.16	< 0.0001[c]
AD	0.055	1	0.055	2465.15	< 0.0001[c]
BC	0.038	1	0.038	1672.12	< 0.0001[c]
BD	2.377E-003	1	2.377E-003	105.86	< 0.0001[c]
CD	0.017	1	0.017	738.38	< 0.0001[c]
A^2	0.71	1	0.71	31755.43	< 0.0001[c]
B^2	0.32	1	0.32	14407.76	< 0.0001[c]
C^2	0.17	1	0.17	7498.87	< 0.0001[c]
D^2	0.26	1	0.26	11636.73	< 0.0001[c]
Residual	3.368E-004	15	2.245E-005		
Lack of Fit	3.368E-004	10	3.368E-005		
Pure Error	0.000	5	0.000		
Cor Total	1.40	29	1.40		

R^2=0.9998; AdjR^2=0.9995; Pred R-Squared=0.9986; C.V. %=0.76; Std. Dev.= 4.738E-003; Mean=0.62; Adeq Precision=214.441;
[c]model terms of significant.

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 where Y1, was cellulase production, X1 pH, X2 Temperature, X3 Peptone, X4 KCl. The Model F-value of 4439.68 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, D, AB, AC, AD, BC, BD, CD, A^2, B^2, C^2, D^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Pred R-Squared" of 0.9986 is in reasonable agreement with the "Adj R-Squared" of 0.9995. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 214.441 indicates an adequate signal. This model can be used to navigate the design space.

This model can be used to predict the cellulase production within the limits of the experimental factors. Figure.1 shows that the actual response values agree well with the predicted response values.

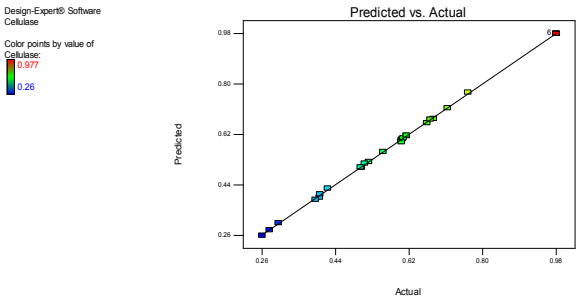

Fig 1 Actual *vs* predicted response values

The interaction effects of variables on cellulase production were studied by plotting 3D surface curves against any two independent variables, while keeping another variables at its central (0) level. Contour plots from the interactions between the variables and the 3D curves of the calculated response (cellulase production) are shown in the figure.2A-F; 3A-F.

The predicted values from the regression equation closely agreed with that obtained from experimental values. 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 obtained from experiments was very close to the actual response predicted by the regression model, which proved the quality of the model. At these optimized conditions the maximum cellulase production was found to be 0.977 IU/ml. The results show a close concordance between the expected and obtained activity level.

The conventional method (i.e., change-one-factor-at-a-time) traditionally used for optimization of multiactor experimental design had limitations because (i) it generates large quantities of data which are often difficult to interpret (ii) it is time consuming and expensive (iii) ignores the effect of interactions among factors which have a great bearing on the response. To overcome these problems, a central composite design (CCD) and RSM were applied to determine the optimal levels of process variables on cellulase production.Only 30 experiments were necessary and the obtained model was adequate (P <0.001). By solving the regression equation, the optimum process conditions were determined. A maximum cellulase yield of 0.977 IU/ml was obtained at the optimized process conditions.

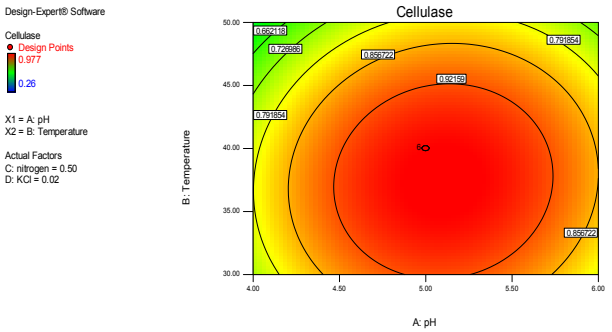

Fig.2A Contour plot showing the effect of pH and temperature on cellulas production

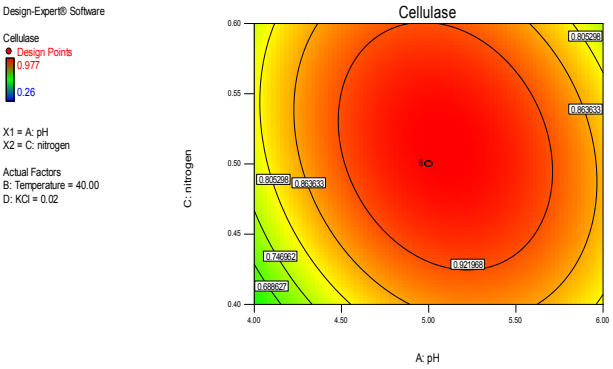

Fig.2B Contour plot showing the effect of pH and peptone on cellulase production

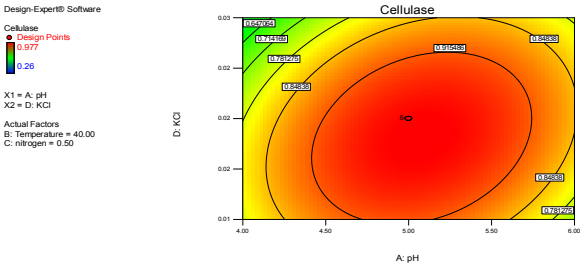

Fig.2C Contour plot showing the effect of pH and KCl on cellulase production

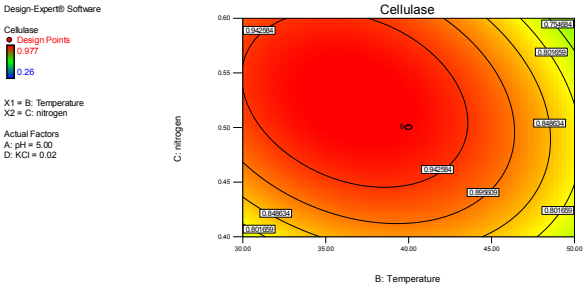

Fig.2D Contour plot showing the effect of temperature and peptone on cellulase production

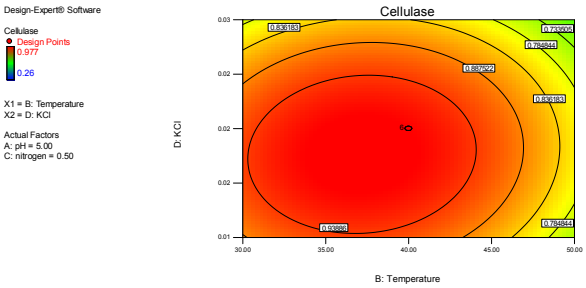

Fig.2E Contour plot showing the effect of temperature and KCl on cellulase production

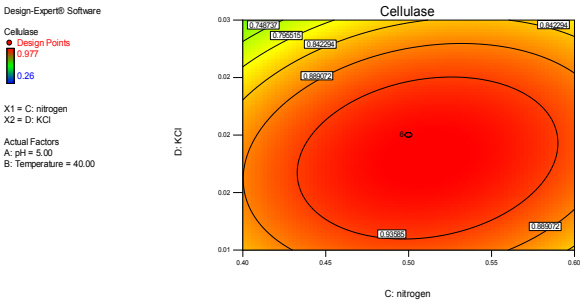

Fig.2F Contour plot showing the effect of peptone and KCl on cellulase production

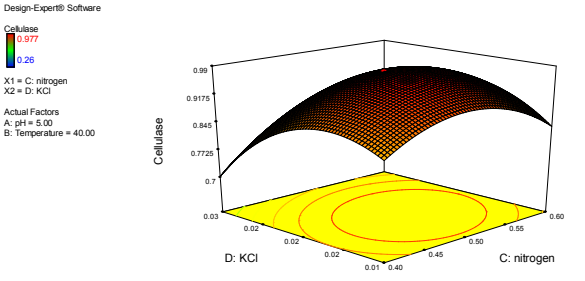

Fig.3A 3D plot showing the effect of peptone and KCl on cellulase production

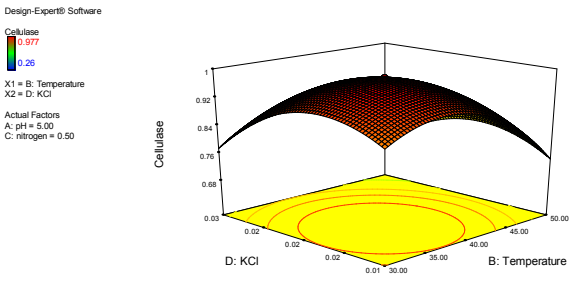

Fig.3B 3D plot showing the effect of temperature and KCl on cellulase production

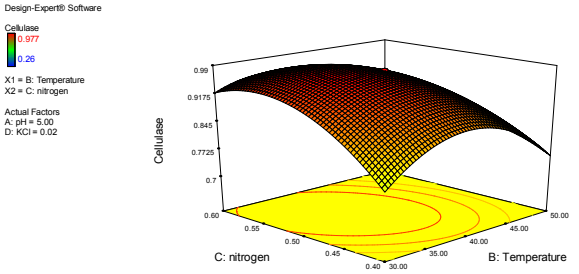

Fig.3C. 3D plot showing the effect of temperature and peptone on cellulase production

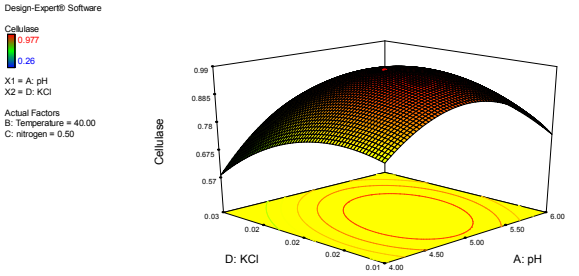

Fig.3D. 3D plot showing the effect of pH and KCl on cellulase production

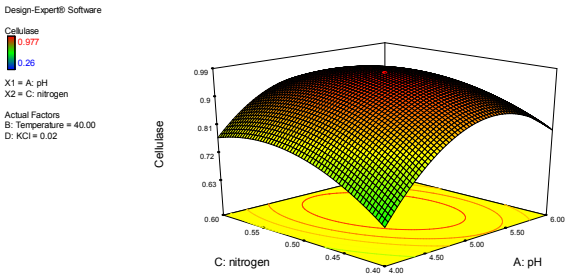

Fig.3E. 3D plot showing the effect of pH and Peptone on cellulase production

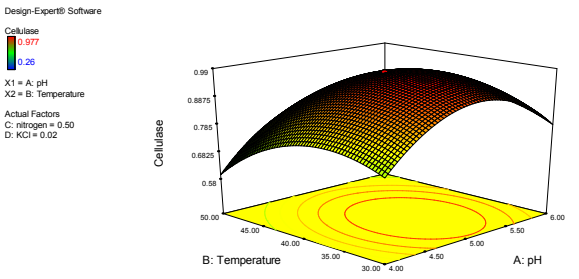

Fig.3F. 3D plot showing the effect of pH and temperature on cellulase production

The research results indicated that RSM not only help us to locate the optimum conditions of the process variables in order

to enhance the maximum cellulase production, but also proves to be well suited to evaluating the main and interaction effects of the process variables on cellulase production from waste agriculture residues.

Mass scale production

After statistical optimization, mass scale production of cellulase enzyme was carried out in laboratory scale fermentor (3L capacity). Cellulase enzyme production of 1.25 IU/ml was obtained using orange peel substrate.

DISSCUSSION

Statistical Optimization

In this study optimum parameters for cellulase production by *Aspergillus niger* using central composite design (CCD) model was analyzed. The optimal level of the key variables (pH, temperature, Peptone and KCl) were used to determine the effect of their interactions on cellulase production using the statistical tool (CCD). The second-order quadratic model with the optimum conditions (pH 5, Temperature 40°C, Peptone 0.5% and KCl 0.02%). The analysis of variance (ANOVA) was employed for the determination of significant parameters. The Model F-value of 1855.84 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. "Pred R-Squared" of 0.9972 is in reasonable agreement with the "Adj R-Squared" of 0.9989. The "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 138.105 indicates an adequate signal. At these optimized conditions the maximum cellulase production was found to be 0.977 IU/ml.This adequately indicated that choosing appropriate cultivation conditions could evidently enhance the yield of cellulase enzyme. Response Surface 3D plots and Contour plots were used to analyse the interactions between the variables.

The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. A circular contour plot of response surfaces indicates that the interaction between the corresponding variables can be ignored, while an elliptical or saddle nature of the contour plot suggests that the interaction between the corresponding variables is significant (Shankar *et al*., 2013).
Similarly, Jabasingh and Nachiyar, (2010) reported that cellulase activity obtained by optimizing the medium contents was found to be significantly affected by the interaction of *A. nidulans* with the designed medium The regression equation showed the cellulase activity as an empirical function in terms of coded factors, where Y_i is the predicted cellulase activity in U/ml. ANOVA for response surface quadratic model gave F value = 23338.52, with p-values of all the coefficients ($p< 0.0001$), implying the significance of the model. The coefficient of variation of the model was (C.V=0.14%). The goodness of fit of the model was examined by determination coefficient (R^2=0.999) which implied that sample variation of more than 99.9% was attributed to the variables and only 0.1% of total variance could not be explained by the model. The adjusted determination coefficient (Adj R^2=0.999) was also satisfactory to confirm the significance of the model. The "Pred R-Squared" of 0.9998 was in reasonable agreement with the "Adj R-Squared" of 0.9999. Adeq precision measured the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 575.478 obtained in this model indicated an adequate signal which could be used to navigate the design space. The results of the response surface quadratic model in the form of analysis of variance (ANOVA) with significance of each coefficient, determined by student's t- test and p- value. At these optimized conditions the model predicted 39.56 U/ml of cellulase activity.

Anwar *et al*. (2016) also reported that minerals were optimized by RSM using *A. niger* under pre-optimized culture conditions (96 h, 30°C and pH, 9.0). Out of total 30 treatments the best treatment, which significantly enhanced the cellulase activity, was T13 (33.6U/mL). The regression coefficients of four variables (C1=N, C2=Ca^{+2}, C3=Mg^{+2} and C4=K^{+}), probability value (p-value) for each variable and p-value for their interactions on the response were used to evaluate the significance levels ($p<0.01$ or $p<0.05$). The p-value suggest that the coefficient for linear effect of calcium, C2and potassium, C4were found to be significant model terms with p values 0 and 0.001, respectively and N*Ca, Ca*K, K*K, N*Mg, Ca*Mg and N*N were significant insight mineral interactions with p values 0, 0, 0.011, 0.001, 0.008 and 0.027, respectively. Whereas, the coefficient of determination (R^2) and R^2-adj were 90.7% and 82%, respectively this ensured the satisfactory adjustment and significance of RSM model to experimental data. The interaction between all four tested mineral variables i.e. N, Ca^{+2}, Mg^{+2} and K^{+2} and their effcet on cellulase production has been displayed in contour plots and response surface 3D curves.

Similarly Sivakumar *et al.* (2011) reported that three factors namely pH, temperature and starch were used for RSM optimization in *Bacillus cereus*. The high degree of similarity was observed between the predicted and experimental values that reflected the accuracy and applicability of RSM to optimize the process for enzyme production. The maximum keratinase enzyme production was 63.01 U/ml by *Bacillus cereus* TS1.The above results favour to correlate this study.

CONCLUSION

The central composite design (CCD) and RSM were applied to determine the optimal levels of process variables on cellulase production. This method is a good substitue for the Conventional method because it have some limitations.

From this research results reported that RSM not only helps locate the optimum conditions for production and also to enhance the maximum cellulase production. At these optimized conditions the maximum cellulase production was found to be 0.977 IU/ml.

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