ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

OPTIMIZING L-ASPARAGINASE PRODUCTION BY *FUSARIUM SOLANI* CLR-36: A STATISTICAL APPROACH USING RESPONSE SURFACE METHODOLOGY

Amani Esmaeil As-Suhbani*, Hemalata Bhosale

Dst-Fist Sponsored School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India

**Corresponding author: amaniesmaeil.7@gmail.com*

ABSTRACT

L-Asparaginase is a component in multi agent chemotherapeutic regiment for treatment of patients with acute lymphoblastic leukaemia (ALL). This study provides data on the scaling up production of L-Asparaginase by *Fusarium solani* CLR-36 under solid state fermentation using low cost materials. A statistical approach for improving L-Asparaginase production was employed using Plackett-Burman experimental design followed by face-centered Central Composite Design of the response surface methodology. Eleven independent variables (viz., temperature, pH, particle size, initial moisture content, incubation time, L-asparagine, ammonium chloride, glucose concentration, inoculum age, inoculum size, and mixed substrates) were evaluated using Plackett-Burman experimental design. The most significant independent variables (glucose concentration, temperature, and inoculum age) which showing positive effect on L-Asparaginase production were selected for further optimization by response surface methodology face-centered central composite design. L-Asparaginase yield of 90.391 IU from response surface methodology optimized bioprocess was close to the predicted activity 89.417 IU with 96.25% model accuracy.

Keywords: L-Asparaginase production, *Fusarium solani* CLR-36, Statistical optimization, Response Surface Methodology

1. INTRODUCTION

L-Asparaginase (EC 3.5.1.1) is a therapeutic enzyme with antitumor activity that has been studied extensively by scientists and researchers [1, 2]. Among the antitumor drugs, L-Asparaginase is the most effective chemotherapeutic agent in the treatment of childhood acute lymphoblastic leukemia (ALL) as well as in many adult treatment protocols [3, 4]. L-Asparaginase acts by catalyzing the L-asparagine deamination into L-aspartic acid and ammonia [5]. Distinct from normal cells, tumor cells need L-asparagine in large amount for protein synthesis and cell division. Therefore, its exhaustion by L-Asparaginase evokes tumor cells destruction [6].

The L-Asparaginases from *E. coli and Erwinia chrysanthemi* have been used commercially for many years as efficient drugs in the chemotherapy of ALL and other types of blood cancer [7]. However, the administration of L-Asparaginases from these sources has been limited due to the high level of long-term use-related hypersensitivity which is partially associated with the enzyme-related Glutaminase activity [3] and also the resistance development that can lead to anaphylactic shock or drug neutralization respectively.

Modified forms of L-Asparaginase (such as L-Asparaginase from other new sources with low or negligible Glutaminase activity, formulations of pegylated L-Asparaginase, and encapsulation of L-Asparaginase into erythrocytes) have been proposed recently to overcome these limitations [8].

Production of L-Asparaginase is considerably influenced by the fermentation medium composition and culture conditions like pH, inoculum age, inoculum size, temperature, and incubation time [9]. For many years, researchers have employed statistical approaches in biotechnology experiments for the optimization strategy that can be maintained on several steps, starting from the screening of the important process parameters and down to the optimizing those parameters [3]. These have several advantages that included minimizing the numbers of experiment, suitability for multiple factor experiments, finding relativity between factors, and searching for of the most suitable conditions and predicting the response [10]. Response surface methodology (RSM) is an efficient strategic experimental tool that can assess the by optimal conditions of a multivariable model [1].

In the present study, strain CLR-36 was identified as *Fusarium solani* strain CLR-36. A statistical approach has been employed for which a Plackett-Burman design (PBD) is used for identifying significant variables influencing glutaminase free L-Asparaginase production by *F. solani* strain CLR-36. Using face centered central composite design (FCCCD) of the response surface methodology (RSM) the levels of the positive significant variables were further optimized.

2. MATERIAL AND METHODS

2.1. L-Asparaginase Producing Microorganisms

Fungal endophyte CLR-36 used in this study was originally isolated from *Curcuma longa* rhizomes collected from Nanded city, India and identified as glutaminase free L-Asparaginase producer strain according to Bhoslae and As-Suhbani, (2019) [11] and was maintained in our laboratory. The strain was kept on Potato Dextrose Agar (PDA) medium. For inoculum development, isolate *CLR-36* was grown on PDA slants at 280°C for 5 days. The active culture was inoculated in 25 mL of McDox broth (g/L): (10 L-asparagine, 2 glucose, 1.52 KH2PO4, 0.52% MgSO4.7H2O, 0.52 KCl, 0.05 ZnSO4.7H2O, 0.03 CuNO3. 3H2O, 0.03 FeSO4.7H2O, and the pH was adjusted to 6) and incubated under shaking conditions. The medium composition and L-Asparaginase activity colorimetric assay protocol were mentioned previously [11].

In brief, the estimation of L-Asparaginase was performed quantitatively by Nesslerization method reported by Imada et al. (1973) [12]**.** In a tube, a prepared mixture was containing 0.5 mL of enzyme, 0.5 mL of 0.05 M L-asparagine, 0.5 mL of 0.5 M Tris-HCl buffer (pH 8.2), and 0.5 mL distilled water and this mixture was kept 30 min at 37°C. To stop the reaction 0.5 mL of 1.5 M trichloroacetic acid (TCA) was added to the mixture. 0.1 mL from the reaction mixture was taken into a tube contain 0.2 mL of Nessler's reagent in 3.7 mL distilled water and incubated for 20 min. At 450 nm, the optical density was read using UV-Visible spectrophotometer [13]. To prepare blank tubes, enzyme was added following the addition of TCA. To get the enzyme activity, ammonium chloride standard curve gradual concentration was prepared and one international unit (IU) of L-Asparaginase was the amount of enzyme which liberates one μmol/min under the assay conditions.

2.2. Fungal Endophyte Isolate Identification

The isolated fungal endophyte was identified by morphological, cultural characteristics together with molecular identification. The morphological and microscopic characteristics of the isolate were identified according to Lacto Phenol Cotton Blue Staining method [14]. For the molecular identification, CLR-36 isolate was identified by 28S rRNA Sequencing. DNA extraction of the isolate was carried out using the method described by Sanger et al., (1977) [15]. PCR product purification was performed by StrataPrep PCR purification kit. (Make-Agilent, Cat no-400771). Using the gene specific sequencing primers (LROR) and ABI BigDye® Terminator v3.1 Cycle Sequencing reaction kit (Applied Biosystems, USA), the purified PCR amplicons was sequenced. The 28S rRNA gene sequence (836 bp) of strain CLR-36 was aligned with the corresponding 28S rRNA sequences of the type strains of representative members of the genus *Fusarium retrieved* from the GenBank and PDB databases by using BLAST program (http://blast .ncbi.nlm.nih.gov/Blast.cgi) [16] and the software package MEGA7 version 7.0.21 [17] was used for multiple alignment and phylogenetic analysis. The phylogenetic tree was constructed via the bootstrap test of neighbor-joining algorithm [18] based on the 28S rRNA gene sequences of strain CLR-36 and related organisms.

2.3. Plackett-Burman Design

The Plackett-Burman Design (PBD) is frequently used to study the effects of medium components on L-Asparaginase production and identify the critical physic-chemical parameters essential to enhance the enzyme production. However, this design does not resource the interaction effect among the different variables. PBD is a two-factorial experimental design (i.e. high and low, denoted −1 and +1, respectively) that detects the most significant independent variables, via screening and evaluation of n independent variables in n+1 experiments, for the enzyme production.

Eleven independent variables (viz., temperature, pH, particle size, initial moisture content, incubation time, L-asparagine, ammonium chloride, glucose concentration, inoculum age, inoculum size, and mixed substrates) were selected based on our previous study on optimization of solid state fermentation process parameters which was carried out using one factor at a time (OFAT) in a sequential approach for the coconut oil cake substrate which was used as a sole carbon source for L-Asparaginase production [19]. All selected variable was represented at these two levels (Table 1). The main effect was principally calculated as the variance between the average measurements of each variable made at a high

level (+1) and a low level (−1). PBD is based on a first-order equation model (Equation 1):

$$Y = \beta_0 + \sum \beta_i |X_i \quad (1)$$

Where Y represents the response (i.e., L-Asparaginase activity in this study), $\beta 0$ represents the model intercept and βi represents linear coefficient, and Xi represents the independent variables level that help in explaining L-Asparaginase activity. All the trials were carried out in triplicate and the average of the enzyme activity was considered as dependent variable or responses [20, 21].

2.4. Response Surface Optimization

Face-centered central composite design (FCCCD) is used to optimize the independent variables levels as well as to describe the interaction effects between the most positive significant variables on the L-Asparaginase production. In the present study, the experimental design was consisting of 20 trials in which the most positive significant independent variables were further studied at three levels (i.e. low, middle, and high, denoted (−1), (0), and (+1) respectively). The experiments were carried out in triplicate. The calculated average of L-Asparaginase activity was considered as the dependent variable or response (Y). Depending on the response surface regression, the second order polynomial equation (Equation 2) was used to fit the experimental results of the FCCCD as follows:

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_{ii} \beta_{ii} X_i^2 + \sum_{ij} \beta_{ij} X_i X_j \quad (2)$$

Where Y presented the predicted response, β_0 presented the regression coefficients, βi presented the linear coefficient, βii presented the quadratic coefficients, βij presented the interaction coefficients, and Xi presented the coded levels of independent variables.

2.5. Statistical Analysis

Minitab statistical software for windows (version 18.0) was used for the experimental designs, statistical analysis. The main effect of the variables was principally calculated as the variance between the average measurements of each variable made at the different levels. Multiple regression analysis was used for analyzing data. To check the goodness of fit of the model, the coefficient of determination (R^2) was used. One way Analysis of Variance (ANOVA), Fisher's F-test and Student's t-test were employed to specify the significance of each coefficient (5% significance level).

3. RESULTS AND DISCUSSION

3.1. Potential Strain Identification

Based on the colony morphology and the lactophenol stained microscopic observation together with 28S rRNA sequence, the potential strain CLR-36 was identified as *Fusarium solani* strain CLR-36. The 28S rRNA gene sequence (836 bp) was determined for that strain and a GenBank database BLAST search for this sequence showed its similarity to that of many species of the *Fusarium* genus.

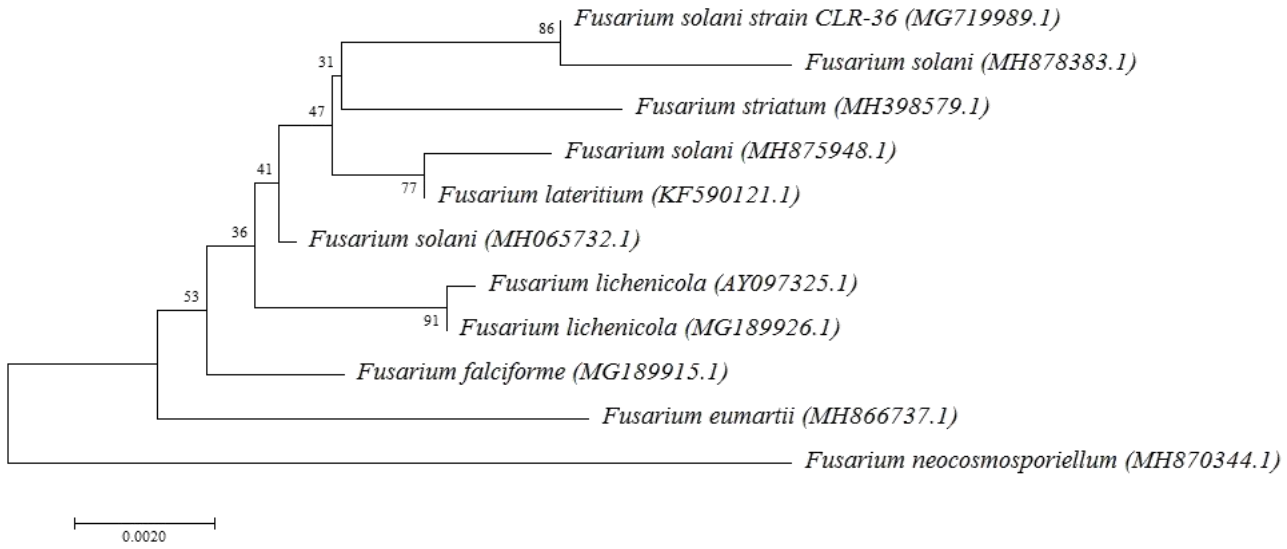


Fig. 1: The phylogenetic tree of the 28S rRNA sequence of strain CLR-36 and related strains. Evolutionary analyses were conducted in MEGA7 [17]

According to the neighbour-joining method of Saitou and Nei, (1987) [18], a phylogenetic tree based on 28S rRNA gene sequences of members of the genus *Fusarium* was constructed using MEGA7. The constructed tree shows a close phylogenetic relationship of strain CLR36 with certain other *Fusarium* species. Phylogenetic analysis shows that the strain CLR36 consistently together with *Fusarium solani* (GenBank accession No. MH878383.1) falls into a clade. On that basis and in view of the cultural and morphological characteristics of isolate No. CLR-36 in relation to the closest related species of the genus *Fusarium*, and it is most closely related to the type strains of *Fusarium solani* strain (GenBank accession No. MH878383.1). Therefore, it was identified as *Fusarium solani* strain CLR36 and its sequencing product was deposited in the GenBank database under accession number MG719989.1 (Fig. 1).

Table 1: Two levels experimental independent variables used for L-Asparaginase production by *Fusarium solani* CLR-36 using Plackett-Burman Design

Code	Variable	Levels	
		-1	+1
A	Temperature (°C)	28	30
B	pH	6	7
C	Incubation time (days)	6	7
D	Initial moisture content (%, w/v)	60	70
E	Particle size (mm)	3	4
F	L-asparagine (g\L)	5	10
G	Inoculum size (%, v/v)	3	4
H	Glucose concentration (g\L)	5	10
J	Inoculum age (days)	4	5
K	Ammonium chloride (g\L)	5	10
L	Mixed substrates of coconut oil cake and wheat bran (ratio)	2:1	1:1

Table 2: Evaluation of the independent variables and the experimental L-Asparaginase activity using Plackett-Burman Experimental Design

Run	A	B	C	D	E	F	G	H	J	K	L	L-Asparaginase activity (IU)
1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	35.409
2	1	1	1	1	-1	-1	1	1	-1	1	1	31.613
3	-1	1	-1	1	-1	1	1	1	1	-1	-1	43.323
4	-1	-1	-1	1	-1	1	-1	1	1	1	1	34.662
5	-1	-1	-1	-1	1	-1	1	-1	1	1	1	28.683
6	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	27.272
7	1	1	-1	1	1	-1	-1	-1	-1	1	-1	19.962
8	1	-1	1	-1	1	1	1	1	-1	-1	1	57.469
9	1	1	-1	-1	1	1	-1	1	1	-1	-1	63.947
10	1	1	1	-1	-1	1	1	-1	1	1	-1	34.838
11	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	14.147
12	1	-1	1	1	1	1	-1	-1	1	1	-1	27.992
13	-1	1	-1	1	1	1	1	-1	-1	1	1	22.812
14	-1	-1	1	-1	1	-1	1	1	1	1	-1	40.544
15	-1	1	1	-1	1	1	-1	-1	-1	-1	1	33.502
16	-1	1	1	1	1	-1	-1	1	1	-1	1	39.733
17	1	1	-1	-1	-1	-1	1	-1	1	-1	1	41.423
18	1	-1	-1	-1	-1	1	-1	1	-1	1	1	46.916
19	1	-1	-1	1	1	-1	1	1	-1	-1	-1	46.240
20	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	18.393

"The "−1" sign represented the minimum value and the "+1" sign represented to the maximum"

3.2. Evaluation of Significant Variables by Plackett-Burman Design

Eleven independent variables including temperature, pH, particle size, initial moisture content, incubation time, L-asparagine, ammonium chloride, glucose concentration, inoculum age, inoculum size, and mixed substrates were evaluated using BPD of 20 runs in order to study the effect of these variables on L-Asparaginase production. Table 2 shows the results of the screened significant variables for production of L-Asparaginase and the related response (Y) using BPD. The data on L-Asparaginase activity using BPD showed a wide variation from 14.147 IU/mL to 63.947 IU/mL in the run numbers 11 and 9 respectively. Such significant variation shows the importance of using statistical optimization for improving medium conditions.

Table 3 shows the Student's t-test and P values which were employed to specify the significance of each coefficient. The corresponding coefficient considered significant when the t- value is large and the P value is small [22].

In the present design, independent variables with P values of less than 0.05 (i.e. the confidence levels above 95%) were considered to have significant effects on the L-Asparaginase production. The value of R^2 is always between 0 and 1and the strong model is having R^2 value close to 1 which indicates the more reliability of the design in predicting the response [23]. The coefficient R^2 value was 0.979 which confirming the strength and significance of the design in predicting the independent variables effect on L-Asparaginase production by *F. solani* CLR-36. Therefore, when neglecting the insignificant factors (p above 0.05), the regression equation of the model for enzyme yield as the independent variable was derived and represented as:

$$Y=35.444+5.137A-3.855D+2.644E+2.517F + 7.728H +3.611J-3.915K+1.788L \quad (3)$$

Where Y is the independent variable or the response (L-Asparaginase production) and *A*, *D*, *E*, *F*, *H*, *J*, *K*, and *L* are temperature, initial moisture content, particle size, L-asparagine, glucose concentration, inoculum age, ammonium chloride, respectively.

L-Asparaginase activity statistical analysis was implemented and represented. Regarding to the main effect of each independent variable, eight variables from the eleven variables (viz., temperature, pH, particle size, L-asparagine, glucose concentration, inoculum age, inoculum size, and mixed substrates) were having a positive effect L-Asparaginase activity, where the other three variables (viz., ammonium chloride, initial moisture content and incubation time) where negatively affecting the L-Asparaginase production (Fig.2). The Pareto chart shows the significance order of the independent variables affecting L-Asparaginase production in PBD. Glucose concentration had the highest positive effect among the eleven variables, followed by temperature, and then inoculum age. Among the three variables, ammonium chloride had the highest negative significance. Next to ammonium chloride, initial moisture content showed negative effect followed by incubation time.

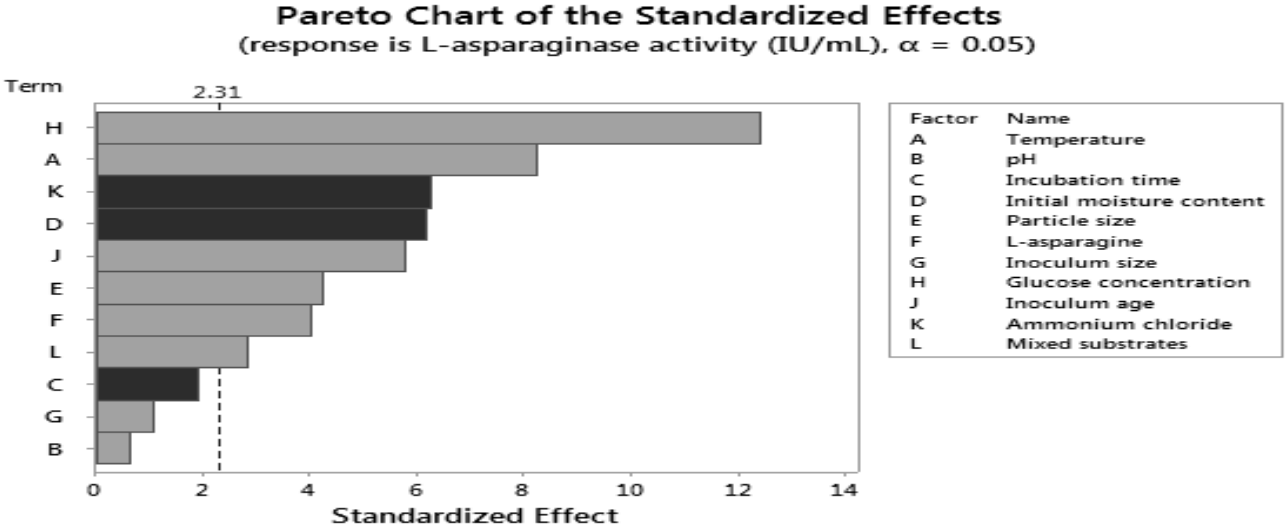


Fig.2: Pareto chart shows the order significance of the independent variables affecting L Asparaginase production by *F. solani* CLR36 (the black color represents positive effects and the grey color represents negative effects)

Table 3: Plackett-Burman Design statistical analysis and analysis of variance

(a)

Term	Effect	Coef	SE Coef	T-Value	P-Value
Constant		35.444	0.623	56.92	0.000
Temperature	10.274	5.137	0.623	8.25	0.000
pH	0.797	0.399	0.623	0.64	0.540
Incubation time	-2.384	-1.192	0.623	-1.91	0.092
Initial moisture content	-7.709	-3.855	0.623	-6.19	0.000
Particle size	5.289	2.644	0.623	4.25	0.003
L-asparagine	5.034	2.517	0.623	4.04	0.004
Inoculum size	1.330	0.665	0.623	1.07	0.317
Glucose concentration	15.456	7.728	0.623	12.41	0.000
Inoculum age	7.223	3.611	0.623	5.80	0.000
Ammonium chloride	-7.829	-3.915	0.623	-6.29	0.000
Mixed substrates	3.556	1.778	0.623	2.86	0.021

(b)

Analysis of variance (ANOVA)

	Df	SS	MS	F-test	Significance F (P value)
Regression	11	2956.86	268.81	34.67	0.000
Residual	8	62.03	7.75		
Total	19	3018.89			

"t: Student's test; P: corresponding level of significance; df: degree of freedom; SS: sum of squares;MS: mean sum of squares; F: Fishers's function; Significance F: corresponding level of significance. R Square 97.95% and adjusted R square 95.12%

3.3. Response Surface Optimization

Based on PBD data, the significant independent variables with positive effect on L-Asparaginase production were selected for optimizing of their levels and the interaction effect of them using FCCCD. The other independent variables were hold at their optimum levels that gave the maximal yield in PBD. For this, different combination of glucose concentration (H), temperature (A), and inoculum age (J) were carried out in with a total of 20 experiments and the experimental response of experiments with the predicted response and the calculated residuals are show in (Table 4). The maximum L-Asparaginase activity (90.391 IU) was achieved in the run number 13 of FCCCD, whereas the minimum L-Asparaginase activity (17.725 IU) was observed in the run number 15.

Multiple regression analysis was used for analyzing data. To check the goodness of fit of the model, the coefficient of determination (R^2) was used. R^2 was 0.929, indicating that the sample variation of 92.9 % was assigned to the variables and only 7.1% of the total variance could not be defined by the model. From the results, it is clear that R^2-value showing a very good fit between the observed and predicted responses and indicating the reliability of the model for L-Asparaginase production. Table 5 presented the analysis of variance (ANOVA) required to study the model adequacy and its significance. The ANOVA of the regression model revealed that the model was highly significant as is shown by the Fisher's F-test (27.8) and the very low probability value (6.4E-06). The t-values and P values listed in Table 5 indicates the significance of each coefficient. The P values represent the significance of the coefficients and are used to understand the mutual interaction patterns between the variables. The data interpretation was based on signs (i.e., the positive or negative effect on the response) and the statistical significance of coefficients (P<0.05) the correlations between two variables could be either a synergistic effect (positive coefficient) or an antagonistic effect (negative coefficient).

From the significance degree it is clear that the linear coefficients of glucose concentration (H), inoculum age (J), interaction between glucose concentration (H) and inoculum age (J) and interaction between temperature (A) and inoculum age (J) and quadratic effect of glucose concentration (H), temperature (A), and inoculum age

(J) are significant. The probability values of the coefficient propose that amongst the three variables studied, H and J illustrates maximum interaction (*P* value 0.0002), indicating that 96.25% of the model is affected by these variables. The temperature (A) linear coefficients and the interaction between A and H were considered not significant (*P* value > 0.05) and that they did not enhance the production of L-asparaginase. The second-order polynomial (equation 5) model, using the multiple regression analysis, was used to estimate the relationship between the dependent and the independent variables and to find out the maximum L-Asparaginase production related to the optimum levels of temperature (A), glucose concentration (H) and inoculum age (J).

$$Y=89.42+5.43H-6.41J-8.63A^2-17.45H^2-9.07J^2+10.52H*J+4.17A*J \quad (4)$$

Where *Y* represents the predicted response, A represents the coded value of temperature, H the coded value of glucose concentration, and J the coded value of inoculum age.

Table 4: Face-Centered Central Composite Design, showing the effect of glucose concentration, temperature, and inoculum age different levels on the response of L-Asparaginase activity along with the predicted L-Asparaginase activity and residuals

(a)

Trial	Glucose concentration (H)	Temperature (A)	Inoculum age (J)	L-Asparaginase activity (IU)		Residual
				Experimental	Predicted	
1	1	0	0	75.185	77.396	-2.211
2	0	-1	0	79.283	77.894	1.389
3	0	0	0	89.391	89.417	-0.026
4	0	1	0	81.861	83.672	-1.811
5	0	0	1	77.721	73.932	3.789
6	0	0	0	89.391	89.417	-0.026
7	0	0	-1	82.55	86.761	-4.211
8	-1	0	0	68.322	66.532	1.79
9	0	0	0	89.391	89.417	-0.026
10	1	-1	-1	53.288	57.135	-3.847
11	-1	1	1	43.173	39.22	3.953
12	-1	-1	-1	71.928	66.775	5.153
13	0	0	0	90.391	89.417	0.974
14	1	1	-1	60.996	54.043	6.953
15	-1	-1	1	17.725	24.572	-6.847
16	0	0	0	89.391	89.417	-0.026
17	0	0	0	89.391	89.417	-0.026
18	1	1	1	65.541	70.588	-5.047
19	1	-1	1	61.15	56.997	4.153
20	-1	1	-1	60.693	64.74	-4.047

(b)

Level	Glucose concentration (g\L)	Temperature (°C)	Inoculum age (days)
-1	5	28	3
0	10	30	4
1	15	35	5

The interaction effects and optimal levels of the variables were determined by plotting the surface plots [Fig. 3 (a)-3(c)] when one of the variables is fixed at optimum value and the other two are allowed to vary.

Figure 3(a) represents the L-Asparaginase activity as a function of temperature (A) and glucose concentration (H) by keeping inoculum age (J) at optimum value. It showed that lower and higher levels of temperature

support relatively low levels of L-Asparaginase activity; the highest value of L-Asparaginase activity was obtained with middle level of temperature and glucose concentration. Further increase of glucose concentration did not result in higher L-Asparaginase activity. Figure 3(b) represents the L-Asparaginase activity as a function of inoculum age (J) and temperature (A) by keeping glucose concentration (H) at optimum value; the maximum L-Asparaginase activity was attained around the middle levels of temperature and around the middle level of inoculum age and further increase in the inoculum age and temperature resulted in a gradual decrease in the L-Asparaginase activity. Figure 3(c) showed that the maximum L-Asparaginase production was achieved with middle level of glucose concentration (H) while lower and higher levels of glucose concentration (H) resulted in a gradual decrease in L-Asparaginase production. Highest value of L-Asparaginase production was obtained with the middle level of inoculum age (J).

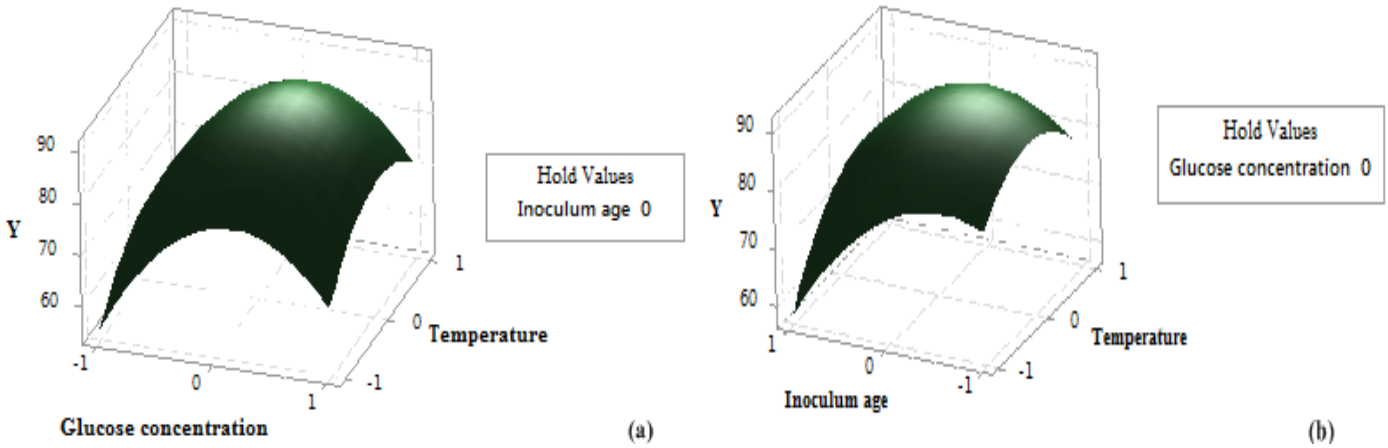
Table 5: Statistical analysis of Face-Centered Central Composite Design and Analysis of Variance

Term	Coef	SE Coef	T-Value	*P*-Value
Constant	89.42	1.74	51.24	0.007
Glucose concentration	5.43	1.61	3.38	0.1021
Temperature	2.89	1.61	1.80	0.0025
Inoculum age	-6.41	1.61	-4.00	0.0002
Glucose concentration*Glucose concentration	-17.45	3.06	-5.70	0.0181
Temperature*Temperature	-8.63	3.06	-2.82	0.0142
Inoculum age*Inoculum age	-9.07	3.06	-2.96	0.8859
Glucose concentration*Temperature	-0.26	1.79	-0.15	0.0002
Glucose concentration*Inoculum age	10.52	1.79	5.86	0.0425
Temperature*Inoculum age	4.17	1.79	2.32	0.007

(b)

Analysis of variance (ANOVA)					
	df	**SS**	**MS**	**F-test**	**Significance *F* (*P* value)**
Regression	9	6445.57	716.17	27.80	6.4E-06
Residual	10	257.64	25.76		
Total	19	6703.22			

t: Student's test; P: level of significance; df: degree of freedom; SS: sum of squares; MS: mean sum of squares; F: Fishers's function; Significance F: corresponding level of significance. R square 0.962, and adjusted R square 0.929



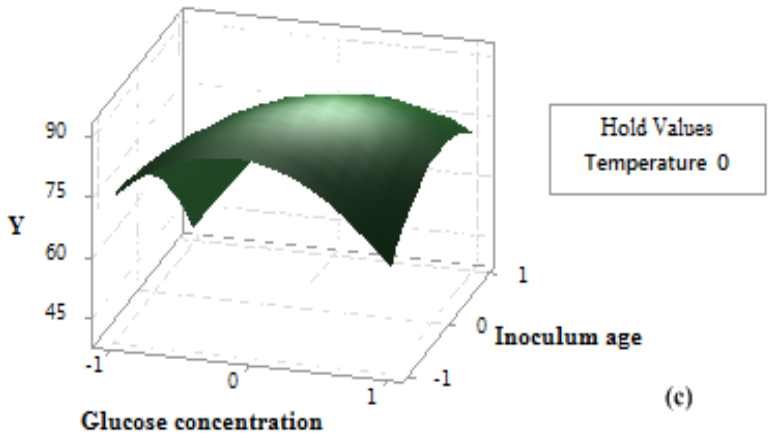


Fig. 3: Surface plots showing the effect and mutual effect of glucose concentration, temperature and inoculum age on the L-Asparaginase production

3.4. Model Verification

A verification experiment was conducted for L-Asparaginase production by *F. solani* CLR-36 using the optimal conditions attained from FCCCD of RSM, and the experimental enzyme activity was compared with the predicted activity. This was performed for determination of the model accuracy and result verification. The experimental L-Asparaginase activity was 90.391 IU, which is very close to the predicted activity (89.417 IU). This indicates a high degree of accuracy of the model (98.29%), which indicates the validation of the model. The variables optimal predicted levels for L-Asparaginase production by *F. solani* strain CLR-36 were temperature (30°C), glucose concentration (10 g/L), and inoculum age (4 days).

4. CONCLUSION

This study provides data on the scaling up L-Asparaginase production by *F. solani* CLR-36. A statistical approach for optimizing L-Asparaginase production from *F. Solani* CLR-36 has been employed using RSM. PBD was used firstly used to identify the independent variables with significant effect on the L-Asparaginase production and followed by FCCCD which was used for further optimization of significant independent variables levels. In this study, L-Asparaginase yield of 90.391 IU from RSM optimized bioprocess parameters is much closer to the predicted RSM activity (89.417 IU) with 98.29% model accuracy. The outcome of this sequential optimization approach offers an effective and rapid screening methodology to identify effect of more than one variable in a single experiment and subsequently fine response regulation.

Competing interests: No competing interests.

5. ACKNOWLEDGEMENT

The authors would sincerely thank the Director of the School of Life Sciences, S. R. T. M. University, Nanded, India for his support and encouragement. This work is a part of the dissertation work in Doctor of Philosophy degree (PhD), by the first author at SRTM University, India.

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