Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. 3**(ISC-2013),** 310-318 **(2014)** *Res. J. Recent. Sci.*

Production and Optimization of L-Glutaminase (EC.3.5.1.2) by *Streptomyces griseus* using Wheat bran under Statistical Designs

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Available online at: www.isca.in, www.isca.me
Received 18th July 2013, revised 22nd December 2014, accepted 4th February 2014

Abstract

L-Glutaminase majorly produced by micro organism including bacteria, yeast and fungi. L-Glutaminase mainly catalyzes the hydrolysis of γ-amido bond of L-Glutamine. In this report, optimization of the culture medium for L-Glutaminase production using Streptomyces griseus was carried out. The optimization of L-Glutaminase production using Wheat bran as substrate was performed with statistical methodology based on experimental designs. The screening of ten nutrients for their influence with Wheat bran on L-Glutaminase production is achieved using Plackett-Burman design. The basal medium contained Peptone 30 g/L, Ferrous sulphate 0.7 g/L, KH_2PO_4 0.7 g/L, NaCl 40 g/L was selected based on their higher influence on L-Glutaminase production. After medium components optimization, the temperature, pH, time, composition of the wheat bran, and inoculum size was optimized using response surface methodology (RSM). The predicted optimum levels are as follows: temperature 30.12 ^{0}C, pH 8.36, time 117.11 h, wheat bran 33.60 g/L and inoculum size 0.90 %. This medium components and parameters were projected theoretically to produce an L-Glutaminase activity of 1959.99 IU/ml. The used methodology was validated using this optimized media components and parameters; the L-Glutaminase activity 1943.5 IU/ml was obtained.

Keywords: L-Glutaminase, Plackett-Burman design, response surface methodology, *streptomyces griseus*, wheat bran.

Introduction

L-Glutaminase or glutaminase (L-Glutamine amido hydrolase EC.3.5.1.2) have identified applications in many fields. This enzymes that take part in the deamidation of L-Glutamine to L-glutamic acid and ammonia. L-Glutaminase plays an important role in all living things like animal, plants and microorganism. L-glutaminase has an important role in cellular nitrogen metabolism[1-4].This enzyme widely used in many industries and pharmaceutical sector as a successful therapeutic agent in the treatment of HIV[5-6] and acute lymphocytic leukaemia[7].The L-Glutaminase having capability leading to the death of glutmine dependent cancer cells by blocking these cells of glutamine. The role of L-Glutaminase to blocking neoplasms of necessary nutrients helps in the treatment of malignancies[7] and also used as an analytical reagent in the determination of glutamate and glutamine[8-9], as a biosensing agent in biosensor[10]. L-Glutaminase used in the food industries to enhance the flavour and aroma of fermented foods due to the increasing nature the glutamic acid level and by this means transmits a delicious taste[11-12]. Now-a-days L-Glutaminase is being used instead of monosodium glutamate to imparting the flavour in Chinese foods[13] and also used in the manufacture of threonine by gamma glutamyl transfer reactions[14]. Its commercial demands gives much attention to search the viable bio processing technology for its large scale production[15].

Hence researchers are concerned in the identification of microbial strains and developing the viable bio processing technique to improved productivity. Bioprocess engineering takes a role in altering the metabolite productivity below the given set of fermentation conditions[16]. Enhancement of metabolic activity is commonly attained by manipulating the dependent and independent variable of the process.

Generally interactive effect of nutrients and incubational parameters with the cell growth to the production of the required enzymes are plentiful, so the best possible process conditions may be identified using an efficient statistical experiment design tools. In recent year, Response Surface Methodology (RSM) mostly used to media components and parameter optimization for their large scale production. These designs show the influence of selected parameters and their interaction with neighbourhood factors of the optimum production. In common practice approach for identification of medium components and parameter cannot show the total interactive effects among the medium components and parameters[17].

Response Surface Methodology (RSM) is a powerful technique where it shows all variables interaction in the processes.RSM relates the optimum level of every factor with its interactive effects along other factors and their influence on the metabolite yield[18-19]. Hence statistical method is suitable to show optimal and exact conditions in a multi factorial designs. RSM decreases the number of trails without avoiding the interaction between

the dependent and independent variables[20]. This design experiments improves statistical interpretation possibilities and identify the significance of all influencing variables even in the presence of complex interactions.

To our knowledge so far no work available on the production of L-Glutaminase by *streptomyces griseus* using wheat bran as substrate under submerged fermentation. *Streptomyces griseus* is an aerobic gram positive filamentous bacterium. In the present investigation, the screening of ten medium components for their influence with wheat bran on L-Glutaminase production is attained using Plackett-Burman experiment and A RSM technique, a face centered central composite design was used to examine the interactions effect of five variables such as temperature, pH, time, concentration of wheat bran, Inoculum size on L-Glutaminase production by *streptomyces griseus* under submerged fermentations.

Material and Methods

Medium components: Nutrient broth, Nesslers's reagent, other nutrients and chemicals were purchased from Hi-Media Limited, Mumbai, India. To measure the optical density, the absorbance was read using UV/Vis Bio Spectrophotometer (EliCoPvt.Ltd., India). Wheat bran was purchased from the local market and it was powdered and dried at 70^{o}C.

Micro organism and culture maintenance: *Streptomyces griseus* NCIM 2622 procured from NCIM, National Chemical Laboratory, India, was used throughout the study. The micro organisms were maintained on Nutrient agar medium slants. Inoculated slants were kept for 4 days in an incubator at 33^{0}C for their growth. Later that incubated slants were stored at 4^{0}C for short period maintenance and sub cultured every 15 days in the above said procedure.

Preparation of inoculum: Inoculum was prepared in 250 ml Erlenmeyer flasks containing 100 ml of nutrient broth liquid medium at pH 7.Prepared nutrient broth liquid medium was autoclaved at 121^{0}C in 15 lb for 20 min and this was inoculated with *Streptomyces griseus* a loop full of culture taken from Nutrient agar slants. The inoculated nutrient broth were kept on a shaker at 150 rpm for 48 h and used as the inoculums throughout the study.

Determination of the significant nutrients using plackett-burman design: To determine what are the nutrients significantly influences the L-Glutaminase production by *Streptomyces griseus;* Plackett-Burman design was used. The Plackett–Burman design is a two factorial design, which detects the important physicochemical parameters mandatory for L-Glutaminase production using Wheat bran as substrate. Ten nutrients were selected in 12 experimental runs. Table-1 shows the higher level (+1) and lower level (−1) of every nutrients. Every runs were carried out in 250ml Erlenmeyer flasks containing 100 ml of working volume with 3% Wheat bran, 1% of inoculum at 33^{0}C in 150 rpm (pH 7) for 24 h and the L-Glutaminase activity were taken as the response (Table- 3). The significant nutrient components for L-Glutaminase production such as peptone 30 g/L, ferrous sulphate 0.7 g/L, KH_2PO_4 0.7 g/L, NaCl 40 g/L were screened and detected by the Plackett-Burman experiment using statistical software package MINITAB (Release 15, PA, USA).

Statistical experimental design: After identifying the nutrients improving L-Glutaminase production by Plackett-Burman design approach, the five most important factors sush as temperature, pH, time, concentration of wheat bran and inoculum size were selected. The selected basal medium contained (g/L) peptone 30, ferrous sulphate 0.7 , KH_2PO_4 0.7 , NaCl 40 at 150 rpm. Response surface method using Face Centered Central Composite design (FCCCD) was used to enhance the L-Glutaminase activity using the software Design-Expert Version 8.0.7.1, Stat-Inc. Minneapolis, USA, to detect the interaction property of five factors. Table-2 shows the central composite design at the particular range of the selected variables in terms of coded and actual values. The average values of L-Glutaminase activities (IU/ml) were taken as responses *Y*. Regression investigation was studied on the data obtained. A second order polynomial equation was then fitted to the data using multiple regression method. This leads to an empirical model that related the responses measured to the independent factors of the design. For a five variable system, the model equation is (1)

$$Y=\beta_0+\beta_1 A+\beta_2 B+\beta_3 C+\beta_4 D+\beta_5 E+\beta_{12} AB+\beta_{13} AC+\beta_{14} AD+\beta_{15} AE+\beta_{23} BC+\beta_{24} BD+\beta_{25} BE+\beta_{34} CD+\beta_{35} CE+\beta_{45} ED+\beta_{11} A^2+\beta_{22} B^2+\beta_{33} C^2+\beta_{44} D^2+\beta_{55} E^2 \quad \textbf{(1)}$$

Where *Y* represent the predicted response in the design ;β_0 is the intercept in the design ; β_1 , β_2 ,β_3 ,β_4 and β_5 represent the linear coefficients in the design ;β_{12} ,β_{13} ,β_{14} ,β_{15} ,β_{23} ,β_{24} ,β_{25} ,β_{34} ,β_{35} and β_{45} represent the interaction coefficients in the design ;β_{11} ,β_{22} ,β_{33} ,β_{44} and β_{55} represent the squared coefficients in the design and A, B, C, D, E, AB, AC, AD, AE, BC, BD, BE, CD, CE, ED, A^2, B^2, C^2, D^2 and E^2 are independent variables in the design. Analysis of variance (ANOVA) was performed. The quantity of variance explained by the polynomial models obtained was given by the multiple coefficient of determination R^2. The fixed polynomial equation was expressed as three-dimensional response contour and surface plots to discover the interaction of every variable for optimal L-Glutaminase activity and picture the correlation between the obtained responses and the experimental levels of each variable used in the design. To predict the optimum level of every variable for maximum response, 'Response optimizer' process using statistical software package MINITAB (Release15, PA, USA) was used. The combined interactive effect of different optimized predicted parameters, which gave maximum L-Glutaminase responses, was examined experimentally to validate the model. All experiments were done in triplicate.

Table-1
High and Low levels of nutrients

S. no	Code	Nutrients (g/L)	High level (+1)	Low level (-1)
1	A	Yeast extract	30	5
2	B	Tryptone	30	5
3	C	Urea	30	5
4	D	Peptone	30	5
5	E	Potassium di hydrogen phosphate	0.7	0.1
6	F	Ferrous sulphate	0.7	0.1
7	G	Manganous sulphate	0.7	0.1
8	H	Sodium chloride	40	5
9	I	Di potassium hydrogen phosphate	0.7	0.1
10	J	Magnesium sulphate	0.7	0.1

Table-2
Range and levels of the selected factors used in FCCCD in terms of actual and coded values

Factors	Levels					
	Code	-2.38	-1	0	1	2.38
Temperature (^oC)	A	20	25	30	35	40
pH	B	6	7	8	9	10
Time (h)	C	72	96	120	144	168
Wheat bran (g/L)	D	10	20	30	40	50
Inoculum size (%)	E	0.5	0.75	1	1.25	1.5

Analytical Experiments: Enzyme separation: At proper time period the fermented broths were harvested for the L-Glutaminase enzyme. The fermented broth was centrifuged at 10000 rpm for 20 min at 4 ^oC in a refrigerated centrifuge and the supernatant collected was used for further L-Glutaminase assay procedures.

Determination of Enzyme activity: L-Glutaminase was assayed method proposed by Imada et al [21]. Initially add 0.5ml of an enzyme preparation, 0.5 ml of L-Glutamine(0.04 M), 0.5 ml of phosphate buffer 0.1 M (pH 8.0), and 0.5 ml of distilled water to a total volume of 2 ml solution. This mixture was incubated at 37°C for 30 min. Then the reaction was stopped by addition of 0.5 ml of 1.5 M Trichloro acetic acid. Further 0.1 ml of the above resultant mixture is added to 3.7 ml of distilled water and 0.2 ml of Nessler's reagent. Finally the developed colour was read after keeping the mixture at 20°C for 20 min at 450 nm in a spectrophotometer. Enzyme and substrate blanks were used as controls. One unit of L-Glutaminase activity was defined as the amount of enzyme that liberated 1μmol of ammonia per 1 min under optimal assay conditions. Assays were done in triplicate and the mean L-Glutaminase activity was defined as International unit per ml (IU/ml).

Results and Discussion

Determination of the significant nutrients using plackett-burman design: The results of Plackett-Burman screening design for enhanced L-Glutaminase production by *Streptomyces griseus*, shown in table 3. The Pareto chart (figure-1) showed the significance of the nutrients with Wheat bran on L-Glutaminase production. From the Pareto chart, the most significant nutrient components for L-Glutaminase production such as peptone 30 g/L, ferrous sulphate 0.7 g/L, KH_2PO_4 0.7 g/L, NaCl 40 g/L were screened and identified.

Statistical optimization of selected factors and their interactive effects: Optimum conditions of the above mentioned important variables and the result of their relations on L-Glutaminase activity were determined by the FCCCD of response surface methodology. Table-4 shows the information of the actual and coded values working in the Central composite design. The results obtained by central composite design were examined by standard analysis of variance and the predicted and observed responses are presented in table-5.

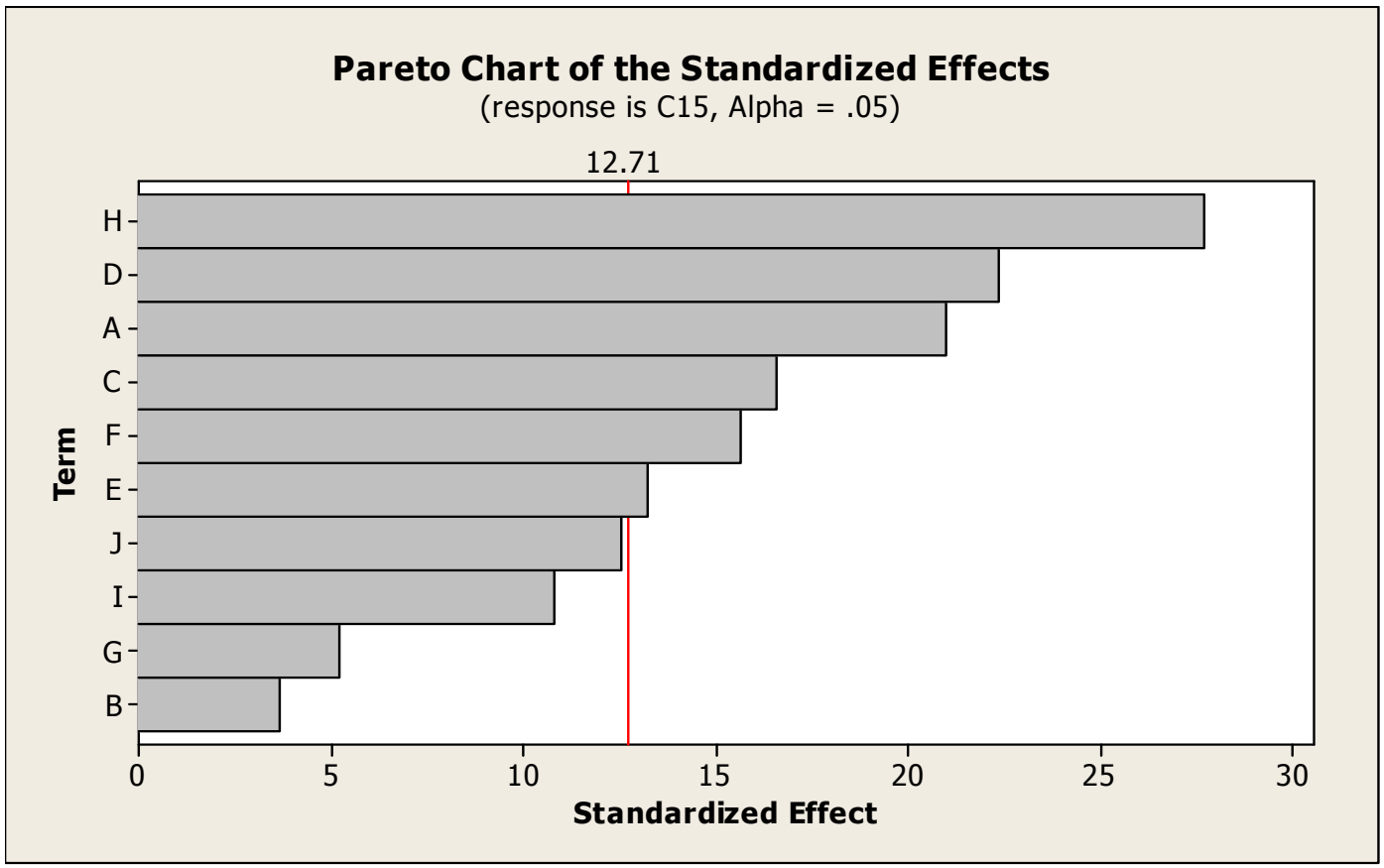


Figure-1
Pareto chart

Table-3
Plackett-Burman design responses for ten nutrients

Run Order	A	B	C	D	E	F	G	H	I	J	Enzyme Activity (IU/ml)
1	-1	-1	1	1	1	-1	1	1	-1	1	49.2
2	1	-1	1	-1	-1	-1	1	1	1	-1	47.3
3	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	56.1
4	-1	1	1	-1	1	-1	-1	-1	1	1	59.8
5	1	-1	-1	-1	1	1	1	-1	1	1	60.0
6	1	1	1	-1	1	1	-1	1	-1	-1	51.2
7	-1	1	-1	-1	-1	1	1	1	-1	1	56.1
8	-1	1	1	1	-1	1	1	-1	1	-1	54.0
9	1	-1	1	1	-1	1	-1	-1	-1	1	51.5
10	1	1	-1	1	-1	-1	-1	1	1	1	49.5
11	-1	-1	-1	1	1	1	-1	1	1	-1	54.7
12	1	1	-1	1	1	-1	1	-1	-1	-1	51.5

Table-4
FCCCD using five selected factors and eight centre points showing observed and predicted responses

Run order	Temp (^{0}C)	pH	Time (h)	Wheat bran (g/L)	Inoculum Size (%)	Enzyme Activity(IU/ml)	
						Observed	Predicted
1	1	-1	-1	1	1	1289.00	1328.28
2	0	0	0	0	2.38	1400.00	1387.12
3	1	-1	1	-1	-1	1355.00	1364.41
4	-1	1	1	1	-1	1530.00	1533.30
5	1	1	1	1	1	1732.00	1733.14
6	0	0	2.38	0	0	1321.00	1315.54
7	-1	1	-1	-1	-1	1051.00	1048.79
8	0	-2.38	0	0	0	1020.00	1010.95
9	1	-1	-1	1	-1	950.00	939.00
10	-1	-1	-1	-1	1	934.00	928.27
11	0	0	0	0	0	1970.00	1969.27
12	-1	-1	1	-1	1	1011.00	1019.57
13	0	0	0	0	-2.38	1360.00	1361.83
14	1	-1	-1	-1	1	1182.75	1174.10
15	-1	-1	1	-1	-1	1257.36	1252.31
16	-1	1	1	1	1	1362.45	1369.04
17	0	0	0	0	0	1970.00	1969.27
18	1	-1	-1	-1	-1	990.73	988.61
19	0	0	0	0	0	1970.00	1969.27
20	0	0	0	0	0	1970.00	1969.27
21	0	0	0	2.38	0	1468.85	1455.89
22	-1	-1	1	1	1	1299.00	1305.89
23	1	-1	1	-1	1	1417.00	1419.74
24	-1	-1	1	1	-1	1329.00	1334.85
25	-1	-1	-1	1	1	1260.00	1254.27
26	1	1	-1	1	1	1517.00	1508.28
27	0	0	0	0	0	1970.00	1969.27
28	1	1	1	1	-1	1600.00	1609.32
29	0	0	0	0	0	1970.00	1969.27
30	-1	1	-1	1	1	1295.93	1298.51
31	-1	1	1	-1	1	921.00	921.13
32	0	0	0	0	0	1970.00	1969.27
33	1	1	-1	-1	1	1191.00	1192.52
34	2.38	0	0	0	0	1247.00	1240.49
35	-1	1	-1	1	-1	1330.75	1332.58
36	-1	-1	-1	-1	-1	1019.00	1030.84
37	0	2.38	0	0	0	1271.00	1268.99
38	1	-1	1	1	1	1543.00	1534.24
39	1	1	-1	-1	-1	1140.00	1142.31
40	-1	-1	-1	1	-1	1151.00	1153.05
41	0	0	-2.38	0	0	790.00	784.41
42	1	1	1	-1	-1	1541.29	1537.02
43	-1	1	-1	-1	1	801.00	810.93
44	0	0	0	0	0	1970.00	1969.27
45	1	1	-1	1	-1	1250.00	1254.29
46	1	-1	1	1	-1	1275.00	1275.13
47	1	1	1	-1	1	1450.00	1457.05
48	-1	1	1	-1	-1	1301.00	1289.16
49	-2.38	0	0	0	0	862.00	857.46
50	0	0	0	-2.38	0	980.00	981.91

Table-5
ANOVA for Response Surface Quadratic Model

Source	Sum of Squares	df	Mean Square	F value	p-value prob> F
Model	5.703E+006	20	2.851E+005	2412.13	< 0.0001
A-A	2.806E+005	1	2.806E+005	2373.37	< 0.0001
B-B	1.273E+005	1	1.273E+005	1077.25	< 0.0001
C-C	5.395E+005	1	5.395E+005	4563.61	< 0.0001
D-D	4.296E+005	1	4.296E+005	3634.29	< 0.0001
E-E	1223.02	1	1223.02	10.35	0.0032
AB	36858.84	1	36858.84	311.81	< 0.0001
AC	47638.58	1	47638.58	403.00	< 0.0001
AD	59042.51	1	59042.51	499.47	< 0.0001
AE	1.660E+005	1	1.660E+005	1403.88	< 0.0001
BC	714.23	1	714.23	6.04	0.0202
BD	52219.42	1	52219.42	441.75	< 0.0001
BE	36606.77	1	36606.77	309.67	< 0.0001
CD	3148.21	1	3148.21	26.63	< 0.0001
CE	33891.06	1	33891.06	286.70	< 0.0001
DE	83054.61	1	83054.61	702.60	< 0.0001
A^2	1.469E+006	1	1.469E+006	12430.32	< 0.0001
B^2	1.193E+006	1	1.193E+006	10093.13	< 0.0001
C^2	1.466E+006	1	1.466E+006	12403.32	< 0.0001
D^2	9.768E+005	1	9.768E+005	8262.98	< 0.0001
E^2	6.136E+005	1	6.136E+005	5191.11	< 0.0001
Residual	3428.12	29	118.21		
Lack of Fit	3428.12	22	155.82		
Pure Error	0.000	7	0.000		
Cor Total	5.706E+006	49			

Table-6
ANOVA for the design

Term	Response of L-Glutaminase activity(IU/ml)	Term	Response of L-Glutaminase activity(IU/ml)
Std. Dev.	10.87	R-Squared	0.9994
Mean	1355.14	Adj R-Squared	0.9990
C.V. %	0.80	PredR-Squared	0.9978
PRESS	12358.40	Adeq Precision	168.128

The second order regression equation gave the stages of L-Glutaminase production as a function of initial values of temperature, pH, time, concentration of wheat bran and Inoculum size, which can be predicted by the following equation (2)

Enzyme ActivityY =1969.08+80.47* A+54.21* B+111.58 * C+99.57 * D+5.31* E +33.94* A*B+38.58* A*C-42.95* A*D+72.01* A*E+4.72* B*C+40.40* B*D-33.82* B*E-9.92* C*D-32.54* C*E+50.95* D*E-162.43* A^2-146.37* B^2-162.26* C^2-132.44* D^2-104.97* E^2 (2)

According to the present designed model, Table.5 shows theF-value of 2412.13 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, E, AB, AC, AD, AE, BC, BD, BE, CD, CE, DE, A^2, B^2, C^2, D^2, E^2 are significant model terms.Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

ANOVA indicated in table.6, the R^2 value of 0.9994 for response *Y*. This again justified the reasonable adjustment of the quadratic model to the experimental data, and showed that the

model could explain 95% of the variability in the response. The "Pred R-Squared" of 0.9978 is in reasonable agreement with the "Adj R-Squared" of 0.9990. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here ratio of 168.128 indicates an adequate signal. This model can be used to navigate the design space. A good association between observed and predicted results reflected the exactness and applicability of the central composite design for process optimization.

L-Glutaminase production for different stages of factors was predicted from the relevant contour and surface plots figure. 2-11. Each contour curve indicated an infinite number of combinations of two selected factors with the other two maintained at their respective central levels. Elliptical nature of the contour in 3D response surface graphs figure. 2-11 showed the shared interactive effects of all the factors. It showed mutual interactive effects between every two variables and gave maximum predicted yield as indicated by the surface confined in the smallest ellipse in the contour plots. Maximum L-Glutaminase production was up to 1970 IU/ml when all the variables were kept at their central code. The model was used for optimization by response optimizer. The model predicted maximum L-Glutaminase production up to 1959.99 IU/ml could be achieved using the medium, wheat bran 33.60 g/L, peptone 30 g/L, ferrous sulphate 0.7 g/L, KH_2PO_4 0.7 g/L, NaCl 40 g/L at pH 8.36 in Temperature of 30.12 ^{0}C for 117.11 h with Inoculum size 0.90 %.Thus, L-Glutaminase production was being predicted after validation of RSM.

Among the five variables tested wheat bran and Inoculum size was the most considerable variable influencing production of L-Glutaminase. It was found that higher wheat bran concentration was inhibitory to L-Glutaminase activity while lower concentration decreased the production of L-Glutaminase. Hence a balance of wheat bran sources enough to attain L-Glutaminase was required. As clear from figure-4, 5, 7-11, the minimum response for enzyme production occurred when Wheat bran and Inoculum size both were in low concentration, while production increased significantly as concentration of Wheat bran and Inoculum size was increased. This implicated the wheat bran and Inoculum size to have a significance interaction on L-Glutaminase production. As the inoculum size increased, the activity depicted a maximum enzyme production of approximately 1970 IU/ml nearly at the middle of Wheat bran concentration; high Wheat bran concentrations beyond this limit minimize L-Glutaminase activity. It showed a tendency towards nutrient limitation. The response also varied noticeably at different levels of Inoculum along the axis. Figure. 5, 8, 10, 11 showed that there is a significant interactive effect of inoculum with wheat bran, temperature, pH and time.

Almost every biological process was pH dependent; a small variation in pH had changed the rate of production. Hence, the optimal pH was very important for maximizing the yield of L-Glutaminase production. Figure 2, 6-8 showed the maximum and minimum pH responses on the L-Glutaminase production.

Incubation temperature also influenced the microbial metabolism, on incubated in different temperature. Figure. 2-5 showed the increased trend of yield from 20°C to 30°C and after that the yield was decreased from 30°C to 40°C. Incubation time was optimized on L-Glutaminase production because the yield of L-Glutaminase was specifically based on substrate utilization and generation time of bacteria. Thus the yield was increased randomly when the incubation time was increased up to 120 h, Figure. 2-5 after that the yields become low due to the competitive between them for the substrate.

Validation was carried out under optimized levels predicted by the design. The theoretically predicted yield was 1959.99 IU/ml. On experimentation, the L-Glutaminase production was about 1943.5 IU/ml was obtained. The experimental values were near to the predicted values. Therefore, the verification and validation of the model was found successful.

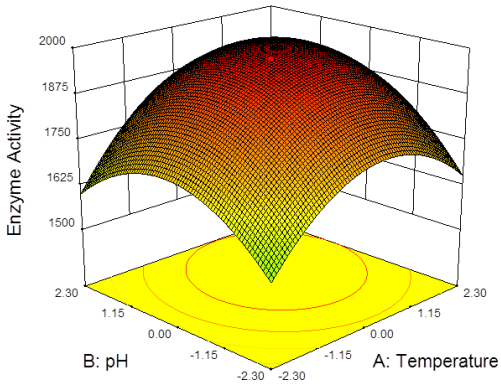


Figure-2

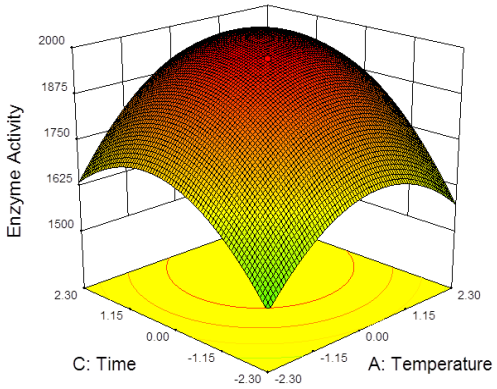


Figure-3

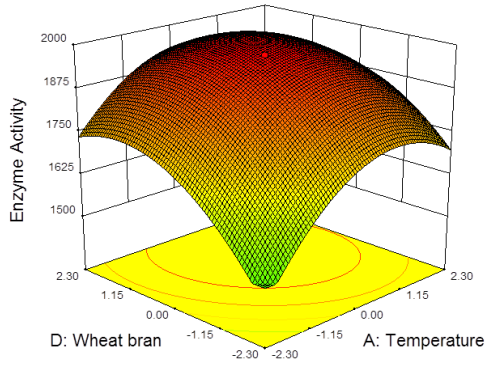
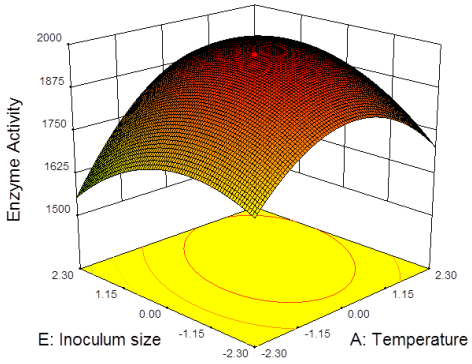


Figure-4



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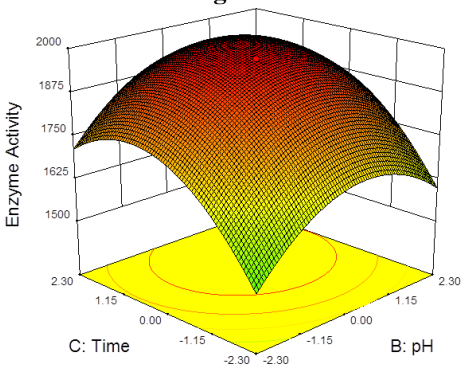

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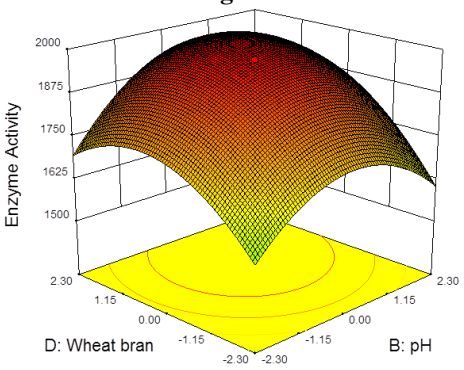

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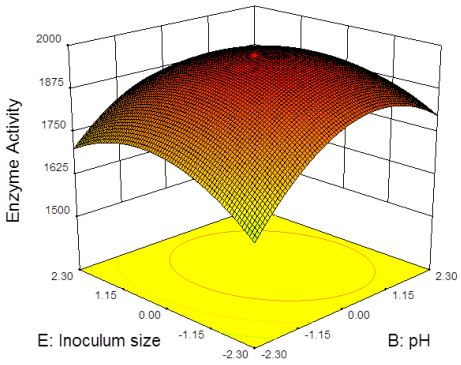
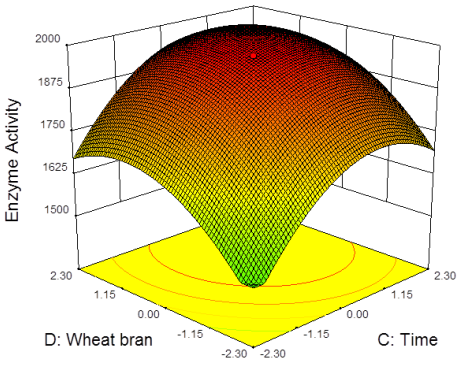


Figure-8



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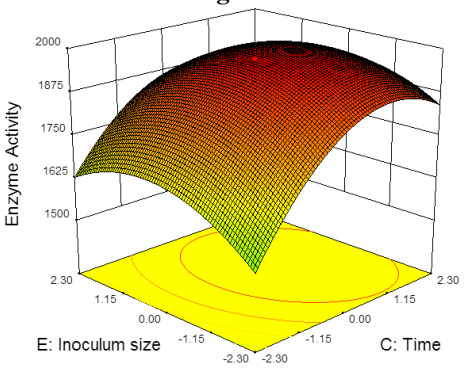

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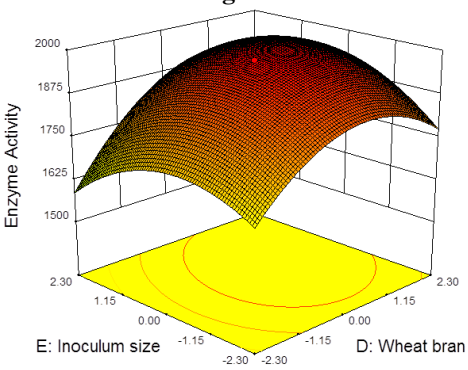

Figure-11

Figure-2 to 11

3 D response surface and contour plots for L-Glutaminase production showing the interaction effects of five selected variables

Conclusion

In this work medium components and process parameters for maximum L-Glutaminase production from *Streptomyces griseus* were optimized by Plackett-Burman design and by RSM. Using Plackett-Burman design approach, Peptone 30 g/L, ferrous sulphate 0.7 g/L, KH_2PO_4 0.7 g/L, NaCl 40 g/L were found to be the most considerable nutrients, which considerably increased L-Glutaminase production. Central composite design was used to study the interactive effects of temperature, pH, time, different concentration of wheat bran and Inoculum size on L-Glutaminase production. The optimal levels of medium components and parameters were obtained as temperature 30.12^0C, pH 8.36, time 117.11 h, wheat bran 33.60 g/L and inoculum size 0.90 %. Using this optimized environment, the produced enzyme activity of L-Glutaminase reaches 1943.5 IU/ml. The results show a close agreement between the expected and obtained production level.

Acknowledgment

The authors thankfully acknowledge UGC, New Delhi, India for providing financial support to carry out this research work under UGC –Non SAP fellowship plan.

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