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OPTIMIZATION OF L- GLUTAMINASE ENZYME PRODUCTION BY *SERRATIA MARCESCENS* USING RICE BRAN UNDER STATISTICAL DESIGNS

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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 *Serratia marcescens* was carried out. The optimization of L-Glutaminase production using Rice bran as substrate was performed with statistical methodology based on experimental designs. The screening of ten nutrients for their influence with Rice bran on L-Glutaminase production is achieved using Plackett-Burman design. The basal medium contained Peptone 30 g/L, $MgSO_4$ 0.7 g/L Manganous sulphate 0.7 g/L, K_2HPO_4 0.7 g/L, NaCl 9 g/L was selected based on their higher influence on L-Glutaminase production. After medium components optimization, the Temperature, pH, Time, composition of the Rice bran, and Inoculum size was optimized using Response Surface Methodology (RSM). The predicted optimum levels are as follows: Temperature 37.04 ^{0}C, pH 7.17, Time 86.41 h, Rice bran 25.84 g/L and Inoculum size 1.29 %. This medium components and parameters were projected theoretically to produce an L-Glutaminase activity of 199.99 IU/ml. The used methodology was validated using this optimized media components and parameters; the L-Glutaminase activity 193.10 IU/ml was obtained.

Keywords: L-Glutaminase, Plackett-Burman Design, Response Surface Methodology, *Serratia marcescens*, Rice Bran

INTRODUCTION

L-Glutaminase or Glutaminase (L-glutamine amido hydrolase EC.3.5.1.2) have identified applications in many fields. This enzymes that catalyze the deamidation of L-

Glutamine to L-Glutamic acid and Ammonia. L-Glutaminase plays an important role in plants, animal tissues and micro organism including bacteria, fungi and yeast. L-Glutaminase has an important role in cellular nitrogen metabolism **[1-4]**. This enzyme widely used in industrial and pharmaceutical sector as an effective therapeutic agent in the treatment of HIV **[5, 6]** and acute lymphocytic leukaemia **[7]**. The L-Glutaminase causes selective death of glutmine dependent tumor cells by blocking these cells of glutamine. The use of L-Glutaminase to blocking neoplasms of essential 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 bio sensing agent in biosensor **[10]**. L-Glutaminase used in the food industries to enhances the flavor and aroma of fermented foods by increasing their glutamic acid level and thereby imparting a delicious taste **[11, 12]**. L-Glutaminase has replaced the use of monosodium glutamate to imparting the flavor 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 **[15]** and cost effective nutrients like agro industrial wastes for its large scale production.

Hence researchers are concerned in the identification of microbial strains and developing the viable bio processing technique to improved cost effective productivity. Bioprocess Engineering takes a role in enhancing the metabolite productivity under a given set of fermentation environment **[16]**.

Enhancement of metabolite production is generally attempted by manipulating the dependent and independent variable of the process.

Generally interactions of medium components and incubational parameters with the cell metabolism to the production of the required compound are plentiful, so the optimum process conditions may be developed using an effective experimental design tools. In recent year, Response Surface Methodology (RSM) has been used to media components and parameter optimization for their large scale production. These designs show the effect of individual factors and their approach with neighborhood factors of the optimum production. In general practice approach for identification of medium components and parameter does not show the net effect of total interactions between various medium components and parameters **[17]**. Response Surface Methodology not only allows the quick screening of a large experimental data, but also shows the each and every

variable interaction in the processes. RSM provides an important relationship regarding the optimum level of each factor along with its interactive effects with other factors and their effects on the metabolite yield **[18]**. Hence statistical method is suitable to show a near optimum conditions and for exact conditions in a multi factorial designs. RSM decreases the number of experiments without neglecting the interaction among the dependent and independent variables **[19]**. This design approach improves statistical interpretation possibilities and identify the significance of all affecting factors even in the presence of complex interactions.

To our knowledge there is no work on the production of L-Glutaminase by serratia marcescens using Rice bran as substrate under submerged fermentation. *Serratia marcescens* is an aerobic a gram-negative bacillus classified as a member of the Enterobacteriaceae. In the present investigation, the screening of ten nutrients for their influence with Rice bran on L-Glutaminase production is achieved using Plackett-Burman design and A RSM technique, a face centered central composite design was used to investigate the interactive effect of five variables viz., Temperature, pH, Time, concentration of Rice bran, Inoculum size on L-Glutaminase production by *Serratia marcescens* under submerged fermentations.

MATERIALS AND METHODS

Medium Components

Nutrient broth, Nesslers's reagent and other media components and chemicals were procured from Hi-Media Limited, Mumbai, India. For optical density measurements, the absorbance was read using UV/Vis Bio Spectrophotometer (EliCoPvt.Ltd., India). Rice bran was collected from the local market and it was powdered and dried at 70°C.

Micro Organism and Culture Maintenance

Serratia marcescens NCIM 2919 procured from NCIM, National Chemical Laboratory, India, was used in the study. The culture was maintained on Nutrient agar medium slants. Inoculated slants were grown in an incubator at 33°C for 4 days. After that the slants were stored at 4°C in a refrigerator for short term preservation and sub cultured every 15 days in the above-mentioned media.

Inoculum Preparation

Inoculum was prepared in 250 ml Erlenmeyer flasks containing 100 ml of nutrient broth liquid medium (pH 7).Prepared medium was autoclaved at 121°C (15 lb) for 20 min and then inoculated with *Serratia marcescens* raised loop full of culture from Nutrient agar slants. The inoculated flasks were kept on a

shaker at 150 rpm for 24 h and used as the inoculum.

Identification of the Significant Nutrients Using Plackett-Burman Design

To determine what are the nutrients significantly influences the L-Glutaminase production by *Serratia marcescens;* Plackett-Burman design was used. The Plackett–Burman experimental design is a two factorial design, which identifies the critical physicochemical parameters required for L-Glutaminase production using Rice bran as substrate. Ten nutrients were screened in 12 experimental runs. **Table 1** shows the low level (−1) and high level (+1) of each nutrients. All experiments were carried out in 250ml Erlenmeyer flasks containing 100 ml of working volume with 3% Rice bran, 1% of inoculum at 33°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, $MgSO_4$ 0.7 g/L, Manganous sulphate 0.7 g/L, K_2HPO_4 0.7 g/L, NaCl 9 g/L were screened and identified by the Plackett-Burman design 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, viz., Temperature, pH, Time, concentration of Rice bran and Inoculum size were selected. The selected basal medium contained (g/L) Peptone 30 , $MgSO_4$ 0.7 ,Manganous sulphate 0.7 , K_2HPO_4 0.7 , NaCl 9 at 150 rpm .Response Surface Method using face centered central composite design (FCCCD) was used to improving L-Glutaminase production using the software Design-Expert Version 8.0.7.1, Stat-Inc.Minneapolis, USA to find the interactive effects of five variables. Central composite design at the given range of the above variables in terms of coded and actual values is presented in **Table 2**. The average maximum L-Glutaminase activities (IU/ml) were taken as responses *Y*. Regression analysis was performed on the resulted data. A second order polynomial equation was then fitted to the data by multiple regression method. This resulted in an empirical model that related the responses measured to the independent factors of the experiment. For a five variable system, the model equation is **(1)**

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

Where *Y* is 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 are 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} are the interaction coefficients in the design ;β_{11} ,β_{22} ,β_{33} ,β_{44} and β_{55} are

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 each variable for maximum L-Glutaminase production and picture the correlation between the responses and the experimental levels of each variable used in the design. To optimize the level of each variable for maximum response, 'Response optimizer' process using statistical software package MINITAB (Release15, PA, USA) was employed. The combination of different optimized parameters, which gave maximum L-Glutaminase responses, was tested experimentally to validate the model. All experiments were done in triplicate.

Analytical Experiments

Enzyme Separation

At proper time intervals the fermentation broths were harvested for the L-Glutaminase enzyme. The broth was centrifuged at 10000 rpm for 20 min at 4°C in a refrigerated centrifuge and the supernatant collected was used for further enzyme assay procedures.

Determination of Enzyme Activity

L-Glutaminase was assayed according to Imada *et al*., **[20]**. The reaction mixture, containing 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 was incubated at 37°C for 30 min. The reaction was stopped by addition of 0.5 ml of 1.5 M Trichloro acetic acid. Then to 3.7 ml of distilled water, 0.1 ml of the above mixture and 0.2 ml of Nessler's reagent were added and color developed 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 enzyme activity was expressed as International unit per ml (IU/ml).

RESULTS AND DISCUSSION

Identification of the Significant Nutrients Using Plackett-Burman Design

The results of Plackett-Burman screening design for enhanced L-Glutaminase production by *Serratia marcescens*, shown in **Table 3**. The Pareto chart (**Figure 1**) showed the significance of the nutrients with Rice bran on L-Glutaminase production. From the Pareto chart, the most

significant nutrient components for L-Glutaminase production such as Peptone 30 g/L, $MgSO_4$ 0.7 g/L, Manganous sulphate 0.7 g/L, K_2HPO_4 0.7 g/L, NaCl 9 g/L were screened and identified.

Statistical Optimization of Screened Variables and Their Interaction Analysis

Optimum levels of the above mentioned important variables and the effect of their interactions on L-Glutaminase production were determined by the face centered central composite design of Response Surface Methodology. **Table 4** shows the information of the actual and coded values working in the FCCCD. The results obtained by FCCCD were analyzed by standard analysis of variance and the mean predicted and observed responses are presented in **Table 5**.

The second order regression equation provided the levels of L-Glutaminase production as a function of initial values of Temperature, pH, Time, concentration of Rice bran and Inoculum size, which can be predicted by the following equation **(2)**,

Enzyme Activity $Y = 217.16-10.09xA+2.62xB-0.21\ C-2.64xD-1.34xE-6.46xA\ xB+9.93xA\ xC+6.11xAxD-3.33xAxE+4.47xBx\ C-6.656E-003x\ B\ x\ D-2.38xBxE+0.77xCxD+0.66xCxE-3.51xDxE-22.53xA^2-29.23xB^2-24.96xC^2-28.07xD^223.75xE^2$ **(2)**

According to the present designed Model, **Table 5** shows theF-value of 238.39 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, D, AB, AC, AD, AE, BC, BE, 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 0.9940 for response *Y*. This again ensured a reasonable adjustment of the quadratic model to the experimental data, and indicated that the model could explain 95% of the variability in the response. The "Pred R-Squared" of 0.9770 is in reasonable agreement with the "Adj R-Squared" of 0.9898."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here ratio of 49.804 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 yield for different levels of factors was predicted from the relevant contour and surface plots **Figure 2-11**. Each contour curve represents an infinite number of combinations of two test factors with the

other two maintained at their respective zero levels. Elliptical nature of the contour in 3D response surface graphs **Figure 2-11** depicted the shared interactions of all the factors. There was a relative significant interaction between every two variables, and there was a maximum predicted yield as indicated by the surface confined in the smallest ellipse in the contour diagrams. Maximum L-Glutaminase production was up to 217.68 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 199.99 IU/ml could be achieved using the medium, Rice bran 25.84 g/L, Peptone 30 g/L, $MgSO_4$ 0.7 g/L Manganous sulphate 0.7 g/L, K_2HPO_4 0.7 g/L, NaCl 9 g/L at pH 7.17 in Temperature of 37.04^0C for 86.41 h with Inoculum size 1.29 %.Thus, L-Glutaminase production was being predicted after validation of RSM.

Among the five variables tested Rice bran and Inoculum size was the most considerable factor influencing production of L-Glutaminase. It was found that high Rice bran concentration was inhibitory to L-Glutaminase production while lower concentration decreased the production of L-Glutaminase. Thus a balance of Rice 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 Rice bran and Inoculum size both were in low concentration, while production increased considerably as concentration of Rice bran and Inoculum size was increased. This suggested the Rice bran and Inoculum size to have a significant effect on enzyme production. L-Glutaminase being a primary metabolite its production was directly related to enzyme being generated. As the Inoculum concentration increased, the response indicated a maximum enzyme production of approximately 217.68 IU/ml nearly at the middle of Rice bran level, higher Rice bran concentrations beyond this limit decreased L-Glutaminase showing a tendency towards nutrient limitation. The response also varied distinctly at different levels of Inoculum along the axis. **Figure 5, 8, 10, 11** suggesting that there is a considerable interaction of Inoculum with Rice 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 25°C to 35°C and after that the yield was decreased from 35°C to 45°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 86.41 h, **Figure 2-5** after that the yields become low due to the competitive between them for the substrate.

Validation was carried out under conditions predicted by the model. The predicted yield was 199.99 IU/ml. On experimentation, the L-Glutaminase production was about 193.10 IU/ml was obtained. The experimental values were found to be very close to the predicted values and hence, the model was successfully validated.

Table 1: High and Low levels of Nutrients

S. No.	Code	Nutrients (g/L)	High level (+1)	Low level(-1)
1	A	Peptone	30	5
2	B	Urea	30	5
3	C	Yeast extract	30	5
4	D	$MgSO_4$	0.7	0.1
5	E	$MnSO_4$	0.7	0.1
6	F	KH_2PO4	0.7	0.1
7	G	Ferrous sulphate	0.7	0.1
8	H	K_2HPO_4	0.7	0.1
9	I	Tryptone	30	5
10	J	Sodium chloride	9	1

Table 2: Experimental Range and Levels of the Independent Factors Used in RSM in Terms of Actual and Coded Factors Working in the FCCCD

Factors	Levels					
	Code	-2.38	-1	0	1	2.38
Temperature (^oC)	A	25	30	35	40	45
pH	B	5	6	7	8	9
Time (h)	C	24	48	72	96	120
Rice bran (g/L)	D	5	15	25	35	45
Inoculum size (%)	E	0.75	1	1.25	1.5	1.75

Table 3: Plackett-Burman Design for Ten Variables.

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	13.59
2	1	-1	1	-1	-1	-1	1	1	1	-1	10.77
3	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	15.41
4	-1	1	1	-1	1	-1	-1	-1	1	1	19.09
5	1	-1	-1	-1	1	1	1	-1	1	1	19.77
6	1	1	1	-1	1	1	-1	1	-1	-1	10.69
7	-1	1	-1	-1	-1	1	1	1	-1	1	15.29
8	-1	1	1	1	-1	1	1	-1	1	-1	13.79
9	1	-1	1	1	-1	1	-1	-1	-1	1	10.99
10	1	1	-1	1	-1	-1	-1	1	1	1	9.29
11	-1	-1	-1	1	1	1	-1	1	1	-1	14.29
12	1	1	-1	1	1	-1	1	-1	-1	-1	11.19

Table 4: Results of FCCCD Using Five Independent Factors and Eight Centre Points Showing Observed and Predicted Responses.

Run order	Temp (^{0}C)	pH	Time (h)	Rice bran (g/L)	Inoculum Size (%)	Enzyme Activity(IU/ml)	
						Observed	Predicted
1	0	0	2.38	0	0	73.27	75.45
2	1	-1	-1	1	-1	85.64	86.28
3	-1	-1	1	-1	-1	77.68	74.46
4	-1	-1	1	-1	1	89.10	91.51
5	1	-1	1	1	-1	90.50	97.02
6	-1	1	-1	-1	1	130	125.45
7	0	0	0	0	0	217.68	217.26
8	-1	1	1	1	-1	95.00	97.38
9	0	0	0	0	0	217.68	217.26
10	-1	-1	1	1	1	68.00	68.57
11	1	-1	-1	-1	1	78.00	74.98
12	1	1	-1	1	-1	70.00	74.39
13	-1	1	-1	1	1	95.00	99.38
14	1	1	-1	1	1	52.00	51.98
15	1	-1	-1	1	1	74.68	73.37
16	0	0	0	-2.38	0	67.00	64.63
17	-1	-1	-1	-1	1	120.00	120.98
18	0	0	0	0	-2.38	93.00	86.01
19	0	0	0	0	0	217.68	217.26
20	1	1	-1	-1	1	42.31	53.61
21	0	0	0	0	0	217.68	217.26
22	0	-2.38	0	0	0	52.00	45.59
23	1	1	1	1	-1	104.21	103.03
24	0	0	0	0	2.38	79.00	79.63
25	-1	-1	-1	-1	-1	98.29	106.56
26	2.38	0	0	0	0	71.00	65.71
27	1	-1	1	-1	-1	83.48	81.51
28	0	0	0	0	0	217.68	217.26
29	1	1	1	1	1	85.88	83.24
30	0	0	0	2.38	0	56.08	52.08
31	0	0	-2.38	0	0	85.00	76.45
32	1	1	-1	-1	-1	65.00	62.01
33	0	2.38	0	0	0	58.00	58.04
34	0	0	0	0	0	217.68	217.26
35	1	1	1	-1	-1	85.80	87.54
36	-1	1	-1	-1	-1	125.00	120.53
37	-2.38	0	0	0	0	114.77	113.69
38	0	0	0	0	0	217.68	217.26
39	0	0	0	0	0	217.68	217.26
40	-1	1	1	-1	1	114.10	113.87
41	-1	1	1	1	1	95.58	90.91
42	1	-1	1	-1	1	85.73	85.25
43	-1	-1	1	1	-1	69.00	65.54
44	1	-1	-1	-1	-1	65.00	73.87
45	1	1	1	-1	1	84.71	81.77
46	-1	-1	-1	1	1	91.88	94.94
47	-1	-1	-1	1	-1	92.83	94.54
48	1	-1	1	1	1	80.68	86.74
49	-1	1	1	-1	-1	100.00	106.32
50	-1	1	-1	1	-1	105.00	108.48

Table 5: ANOVA for Response Surface Quadratic Model

Source	Sum of Squares	df	Mean Square	F value	p-value prob> F
Model	1.347E+005	20	6735.19	238.39	< 0.0001
A-A	4407.78	1	4407.78	156.01	< 0.0001
B-B	296.77	1	296.77	10.50	0.0030
C-C	1.90	1	1.90	0.067	0.7975
D-D	301.68	1	301.68	10.68	0.0028
E-E	77.85	1	77.85	2.76	0.1077
AB	1335.01	1	1335.01	47.25	< 0.0001
AC	3158.24	1	3158.24	111.79	< 0.0001
AD	1193.66	1	1193.66	42.25	< 0.0001
AE	354.43	1	354.43	12.54	0.0014
BC	640.45	1	640.45	22.67	< 0.0001
BD	1.418E-003	1	1.418E-003	5.018E-005	0.9944
BE	180.70	1	180.70	6.40	0.0171
CD	19.21	1	19.21	0.68	0.4164
CE	13.81	1	13.81	0.49	0.4901
DE	393.16	1	393.16	13.92	0.0008
A^2	28206.60	1	28206.60	998.37	< 0.0001
B^2	47468.83	1	47468.83	1680.15	< 0.0001
C^2	34620.91	1	34620.91	1225.40	< 0.0001
D^2	43786.90	1	43786.90	1549.83	< 0.0001
E^2	31336.93	1	31336.93	1109.16	< 0.0001
Residual	819.33	29	28.25		
Lack of Fit	819.33	22	37.24		
Pure Error	0.000	7	0.000		
Cor Total	1.355E+005	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.	5.32	R-Squared	0.9940
Mean	105.81	Adj R-Squared	0.9898
C.V. %	5.02	PredR-Squared	0.9770
PRESS	3114.26	Adeq Precision	49.804

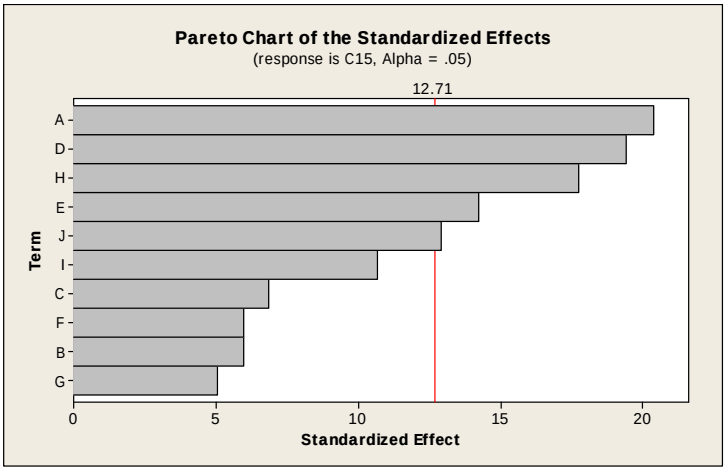

Figure 1: Pareto Chart

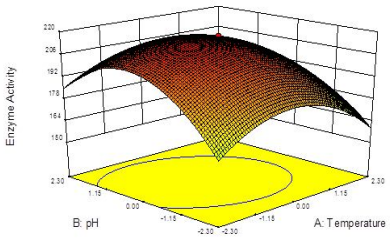

Figure 2

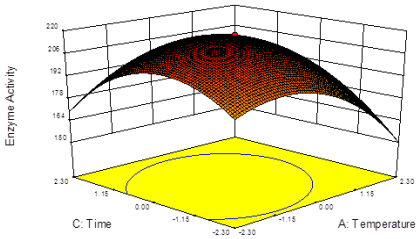

Figure 3

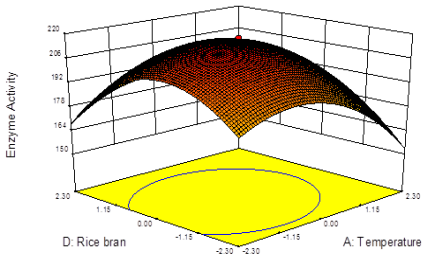

Figure 4

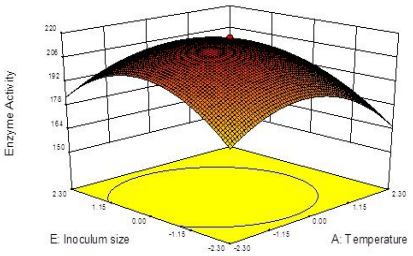

Figure 5

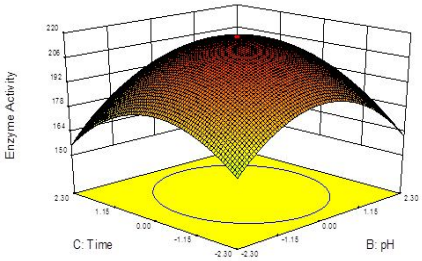

Figure 6

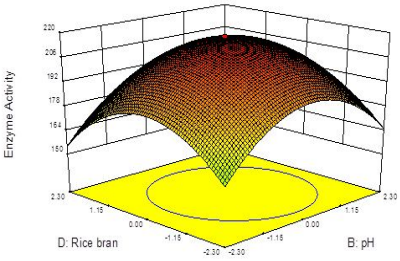

Figure 7

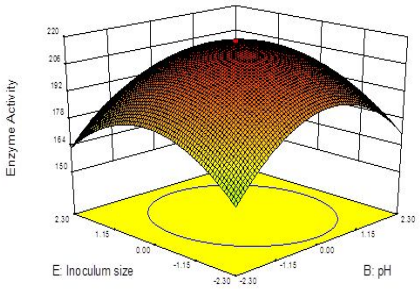

Figure 8

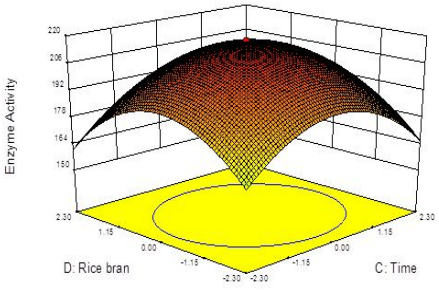

Figure 9

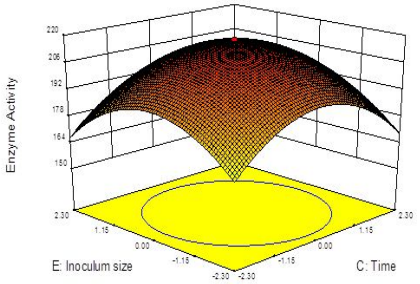

Figure 10

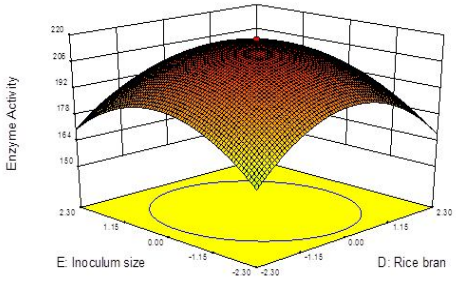

Figure 11

Figure 2-11:Three-Dimensional Response Surface and Contour Plots for L-Glutaminase Production Showing the Interactive Effects of Five Selected Variables

CONCLUSION

In this work medium components and process parameters for maximum L-Glutaminase production from *Serratia marcescens* were optimized by Plackett-Burman design and by RSM. Using Plackett-Burman design approach, Peptone 30 g/L, $MgSO_4$ 0.7 g/L, Manganous sulphate 0.7 g/L, K_2HPO_4 0.7 g/L, NaCl 9 g/L were found to be the most considerable variables, which significantly enhanced L-Glutaminase production. Central composite design was used to study the interactive effects of Temperature, pH, Time, different concentration of Rice bran and Inoculum size on L-Glutaminase production. The optimal levels of medium components and parameters were obtained as Temperature 37.04^0C, pH 7.17, Time 86.41 h, Rice bran 25.84 g/L and Inoculum size 1.29 %. Using this optimized environment, the produced enzyme activity of L-Glutaminase reaches 193.10 IU/ml. The results show a close agreement between the expected and obtained production level.

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