IJPSR (2022), Volume 13, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 June 2021; received in revised form, 26 July 2021; accepted, 27 July 2021; published 01 April 2022

ADVANCEMENT OF GLYCOSIDE HYDROLASE PRODUCTION FROM BACILLUS CEREUS KKSJ 1981 BY STATISTICAL METHOD OF OPTIMIZATION

Siva J. Jyothi [*1], Emandi Hemalatha [2] and Kishore K. Kumar [3]

Hindu college of Pharmacy [1], Amaravathi Road, Guntur - 522002, Andhra Pradesh, India.
Sri Vishnu College of Pharmacy [2], Bhimavaram - 534202, Andhra Pradesh, India.
Narayana Pharmacy College [3], Chintareddy Palem, Nellore, JNTUA, Ananthapuramu - 524002, Andhra Pradesh, India.

Keywords:

Pullulan, Glucanohydrolase, Nutraceuticals, Central composite design.

Correspondence to Author:
Ms. Siva J. Jyothi

Assistant Professor,
Hindu College of Pharmacy,
Amaravathi Road, Guntur - 522002,
Andhra Pradesh, India.

E-mail: jsjbiotech81@gamil.com

ABSTRACT: Pullulanase (Pullulanase-α-glucanohydrolase EC 3.2.1.41) is a glucanohydrolase enzyme that can break down the pullulan and allied oligosaccharides into glucose. Promising of starch-based industries are biofuels, starch syrup, nutraceuticals, *etc.*, increase the glucanohydrolases demand. Present investigation revealing that the Bacillus cereus KKSJ 1981 is enhanced the production of pullulanase through the optimization of temperature, agitation speed, pH, soluble starch concentration, $MnSO_4$ and yeast extract. Response surface methodology (RSM) was utilized to optimize the selected six parameters. A central composite design with 50 experiments was performed in this investigation. The analyzed data depicts that the correlation coefficient (R^2) value was 0.9873, and the adjusted R2 value was 0.9717 indicates the high significance of the model. Further, the selected parameters in optimum conditions were predicted through the developed mathematical model, and validation experiments were performed at these conditions. Overall, 1.2 folds of pullulanase were improved through the RSM based optimization.

INTRODUCTION: Pullulanase (EC 3.2.1.41) is a pullulan α-glucanohydrolase enzyme widely used in de-branching of α-1, 6 glycosidic linkages of pullulan, starch, amylopectin, and other related oligosaccharides by hydrolysis [1]. With the combination of amylolytic enzymes, pullulanase permits absolute and efficient conversion of starch and other polysaccharides into small fermentable sugars, *i.e.*, fructose and maltose. During the saccharification process addition of pullulanase accelerates the sugars production and reduces the manufacturing cost of sugars from starch [2, 3]. Pullulanase is also utilized for several applications, *i.e.*, plaque controlling agent, in detergent industry [4], in an escalation of cyclodextrin production, *etc.* [5].

Among all, pullulanase microbial enzymes have gained industrial importance because of their precise action on the substrates. Various microbial sources such as B. acidopullulyticus, B. cereus FDTA-13, Klebsiella planticola, Geobacillus stearothermophilus, B. deramificians, *etc.*, Hii *et al*. reported that the pullulanase production by Bacillus Sp was more suitable for the industrial production

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(4).1624-31

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(4).1624-31

because it is easy to cultivate at a larger scale, less doubling time, not much complex media to grow and easy to purify [6, 7, 8]. Currently, the enzymes industry has many challenges. The main one is production at a lower cost because many enzymes use low-value precursors such as ethanol, sugar syrups, *etc*. Enhancement of final enzyme yields could mitigate this problem. Recombinant organisms could enhance the yields; however, purification procedures were intricate and used costly resins for purification, which leads to the rise of the final product. So that enhancement of the yield is important by varying the procedure and nutritional parameters. Many researchers enhanced the production of various microbial enzymes by optimizing nutritional, environmental parameters in shake flask levels and reactor levels [9, 10].

The fundamental general methodology of optimization is the one-factor-at-a-time strategy. In this technique, one factor is changing at different levels, and all other parameters are kept constant. It is easy to work, not required any special skills and special software to interpret the data [11]. However, this method has many limitations. This technique takes involves a lot of experimental work and lacks the interaction between parameters, estimation of these interactions is also difficult. To overcome these problems, various statistical and artificial intelligence methods were evolved.

The factorial methods are useful to screen the parameters and advance optimization. On the other hand, these methods are limited with levels. The Placket-Burman technique was evolved to screen the various parameters [12]. Response surface method (RSM) was found to be superior to factorial methods. RSM was widely used in different industries, including enzyme industries [13, 14]. However RSM also has limitations, it can be applied for less number of variables; higher the variables make model complex and need many experiments, which is difficult to conduct. RSM needs minimal mathematical knowledge and software. Currently, many commercially available statistical software's makes RSM user-friendly. The artificial intelligence methods *i.e.,* neural networks, fuzzy logic, genetic algorithm, swam & ant algorithms, *etc.*, have proven superior to statistical methods [14, 15]. Even though artificial intelligence methods are proven superior to RSM, employing these methods in optimization is difficult because it needs a large data for conquer the learning bias and needs special software skills and the user should have mathematical knowledge [14]. Based on these constraints, artificial intelligence techniques were not widely used in biotech industries for optimization. At present many researchers are following the RSM. In the present study RSM with central composite design (CCD) was used to optimize the several parameters for enhanced production of pullulanase by Bacillus cereus KKSJ 1981.

MATERIALS AND METHODS:

Microorganism and Inoculum Preparation: The isolated B. cereus KKSJ 1981 (MN592984) was used in this study. The isolated bacterial culture is streaked on nutrient agar slants and was stored. A routine subculturing process is performed periodically. The microorganism was transferred (a loop full) into the nutrient broth (50 mL) and was incubated at 37 °C on a shaker incubator. Once the culture achieves 0.8 OD at 600 nm (~10^8 cells/ml) it is used to prepare the inoculum. An 18 to 24 h actively growing culture (~10^8 cells/ml) was used for optimization studies.

Pullulanase Production: The pullulanase production using B. cereus KKSJ 1981 was performed in pullulan media 16. The media consist of pullulan 10 g/L, Nacl 2 g/L, $MgSO_4$ 0.1 g/L, K_2HPO_4 0.17 g/L, KH_2PO_4 0.12 g/L and pH 7.5. The media was sterilized at 121 °C for 20 min 1% inoculum was inoculated and at 37 °C for 72 h incubated. After incubation, the broth was centrifuged at 10,000 rpm for 10 min at 4 °C temperature. The cell's free broth was considered a crude enzyme and pullulanase activity was assayed.

Assay of Pullulanase Enzyme Activity: The activity of pullulanase was estimated according to Ara *et al.* [17] In brief, the pullulan was used as a substrate for measuring the activity. It contains 1% pullulan in 460 µL of 0.05 M phosphate buffer with pH 7, to this 40 µL of crude extract was added and incubated at 40 °C for 10 min. The DNS (Dinitrosalicylic acid) method [18] was used for the estimation of released reducing sugars. Pullulanase activity was estimated as the amount of D-glucose released in µmols per min under assay condition and it expressed in U/ml.

Optimization by Pullulanase Production Using Response Surface Methodology (RSM): In this investigation, a CCD (central composite design) was utilized to establish optimum response for Pullulanase production by B. cereus KKSJ 1981. The factors studied were temperature, pH, agitation speed, the concentration of soluble starch, yeast extract, and $MnSO_4$. A 50 experimental CCD was employed and depicted in **Table 1.** Selected parameters levels and CCD plan. All factors levels Xi were coded as xi and depicted in expression 1.

$$X_i = x_i – x_0 /\Delta x_i \; i = 1, 2, 3,.....k$$

Where xi is the coded value of a selected variable, Xi real value of a selected variable, X_0 real value of variable at these central point and Δ Xi is step change. A second-order polynomial model fits the obtained activity of pullulanase to correlate the selected parameters. General second-degree polynomial equation 2 is shown.

$$Y_i = \beta_0 + k/\beta_{ii}\, x_i^2 + ij/i\, j/j\, \beta_{ij}\, x_i x_j + e \text{ ------2}$$

Where Yi is the predicted Pullulanase yield, xi, xj are selected parameters; β_0 =offset term; β_l & βii is the ith linear and quadratic coefficients, βij the interaction coefficient, and 'e'= considered as error. Analysis of variance was tested by the statistical model analysis (ANOVA). The lack of fit test was used to establish whether the constructed model was adequate to illustrate the data observed. Correlation coefficient (R^2) was used to determine the percentage of variability that could be explained by selected variables in the model. Contour plots were generated to demonstrate the collective impacts of various independent variables on pullulanase production. Design Expert 13 (trail version) software was used in this study.

RESULTS AND DISCUSSION: Based on the one-factor-at-a-time method and PBD, various factors, i.e., pH, temperature, agitation speed, the concentration of soluble starch, yeast extract, and $MnSO_4$ which are considerably enhanced the pullulanase production by Bacillus cereus KKSJ 1981 using by RSM for optimization. The obtained low (5.43 U/ml) and high (16.29 U/ml) pullulanase yield **Table 1** indicated that the variables and their levels have the significant achievement of glycoside hydrolase production using B. cereus KKSJ 1981. The data were analyzed by linear regression analysis, and obtained coefficients of the model were examined for significance. The calculated coefficient of determination (R^2) is 0.9873, and it is denoted that 98.73 % of variability could be explained. The adjusted R2 value of 0.9717 and the predicted R2 value of 0.9159 are nearer to the R2 value, indicating the model is highly appreciable [13, 19, 21]. The observed less variation between the experimental model and predicted pullulanase values indicating the accuracy of conducted experimentation. **Fig. 1** depicted the correlation linking of the experimental and model-predicted values. In this graph, all data points are concentrated nearer to the fitted line implies that the model envisaged values are comparable to the values attained experimentally. A relatively lower value of the coefficient of variation (CV=3.49%) indicated a better precision and reliability of experimentations carried out. In a second-order regression equation 3 the pullulanase activity was presented as a function of specified parameters. In equation 3 all factors are explained as coding standards. This mathematical equation denotes an empirical relationship between glycoside hydrolase yield and test variables.

Pullulanase activity (u/ml) = $15.885 – 0.7765 \times X_2 - 0.2330 \times X_3 + 0.4335 \times X_4 + 0.0805 \times X_5 + 0.6785 \times X_6 – 0.5942 \times X_1 \times X_1 – 0.7268 \times X_2 \times X_2 - 0.6863 \times X_1 \times X_3 – 0.5268 \times X_4 \times X_4 – 0.8342 \times X_5 \times X_5 – 1.4355 \times X_6 \times X_6 – 0.6219 \times X_1 \times X_2 – 0.6863 \times X_1 \times X_3 – 0.5775 \times X_1 \times X_4 + 0.3113 \times X_1 \times X_5 – 0.3494 \times X_1 \times X_6 – 0.3619 \times X_2 \times X_3 - 0.1544 \times X_2 \times X_4 – 0.2431 \times X_2 \times X_5 + 0.7288 \times X_2 \times X_6 – 0.3263 \times X_3 \times X_4 + 0.3787 \times X_3 \times X_5 + 0.3519 \times X_3 \times X_6 + 0.7288 \times X_2 \times X_6 – 0.3263 \times X_3 \times X_4 + 0.3787 \times X_3 \times X_5 + 0.3519 \times X_3 \times X_6 + 0.0838 \times X_4 \times X_5 – 0.1894 \times X_4 \times X_6 + 0.4831 \times X_5 \times X_6.$

Table 2 depicts the effects, coefficients along with the respective t, F, and *p-values* of selected variables at their linear, square, interaction levels, and ANOVA of the model. It was observed that, among six variables, the quadratic term of $MnSO_4$ concentration showed the uppermost effect (-2.871) and lowest *p-value* (2.9 × 10-15) followed by the yeast extract concentration of quadratic term (effect = -1.6685, p = 1.48 × 10-10). In linear terms, temperature followed by pH has a higher effect than the others. The linear term of yeast extract was

insignificant. Among all interactions, pH with $MnSO_4$ concentration has a highest effect (1.4575) followed by temperature with agitation speed (-1.3725). The interaction of soluble starch with pH and yeast extract has higher *p-values* ($p>0.05$), indicating that these two interaction terms are insignificant. The quadratic terms of concentrations of $MnSO_4$, yeast extract, soluble starch, and agitation speed have the highest effect than their linear terms. It indicates that these variables and their concentrations are important for pullulanase secretion by B. cereus KKSJ 1981, a small variation of these parameters shows the significant impact on the glycoside hydrolase production. Yeast extract concentration was significant only at quadratic term, which indicates that the selected nitrogen source could act as a limiting parameter for pullulanase production using isolated bacteria. Based on p values, the insignificant coefficients ($p>0.05$) were eliminated from Eq. 3. The final response function to predict the pullulanase activity after eliminating the insignificant terms was as follows (equation 4).

Pullulanase activity (u/ml) = $15.885 - 0.7765 \times X_2 - 0.2330 \times X_3 + 0.4335 \times X_4 + 0.0805 \times X_5 + 0.6785 \times X_6 - 0.5942 \times X_1 \times X_1 - 0.7268 \times X_2 \times X_2 - 0.6863 \times X_1 \times X_3 - 0.5268 \times X_4 \times X_4 - 0.8342 \times X_5 \times X_5 - 1.4355 \times X_6 \times X_6 - 0.6219 \times X_1 \times X_2 - 0.6863 \times X_1 \times X_3 - 0.5775 \times X_1 \times X_4 + 0.3113 \times X_1 \times X_5 - 0.3494 \times X_1 \times X_6 - 0.3619 \times X_2 \times X_3 - 0.1544 \times X_2 \times X_4 - 0.2431 \times X_2 \times X_5 + 0.7288 \times X_2 \times X_6 - 0.3263 \times X_3 \times X_4 + 0.3787 \times X_3 \times X_5 + 0.3519 \times X_3 \times X_6 + 0.7288 \times X_2 \times X_6 - 0.3263 \times X_3 \times X_4 + 0.3787 \times X_3 \times X_5 + 0.3519 \times X_3 \times X_6 + 0.0838 \times X_4 \times X_5 - 0.1894 \times X_4 \times X_6 + 0.4831 \times X_5 \times X_6$.

The results obtained are in accordance with Nair *et al.* [22] the authors observed the highest enzyme production at incubation temperature at 32 ± 2 °C and pH 6.5 by B. cereus. However, the results vary from Waleed *et al.* [16] reports they noticed incubation temperature at 37 °C and pH 7.5 from B. cereus. From this, it was noticed that similar species of microorganisms also produce the desired enzyme at various conditions. Asha *et al.* [23] and Prabhu *et al.* [24] reported that 37 °C is optimum for pullulanase production by B. halodurans & Klebsiella aerogenes NCIM 2239 respectively. The pH 7 was noticed as optimum for pullulanase production by Clostridium thermo sulfur genes SVM17&Klebsilla aerogenes NCIM 2239 [25, 24]. The secondary metabolites productions were purely microbial-specific. Optimizations of various conditions are critical to achieve maximum production of the enzyme. 2D contour plots were drawn by using equation 4. All the contours showed circles or elliptical indicates that there is the absence of interaction or minimal interaction among selected parameters. **Fig. 2A** shows the interaction influence of temperature with yeast extract; this graph showed that the contours are circular, indicating no interaction between temperature and yeast extract. The interaction between rpm and pH is shown in **Fig. 2B** from this, it was observed that pH is independent of rpm. In **Fig. 2C** the contours are slightly elliptical and inclined towards pH, which implies that $MnSO_4$ concentration slightly influences the medium pH. **Fig. 2D** & **2E** represent the interaction of soluble starch with yeast extract and $MnSO_4$; from these plots, it was noticed that soluble starch concentration is not influenced by yeast extract and mineral salt. Similarly, no interaction was noticed between the yeast extract and $MnSO_4$ **Fig. 2F.**

A numerical method was used to solve equation 4 to predict the most favorable environments for pullulanase production. The optimum conditions obtained are declared as temperature 30. 4 °C, pH 7.1, agitation speed 215 rpm, soluble starch concentration 12.71g/L, yeast extract concentration 5.0 g/L, and MnSO4 concentration 6.3 mM. At the conditions, the predicted pullulanase activity was 16.83 U/ml; however, 17.10 U/ml of enzyme activity was obtained by performing the experiments.

The experimental validation values were found that nearer to the software predicted values and hence, the model was successfully validated. Overall, 1.2 fold enhancement of enzyme activity was achieved by means of RSM. The final yield of pullulanase by Bacillus cereus KKSJ 1981 after RSM optimization is much higher than other Bacillus cereus species reported by Nair *et al.* 22 and Waleed *et al.* [16]. Statistical methods have also been effectively using to optimize the nutrients levels in submerged and solid-state fermentations for pullulanase production. Hii *et al*. used CCD as a tool for the optimization of pullulanase production using R.

planticola DSMZ 4617 [26]. By sequential optimization methods *viz* PBD-CCD, 3 folds of pullulanase production was enhanced by Bacillus subtilis MF467279 [27].

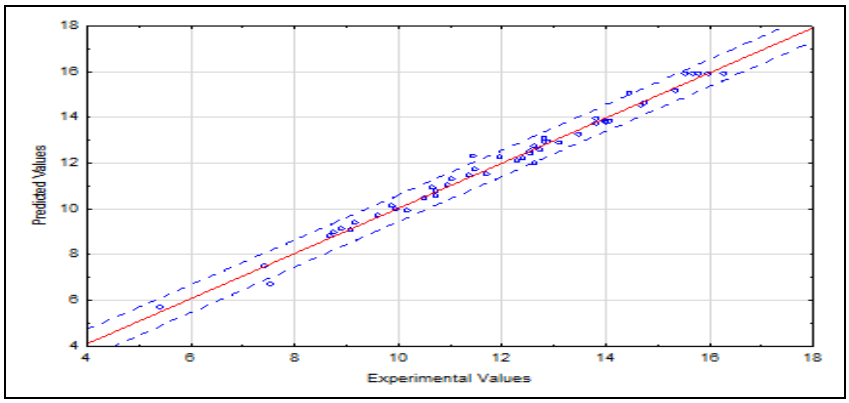

FIG. 1: CORRELATION BETWEEN EXPERIMENTAL AND PREDICTED PULLULANASE ACTIVITY BY B. CEREUS KKSJ 1981

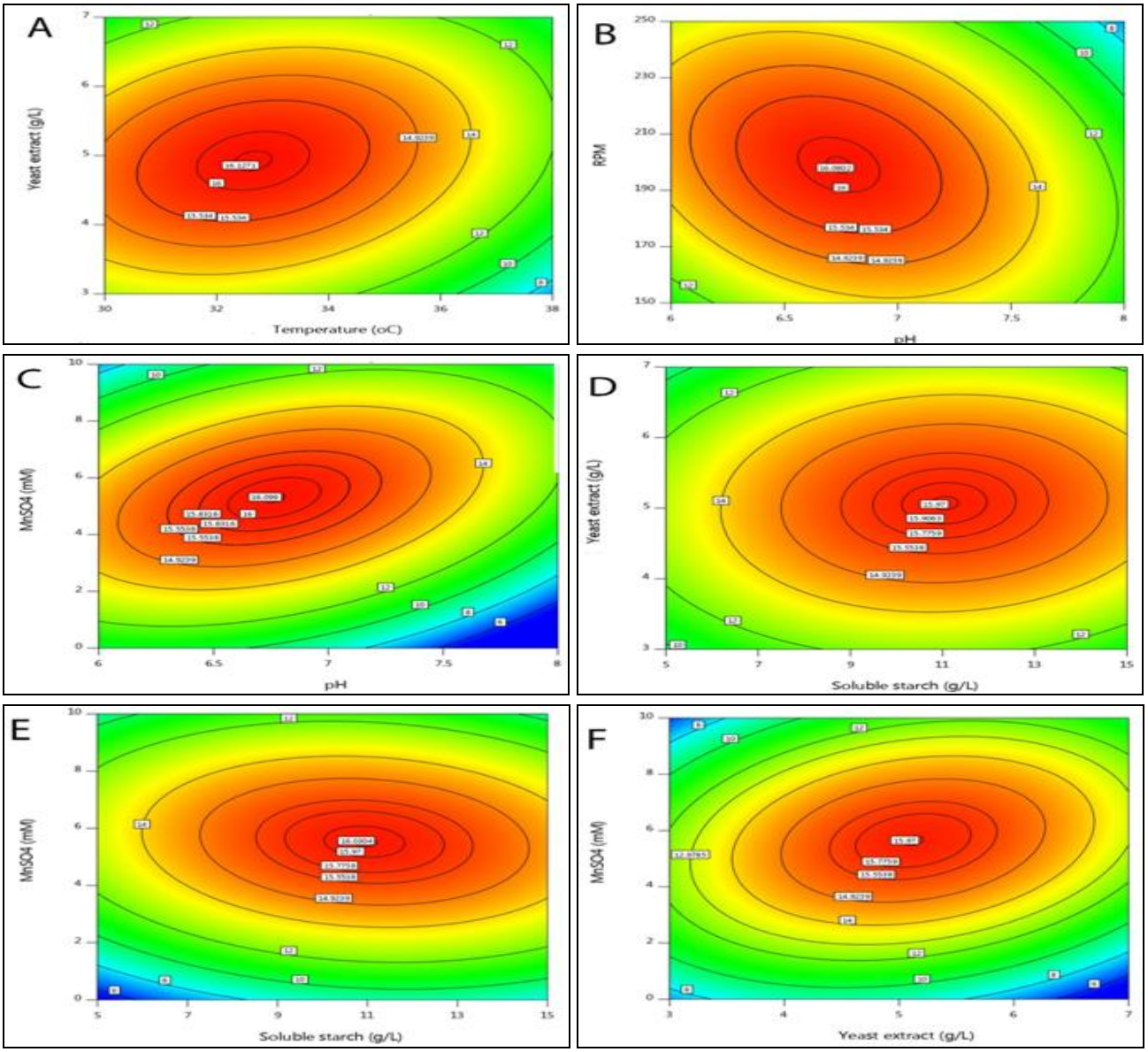

FIG. 2: INTERACTION INFLUENCE OF SELECTED PARAMETERS ON PULLULANASE PRODUCTION BY B. CEREUS KKSJ 1981. A) TEMPERATURE VS YEAST EXTRACT, B) P^H VS RPM, C) P^H VS $MNSO_4$, D) SOLUBLE STARCH VS YEAST EXTRACT E) SOLUBLE STARCH VS $MNSO_4$ AND F) YEAST EXTRACT VS $MNSO_4$

TABLE 1: CCD MATRIX ALONG WITH EXPERIMENTAL AND PREDICTED PULLULANASE ACTIVITY BY B. CEREUS KKSJ 1981. VALUES IN THE BRACKETS ARE CODED VALUES

S. no	Temperature (°C) (X1)	pH (X2)	Agitation speed (RPM) (X3)	Soluble starch (g/L) (X4)	Yeast extract (g/L) (X5)	$MnSO_4$(mM) (X6)	Pullulanase activity (U/ml)		
							Experimental	Predicted	Error
1	32(-1)	6.5(-1)	175(-1)	7.5(-1)	4(-1)	2.5(-1)	10.75	10.55	0.20
2	32(-1)	6.5(-1)	175(-1)	7.5(-1)	6(1)	7.5(1)	10.19	9.92	0.27
3	32(-1)	6.5(-1)	175(-1)	12.5(1)	4(-1)	7.5(1)	11.43	12.30	-0.87
4	32(-1)	6.5(-1)	175(-1)	12.5(1)	6(1)	2.5(-1)	12.42	12.21	0.21
5	32(-1)	6.5(-1)	225(1)	7.5(-1)	4(-1)	7.5(1)	12.3	12.09	0.21
6	32(-1)	6.5(-1)	225(1)	7.5(-1)	6(1)	2.5(-1)	10.96	11.02	-0.06
7	32(-1)	6.5(-1)	225(1)	12.5(1)	4(-1)	2.5(-1)	13.50	13.26	0.24
8	32(-1)	6.5(-1)	225(1)	12.5(1)	6(1)	7.5(1)	15.35	15.14	0.21
9	32(-1)	7.5(1)	175(-1)	7.5(-1)	4(-1)	7.5(1)	12.75	12.54	0.21
10	32(-1)	7.5(1)	175(-1)	7.5(-1)	6(1)	2.5(-1)	7.43	7.48	-0.05
11	32(-1)	7.5(1)	175(-1)	12.5(1)	4(-1)	2.5(-1)	13.12	12.90	0.22
12	32(-1)	7.5(1)	175(-1)	12.5(1)	6(1)	7.5(1)	14.01	13.79	0.22
13	32(-1)	7.5(1)	225(1)	7.5(-1)	4(-1)	2.5(-1)	9.64	9.69	-0.05
14	32(-1)	7.5(1)	225(1)	7.5(-1)	6(1)	7.5(1)	13.82	13.93	-0.11
15	32(-1)	7.5(1)	225(1)	12.5(1)	4(-1)	7.5(1)	14.09	13.84	0.25
16	32(-1)	7.5(1)	225(1)	12.5(1)	6(1)	2.5(-1)	9.96	9.98	-0.02
17	36(1)	6.5(-1)	175(-1)	7.5(-1)	4(-1)	7.5(1)	10.75	10.76	-0.01
18	36(1)	6.5(-1)	175(-1)	7.5(-1)	6(1)	2.5(-1)	11.95	12.22	-0.27
19	36(1)	6.5(-1)	175(-1)	12.5(1)	4(-1)	2.5(-1)	13.82	13.73	0.09
20	36(1)	6.5(-1)	175(-1)	12.5(1)	6(1)	7.5(1)	12.56	12.53	0.03
21	36(1)	6.5(-1)	225(1)	7.5(-1)	4(-1)	2.5(-1)	10.68	10.92	-0.24
22	36(1)	6.5(-1)	225(1)	7.5(-1)	6(1)	7.5(1)	12.82	13.07	-0.25
23	36(1)	6.5(-1)	225(1)	12.5(1)	4(-1)	7.5(1)	9.09	9.06	0.03
24	36(1)	6.5(-1)	225(1)	12.5(1)	6(1)	2.5(-1)	11.50	11.73	-0.23
25	36(1)	7.5(1)	175(-1)	7.5(-1)	4(-1)	2.5(-1)	9.89	10.13	-0.24
26	36(1)	7.5(1)	175(-1)	7.5(-1)	6(1)	7.5(1)	11.03	11.29	-0.26
27	36(1)	7.5(1)	175(-1)	12.5(1)	4(-1)	7.5(1)	10.5	10.46	0.04
28	36(1)	7.5(1)	175(-1)	12.5(1)	6(1)	2.5(-1)	8.9	9.14	-0.24
29	36(1)	7.5(1)	225(1)	7.5(-1)	4(-1)	7.5(1)	8.76	8.99	-0.23
30	36(1)	7.5(1)	225(1)	7.5(-1)	6(1)	2.5(-1)	7.52	6.68	0.84
31	36(1)	7.5(1)	225(1)	12.5(1)	4(-1)	2.5(-1)	5.43	5.72	-0.29
32	36(1)	7.5(1)	225(1)	12.5(1)	6(1)	7.5(1)	9.16	9.38	-0.22
33	30(-2)	7(0)	200(0)	10(0)	5(0)	5(0)	14.48	15.06	-0.58
34	38(2)	7(0)	200(0)	10(0)	5(0)	5(0)	12.63	11.95	0.68
35	34(0)	6(-2)	200(0)	10(0)	5(0)	5(0)	14.69	14.51	0.18
36	34(0)	8(2)	200(0)	10(0)	5(0)	5(0)	11.36	11.44	-0.08
37	34(0)	7(0)	150(-2)	10(0)	5(0)	5(0)	14.02	13.84	0.18
38	34(0)	7(0)	250(2)	10(0)	5(0)	5(0)	12.82	12.91	-0.09
39	34(0)	7(0)	200(0)	5(-2)	5(0)	5(0)	12.89	12.91	-0.02
40	34(0)	7(0)	200(0)	15(2)	5(0)	5(0)	14.76	14.64	0.12
41	34(0)	7(0)	200(0)	10(0)	3(-2)	5(0)	12.56	12.39	0.17
42	34(0)	7(0)	200(0)	10(0)	7(2)	5(0)	12.63	12.71	-0.08
43	34(0)	7(0)	200(0)	10(0)	5(0)	0(-2)	8.69	8.79	-0.10
44	34(0)	7(0)	200(0)	10(0)	5(0)	10(2)	11.69	11.50	0.19
45	34(0)	7(0)	200(0)	10(0)	5(0)	5(0)	16.29	15.88	0.41
46	34(0)	7(0)	200(0)	10(0)	5(0)	5(0)	15.82	15.88	-0.06
47	34(0)	7(0)	200(0)	10(0)	5(0)	5(0)	15.56	15.88	-0.32
48	34(0)	7(0)	200(0)	10(0)	5(0)	5(0)	15.98	15.88	0.10
49	34(0)	7(0)	200(0)	10(0)	5(0)	5(0)	15.68	15.88	-0.20
50	34(0)	7(0)	200(0)	10(0)	5(0)	5(0)	15.79	15.88	-0.09

TABLE 2: EFFECT OF COEFFICIENTS AND ANALYSIS OF VARIANCE; SS = SUM OF SQUARES; DF= DEGREE OF FREEDOM; MS= MEAN SQUARE; * INDICATES INSIGNIFICANT TERMS

Factor	Effect	Coefficients	SS	df	MS	F-value	t-value	p-value
Intercept	15.8848	15.8848					100.719	0.000
X1	-1.5530	-0.7765	24.1181	1	24.1181	135.7457	-11.651	0.000

X2	-1.5360	-0.7680	23.5930	1	23.5930	132.7901	-11.523	0.000
X3	-0.4660	-0.2330	2.1716	1	2.1716	12.2224	-3.496	0.002
X4	0.8670	0.4335	7.5169	1	7.5169	42.3079	6.504	0.000
X5*	0.1610	0.0805	0.2592	1	0.2592	1.4589	1.208	0.240
X6	1.3570	0.6785	18.4145	1	18.4145	103.6437	10.181	0.000
X1*X1	-1.1885	-0.5943	11.3003	1	11.3003	63.6021	-7.975	0.000
X2*X2	-1.4535	-0.7268	16.9013	1	16.9013	95.1269	-9.753	0.000
X3*X3	-1.2560	-0.6280	12.6203	1	12.6203	71.0317	-8.428	0.000
X4*X4	-1.0535	-0.5268	8.8789	1	8.8789	49.9738	-7.069	0.000
X5 *X5	-1.6685	-0.8343	22.2711	1	22.2711	125.3504	-11.196	0.000
X6*X6	-2.8710	-1.4355	65.9411	1	65.9411	371.1416	-19.265	0.000
X1*X2	-1.2438	-0.6219	12.3753	1	12.3753	69.6529	-8.346	0.000
X1*X3	-1.3725	-0.6863	15.0701	1	15.0701	84.8199	-9.210	0.000
X1*X4	-1.1550	-0.5775	10.6722	1	10.6722	60.0672	-7.750	0.000
X1*X5	0.6225	0.3113	3.1001	1	3.1001	17.4483	4.177	0.000
X1*X6	-0.6987	-0.3494	3.9060	1	3.9060	21.9845	-4.689	0.000
X2*X3	-0.7238	-0.3619	4.1905	1	4.1905	23.5858	-4.857	0.000
X2*X4*	-0.3088	-0.1544	0.7626	1	0.7626	4.2923	-2.072	0.050
X2*X5	-0.4863	-0.2431	1.8915	1	1.8915	10.6461	-3.263	0.004
X2*X6	1.4575	0.7288	16.9945	1	16.9945	95.6512	9.780	0.000
X3*X4	-0.6525	-0.3263	3.4061	1	3.4061	19.1705	-4.378	0.000
X3*X5	0.7575	0.3788	4.5905	1	4.5905	25.8368	5.083	0.000
X3*X6	0.7038	0.3519	3.9621	1	3.9621	22.3003	4.722	0.000
X4*X5*	0.1675	0.0838	0.2245	1	0.2245	1.2633	1.124	0.273
X4*X6	-0.3788	-0.1894	1.1476	1	1.1476	6.4592	-2.541	0.019
X5*X6	0.9662	0.4831	7.4691	1	7.4691	42.0390	6.484	0.000
Error			3.9088	22	0.1777			
Total SS			307.6575	49				

CONCLUSION: Pullulanase is an important high demanding industrial glycoside hydrolase that is using to produce glucose using pullulan and other similar oligosaccharides.

Economically and industrially valuable pullulanase production is the main Constraint. In the present study, pullulanase production using B. cereus KKSJ 1981 was enhanced by optimizing various processes and nutritional conditions.

In this investigation, the yield was enhanced by altering the conditions of the existing parameters without the addition or replacement of nutrients. By using RSM with 6 parameters each variable on 5 levels were optimized by 50 experiments. The analysis of results indicated that the accuracy and precision of experiments were conducted. Overall this study depicts 120 % of pullulanase production by B. cereus KKSJ 1981 was enhanced by optimizing the important parameters by statistical methods.

REFERENCES:

1. Duan X and Wu J: Enhancing the secretion 1 efficiency and thermostability of Bacillus deramificans pullulanase mutant D437H/ D503Y by N-terminal domain truncation. Applied and Environmental Microbiology 2015; 6: 1926-31.
2. Douglas CW and Mitchinson C: Enzymes involved in the processing of starch to sugars. Trends in Biotechnology 1997; 15: 349-52.
3. Norman B: A novel debranching enzyme for application in the glucose syrup industry. Starch 1982; 34: 340-46.
4. Adrio J and Demain A: Microbial enzymes: tools for biotechnological processes. Bio Molecules 2014; 4: 117-39.
5. Nishank P, Sweta G, Moinuddin A, Zaved AK and Suneetha V: Production of economically important products by the use of pullulanase enzyme. International Journal of Science Innovations and Discoveries 2012; 2: 266-73.
6. Hii LS, Rosfarizan M, Ling TC and Ariff AB: Statistical optimization of pullulanase production by raoultella planticola dsmz 4617 using sago starch as carbon and peptone as nitrogen sources. Food and Bioprocess Technology 2012; 5: 729-37.
7. Takasaki Y: Purifications and enzymatic properties of □-amylase and pullulanase from bacillus cereus var rnycoidest. Agricultural and Biological Chemistry 1976; 40: 1523-30.
8. Takasaki Y: Pullulanase-amylase complex enzyme from bacillus subtilis. Agricultural and Biological Chemistry 1987; 51: 9-16.
9. Sathish T, Lakshmi GS, Rao Ch S, Brahmaiah P and Prakasham RS: Mixture design as first step for improved glutaminase production in solid-state fermentation by isolated Bacillus sp. RSP-GLU. Letters in Applied Microbiology 2008; 47: 256-62.
10. Sathish T and Prakasham RS: Modeling the effect of l-glutamine, aeration and agitation regimes on production of l-glutaminase in stirred tank reactor using bacillus subtilis

RSP-GLU. International Journal of Industrial Biotechnology 2011; 1: 27-33.
11. Laxmi GS, Sathish T, Subba Rao Ch, Brahmaiah P, Hymavathi M and Prakasham RS: Palm fiber as novel substrate for enhanced xylanase production by isolated aspergillus sp. RSP-6. Current Trends of Biotechnology and Pharmacy 2008; 2: 447-55.
12. Hymavathi M, Sathish T, Brahmaiah P and Prakasham R S: Impact of carbon and nitrogen sources on L-asparaginase production by isolated Bacillus circulans (MTCC 8574): Application of saturated Plackett–Burman design. Chemical and Biochemical Engineering Quarterly 2010; 24: 473-80.
13. Hymavathi M, Sathish T, Subba Rao Ch and Prakasham RS: Enhancement of l-asparaginase production by isolated bacillus circulans (mtcc 8574) using response surface methodology. Applied Biochemistry and Biotechnology 2009; 159; 191-98.
14. Sathish T and Prakasham RS: Enrichment of glutaminase production by Bacillus subtilis RSP-GLU in submerged cultivation based on neural network–Genetic algorithm approach. Journal of Chemical Technology and Biotechnology 2010; 85: 50-58.
15. Chiranjeevi PV, Pandian MR and Sathish T: Integration of artificial neural network modeling and genetic algorithm approach for enrichment of lactase production in solid state fermentation by pleurotus ostreatus. Bio Resources 2014; 9: 2459-70.
16. Waleed M, Faiza AA and Nizar I: Production optimization of pullulanase enzyme produced by bacillus cereus isolated from syrian sources. International Food Research Journal 2015; 22: 1824-30.
17. Ara K, Saeki K, Igarashi K, Takaiwa M, Uemura T, Hagihara H, Kawai S and Ito S: Purification and characterization of an alkaline amylopullulanase with both alpha-1,4 and alpha-1,6 hydrolytic activity from alkalophilic Bacillus sp KSM-1378. Biochimica Biophysica Acta 1995; 1243: 315-24.
18. Miller GL: Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 1959; 31: 426-28.
19. Chiranjeevi PV, Pandian MR and Sathish T: Enhancement of Lactase production from pleurotus ostreatus pvcrsp-7 by altering the nutritional conditions using response surface methodology. Bioresource Technology 2014; 9: 4212-25.
20. Sathish T, Uppuluri KB, Chari PVB and Kezia D: Sequential optimization methods for augmentation of marine enzymes production in solid-state fermentation: l-glutaminase production a case study. in (eds) kim se-k and toldrá f. Advances in Food and Nutrition Research Burlington Academic Press 2016; 78: 95-114.
21. Sathish T, Kezia D, Bramhachari PV and Prakasham RS: Multi-objective based superimposed optimization method for enhancement of L-glutaminase production by bacillus subtilis RSP-GLU. Karbala International Journal of Modern Science 2018; 4: 50-60.
22. Nair SU, Singhal RS and Kamat MY: Enhanced production of thermostable pullulanase type 1 using bacillus cereus fdta 13 and its mutant. Food Technology and Biotechnology 2006; 2: 275-82.
23. Asha R, Niyonzima FN and Sunil SM: Purification and properties of pullulanase from bacillus halodurans. International Research Journal of Biological Sciences 2013; 2(3): 35-43.
24. Prabhu N, Maheswari RU, Singh MVP, Karunakaran S, Kaliappan C and Gajendran T: Production and purification of extracellular pullulanase by klebsilla aerogenes NCIM 2239. African Journal of Biotechnology 2018; 17: 486-94.
25. Mrudula S, Reddy G and Seenayya G: Screening of Various Raw Starches on production of thermostable amylo pullulanase by clostridium thermosulfurogenes SVM17. World Applied Sciences Journal 2011; 15: 996-1001.
26. Hii SL, Tan JS, Ling TC and Ariff AB: Pullulanase: role in starch hydrolysis and potential industrial applications. Enzyme Research 2012; 1-14.
27. Aty AAE, Shehata AN and Shaheen TI: Production and sequential optimization of Bacillus subtilis MF467279 pullulanase by statistical experimental designs and evaluation of its desizing efficiency. Biocatalysis and Agricultural Biotechnology 2018; 14: 375-85.

How to cite this article:
Jyothi JS, Emandi H and Kumar KK: Advancement of glycoside hydrolase production from bacillus cereus kksj 1981 by statistical method of optimization. Int J Pharm Sci & Res 2022; 13(4): 1624-31. doi: 10.13040/IJPSR.0975-8232.13(4).1624-31.