Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

OPTIMIZATION OF MINERAL SUPPLEMENTS FOR THE PRODUCTION OF ALPHA AMYLASE FROM RICE BRAN USING *Aspergillus Oryzae* THROUGH SUBMERGED FERMENTATION

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Received – April 30, 2020; Revision – June 25, 2020; Accepted – August 06, 2020

Available Online – August 25, 2020

DOI: [http://dx.doi.org/10.18006/2020.8\(4\).456.468](http://dx.doi.org/10.18006/2020.8(4).456.468)

KEYWORDS

Alpha amylase

Rice Bran

Aspergillus oryzae

Submerged fermentation

Mineral supplements
Optimization

Plackett-Burman Design

ABSTRACT

This study is aimed to investigate the optimum level of mineral supplements for the maximum production of fermentative α -amylase by using cheap substrate rice bran. The fungal strain *Aspergillus oryzae* MTCC-8624 is used to investigate the α -amylase production capability. The culture is maintained on potato dextrose agar (PDA) and sub-cultured at an interval of three months. The culture is initially screened for amylase production by starch agar plate assay on standard media. The report concludes that the optimization of mineral supplements for the fermentative α -amylase production and its suitability for a large-scale production using cheap and easily available substrate rice bran. The significant media components identified by Plackett-Burman design are $\text{KH}_2\text{PO}_4 = 2.69 \text{ g/L}$; $\text{MgSO}_4 = 1.70 \text{ g/L}$; $\text{CaCl}_2 = 0.53 \text{ g/L}$; $\text{FeSO}_4 = 0.5 \text{ g/L}$ and $(\text{NH}_4)_2\text{SO}_4 = 4.95 \text{ g/L}$ for Rice Bran

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
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1 Introduction

Application of amylase enzyme is versatile and its demand in the various industries such as food, fermentation, textile, paper, detergent, pharmaceutical, and sugar are paramount (Asrat & Girma, 2018). Further, the economic and technological significance forced to pay great attention to the production of amylase enzyme. Its role in the hydrolysis of α -D-(1,4) glycosidic linkage in starch components and related polysaccharides to release maltose and disaccharide is the main reason for the continuous intensive research (Shah et al., 2014; Avwioroko & Tonukari, 2015; Bharathiraja et al., 2016; Subash et al., 2017; Asrat & Girma, 2018). Selection of substrate, the potential of microorganism, methods of cultivation, cell growth, nutrient requirement, metal ions, pH, temperature, incubation period and thermostability are the main factors which directly influence the rate of amylase production (Pankaj et al., 2015). The utilization of agriculture waste is a promising one for the sustainable production of amylase. Cheaper cost and abundant availability are the supporting thoughts for the above statement (Bharathiraja et al., 2016). Due to the increasing demand for alpha-amylase in various industries, there is enormous interest in developing enzymes with better properties such as raw starch degrading amylases suitable for industrial applications and there cost-effective production techniques (Konsula & Liakopoulou-Kyriakides, 2004; Shivaramakrishnam et al., 2006). The selection of appropriate carbon and nitrogen sources or other nutrients is one of the most critical stages in the development of an efficient and economic process (Jiby et al., 2016). Further, Asrat & Girma (2018) investigated the potential of newly isolated strain *Aspergillus niger* FAB-211 using fruit peel wastes for the production of amylase. The report infers the maximum amylase activity of 1,241 U/ml at a pH of 6.0 and temperature at 45°. Further, Elmansy et al. (2018) studied the effect of various fermentation conditions on α -amylase production through shake-flask culture *Bacillus* sp. NRC22017 and the maximum yield of α -amylase is inferred to be 15.15 $\pm$ 0.47 U/ml at a pH of 6.0 with an inoculum size of 500 μ l at 45 °C and anaerobic incubation period of 72 h. Physical factors that are affecting the production of the α -amylase from a newly isolated *Bacillus* sp. M10 strain. lus sp. M-10 namely temperature, pH, aeration, inoculum size, and inoculum age are optimized by Demirkan et al. (2017). Among the important factors, 37°C temperature, pH 7.0, 150 rpm for aeration, 2.5% (v/v) inoculation size and 2 days for inoculation age are found to be the optimum rate for maximum α -amylase production of 30 U/mL at the hour of 48. Pathania et al., (2017) optimized the process variables included pH, temperature, inoculum size, incubation days and substrate concentration for production of amylase by *B. amyloliquifaciens* SH8 using response surface methodology and achieved the maximum amylase activity of 16.07 IU/ml at the optimum condition of pH 5, temperature 45°C, inoculum size 5%, incubation day of 5, and substrate concentration of 0.60%. Similarly, Jiby et al. (2016) tested the activity of α -

amylase from *A. niger* utilizing coconut water, tapioca water, rice water, and white yam. The maximum activity of α -amylase is recorded as 0.29 x 10⁻³ μ moles/sec after 7 days of submerged fermentation on white Yam water at pH 7.0 and 28 °C. Among the three medium, rice water recorded as second (0.09 x 10⁻³ μ moles/sec) and tapioca water (0.06 x 10⁻³ μ moles/sec) as third position. Vimal et al. (2015) identified as *B.amyloliquefaciens* KCP2 using 16S rDNA gene sequencing data yielded the maximum amylase activity of 63.12 U/gds. Similarly, Ahmed et al. (2015) optimized the experimental parameters for alpha-amylase production by *A. fumigatus* in submerged fermentation, these researchers used sunflower waste, cotton stalk, rice husk, date syrup, and molasses are tested as carbon source. The maximum production of α -amylase is found to be 7.01 U/mL by *A. fumigates* are 72 h of incubation period at initial pH 5.5, temperature 35° C, inoculum size of 6x10⁶ conidia in 50 mL of culture medium and agitation rate of 150 rev/min. Shah et al. (2014) isolates a total of 17 fungal cultures from soil samples. Out of which IP31, *A.oryzae*, exhibited good amyolytic enzymes production. After parametric optimization maximum amyolytic activity was observed, when pH of the mineral salt medium was 7.0, incubation temperature of 45°C, after incubation of 72 hrs by using 50 mL of starchy wastewater as sole carbon source with 5 discs of *A. oryzae*. After studying the kinetic properties of α -amylase, its maximum activity was found at pH 6, the temperature of 50°C, with 1.5% of substrate concentration. The Vmax and Km value was 37.037 IU/mL and 1.4 mg/mL respectively for α -endocellulase. Submerged fermentation has been defined as fermentation in the presence of excess water. Almost all the large-scale enzyme-producing facilities are using the proven technology of submerged fermentation due to better monitoring and ease of handling (Singhania, 2011). It is the preferred technology for industrial enzyme production due to ease of handling at large-scale when compared to SSF. Conventional fermenters for submerged fermentation technology are properly advanced and offer online manage over several parameters together with pH, temperature, dissolved oxygen, and froth formation with easy mass transfer and heat removal. These benefits make submerged fermentation technology superior to solid-state. It is widely conformist for industrial metabolites production. The choice of an appropriate condition for the substrate is a key aspect of submerged fermentation. It depends on various factors, including cost, availability, particle size, and moisture content. This screening process involves, avoiding the formation of several by-products and the substrate of choice being the one that not only serves as the best nutrient source but also acts as the best support for cell growth (Rajagopalan & Krishnan, 2008). The medium in submerged fermentation is liquid, which remains in contact with the microorganisms (Singhania et al., 2015). With this few introductions, this investigation is aimed to optimize the mineral supplements for the production of alpha-amylase from *A.oryzae* using rice bran through submerged fermentation.

2 Materials and Methods

2.1 Microorganism and its Maintenance

The strain of *A.oryzae* MTCC-8624 used in this investigation was obtained from MTCC, Institute of Microbial Technology (IMTECH), Chandigarh, India. The culture is maintained on potato dextrose agar (PDA) and sub-cultured at an interval of three months (Ellaiah et al., 2002). The culture is initially screened for amylase production by starch agar plate assay on standard media. The inoculated plates, containing media, supplemented with starch is stained with Gram's iodine reagent, after 72 hr of incubation. The plates are then flooded with iodine solution for 15 minutes and washed with warm water to remove the excess colour (Mabel et al., 2006).

2.2 Inoculum Preparation

The strain is sub-cultured on PDA slants and incubated for 72 hr at a temperature of 25°C. After the incubation period, the spore suspensions are prepared by adding 10 ml of sterile water to the PDA slant containing sporulated slant cultures. The spores on the surface of the medium were dislodged using inoculation needle under aseptic conditions. The spore suspensions are filtered using sterile muslin cloth into sterile flasks. The filtered spore suspension is transferred into 250 ml Erlenmeyer flask containing 100 ml of potato dextrose broth and incubated for three days at 25°C. Appropriate volumes of inoculums (% v/v) are used to inoculate the production medium (Tanyildizi et al., 2005).

2.3 Fermentation Medium

In this investigation, rice bran is utilized as a substrate. It is collected from nearby areas of Chidambaram, Tamil Nadu, India. Since this agricultural by-product is not available in completely dried form, it is necessary to dry this substrate prior to use them in the fermentation process. In the present study, the substrate is dried by keeping them in the oven at 80°C for 12 hr. After drying, the substrates are powdered in a laboratory grinder and sieved using a 40mm sieve. An adequate amount of the powdered substrate is mixed with 100ml of the corresponding mineral salt media in a 250ml Erlenmeyer flask. After adjusting the pH, the contents of the flask were sterilized in an autoclave at 121°C and 15 psi pressure for 15 minutes. Appropriate volumes of inoculums are added to the flasks after cooling it down to room temperature. All the experiments for media optimization were carried out with a substrate concentration of 20g/L, inoculum size of 5% (v/v), and fermentation time of 72 hr. The pH and temperature are maintained at 5 and 25°C respectively (Sarraf et al., 1993).

2.4 Extraction of amylase from the fermentation medium

At the end of the fermentation period, contents of the flask are filtered using a Whatman No.44 filter paper followed by filtration

through a muslin cloth. The filtrate is then centrifuged at 10,000 rpm for 10 min and the supernatant was used as the source of enzyme for assay (Ahuja et al., 2004).

2.5 Assay of amylase

Assay system for amylase activity is carried out by measuring the amount of reducing sugar according to DNS method (Fogarty, 1983). Amylase activity is determined by incubating a mixture of 1 ml of the aliquot of each enzyme source and 1% soluble starch dissolved in 0.1 M phosphate buffer, at pH 7, at 55°C for 15 min. The reaction is arrested by adding 1 ml of 3, 5 DNS Acid followed by 10 minutes boiling. The final volume is made up of 12 ml with distilled water and the reducing sugar released was measured at 540 nm. Reducing sugar (Glucose or maltose) concentration is determined from a standard curve under the same condition using glucose. Figure 1 is used to represent the calibration curve for glucose concentration using Bio-spectrophotometer (Ellaiah et al., 2002; Mabel et al., 2006).

Figure 1 Calibration chart for glucose concentration using Bio-spectrophotometer

3 Results and Discussion

3.1 Screening of media components for *Aspergillus oryzae*

Fifteen variables were screened in twenty trials, each variable being a media constituent. The medium constituents used for *A. oryzae* MTCC-8624 are KH_2PO_4 (A), $(\text{NH}_4)_2\text{SO}_4$ (B), KCl(C), MgSO_4 (D), CaCl_2 (E), Urea(F), FeSO_4 (G), ZnCl_2 (H), NH_4NO_3 (J), NaCl(K), MnSO_4 (L), K_2HPO_4 (M), CoCl_2 (N), NaNO_3 (O) and FeCl_3 (P). Two level design (-1 indicates the lower level and +1 indicates the higher level) are generated for fifteen variables with one replicate. The coded and actual values of the variables are shown in Table 1. Table 2 shows Plackett-Burman design for conducting 20 trials and the corresponding amylase production was noted. The effect of the variables and their significance on

Table 1 Variables screened in Plackett–Burman design for amylase activity using *A. oryzae* MTCC-8624

Variables	Low level (–) values (g/L)	High level (+) values (g/L)
KH ₂ PO ₄	1	5
MgSO ₄	1	3
Peptone	1	10
Yeast extract	2	8
FeSO ₄	2	5
CaCl ₂	0.2	2
NaCl	1	3
NaNO ₃	0.5	1.5
Urea	0.5	5
MnSO ₄	0.1	0.9
(NH ₄) ₂ SO ₄	1	5
CoCl	0.005	0.5
ZnSO ₄	1	5
ZnCl ₂	0.1	1
KCl	1	7

Table 2 Plackett–Burman experimental design matrix for screening of important variables for *A. oryzae* MTCC-8624 utilizing rice bran

Runs	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Amylase Activity (U/ml) (mol H ₂ /mol glucose)	
																Exp.	Pred.
1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	9.60	9.647
2	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	9.87	9.837
3	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	8.93	8.877
4	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	4.62	4.487
5	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	9.15	9.103
6	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	7.98	7.669
7	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	4.62	5.011
8	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	9.65	10.041
9	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	4.63	4.339
10	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	7.54	7.507
11	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	5.97	6.003
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	9.03	9.241
13	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	7.98	8.271
14	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	9.80	9.489
15	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	10.17	9.779
16	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	8.73	8.783
17	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	8.43	8.219
18	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	9.79	9.843
19	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	6.72	6.931
20	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	6.49	6.623

Table 3 Estimated effects and coefficients of the Plackett–Burman design for *A. oryzae* MTCC-8624 utilizing rice bran

Terms	Effect	Coeffi.	SE Coeffi.	T	P
Constant		7.985	0.1122	71.19	0.000
A	-2.046	-1.023	0.1122	-9.12	0.001
B	1.394	0.697	0.1122	6.21	0.003
C	0.552	0.276	0.1122	2.46	0.070
D	-1.850	-0.925	0.1122	-8.25	0.001
E	-0.870	-0.435	0.1122	-3.88	0.018
F	-0.310	-0.155	0.1122	-1.38	0.239
G	1.020	0.510	0.1122	4.55	0.010
H	-0.206	-0.103	0.1122	-0.92	0.410
J	0.416	0.208	0.1122	1.85	0.137
K	0.182	0.091	0.1122	0.81	0.463
L	0.590	0.295	0.1122	2.63	0.058
M	-0.484	-0.242	0.1122	-2.16	0.097
N	-0.344	-0.172	0.1122	-1.53	0.200
O	0.050	0.025	0.1122	0.22	0.835
P	-0.606	-0.303	0.1122	-2.70	0.054

Figure 2 Parity plot between the experimental and predicted values of important variables for *A. oryzae* MTCC-8624 utilizing rice bran

the enzyme activity is found by analyzing the responses statistically. Variables with P value <0.05 are significant. The effect estimates of amylase activity from the result of Plackett–Burman design for rice bran utilizing *A. oryzae* MTCC-8624 was represented in Table 3. The Parity plot between the

experimental and predicted values is shown in Figure 2. Figure 3 shows the Pareto chart for rice bran. From the Pareto chart KH_2PO_4 , $(\text{NH}_4)_2\text{SO}_4$, MgSO_4 , CaCl_2 and FeSO_4 were found to be significant. It is reached based on the p values noted in Table 3.

Figure 3 Pareto chart showing the effect of media components on amylase production using *A. oryzae* MTCC-8624 utilizing rice branTable 4 Actual and coded values employed in CCD for media optimization of *A. oryzae* MTCC-8624 utilizing rice bran as substrate

Variables	Symbols	Coded levels				
		-2.38	-1	0	+1	+2.38
KH ₂ PO ₄ (g/L)	A	1	2	3	4	5
(NH ₄) ₂ SO ₄ (g/L)	B	1	3	5	7	9
MgSO ₄ (g/L)	C	0.5	1	1.5	2	2.5
CaCl ₂ , (g/L)	D	0.1	0.3	0.5	0.7	0.9
FeSO ₄ (g/L)	E	0.1	0.3	0.5	0.7	0.9

3.2 Optimization of media composition of *Aspergillus oryzae* MTCC-8624 using CCD for rice bran

The optimum levels of significant variables obtained from the Plackett-Burman design for *A. oryzae* MTCC-8624 is determined by the central composite design of RSM. Table 1 gives the detail of the actual and coded values employed in the design for the substrate rice bran. The 52 run design matrixes using the five independent variables with the experimental and predicted responses are shown in Table 2. The second-order polynomial Equation 2 for amylase production (Y) for *A. oryzae* MTCC-8624 utilizing rice bran as substrate was found as follows:

$$\begin{aligned}
 Y = & 13.5532 + 0.0420755A - 0.00609608B + 0.248739C \\
 & + 0.273624D - 0.166398E \\
 & - 0.648658A^2 - 0.241188B^2 \\
 & - 0.596509C^2 - 0.341067D^2 \\
 & - 0.500166E^2 - 0.279688AB \\
 & + 0.199062AC - 0.212813AD \\
 & + 0.300938AE + 0.369063BC \\
 & + 0.134063BD - 0.154063BE \\
 & - 0.0209375CD - 0.170313CE \\
 & + 0.252188DE \quad \text{---} \rightarrow (2)
 \end{aligned}$$

Where A - KH₂PO₄; B - (NH₄)₂SO₄; C - MgSO₄; D - CaCl₂ and E - FeSO₄

The parameters estimated and the corresponding P-values are shown in Table 3. From the data the terms A, D, E, B², C², D², E², AB, AC, AE, BC, BE, and DE were found to be significant for rice bran. Here the R² values of 0.9833 indicate a good agreement between the experimental and predicted values for rice bran. The R² predicted values of 0.9353 were also in good agreement with R² adjusted values of 0.9725 correspondingly. The parity rice bran is represented in Figure 4.

For rice bran, the minimum and maximum production was 10.41 U/ml and 14.71 U/ml respectively for Run No. 25 and Run No. 49. The results obtained by CCD are analyzed by standard analysis of variance (ANOVA) are shown in Table 4. The central composite experimental design with five independent variables for media optimization of *A. oryzae* MTCC-8624 utilizing rice bran as a substrate is shown in Table 5.

The three-dimensional response surface curves constructed by the regression model are shown in Figure 5.1 to 5.10 for rice bran. The

Figure 4 Parity plot between the experimental and predicted values of important variables for *A. oryzae* MTCC-8624 utilizing rice bran

Table 5 The Central Composite experimental design with five independent variables for media optimization of *A. oryzae* MTCC-8624 utilizing rice bran as substrate

Run No	Coded Values					Amylase Activity (U/ml)	
	A	B	C	D	E	Exp.	Pred.
1	0	0	0	0	0	14.71	14.701
2	1	1	1	1	-1	12.09	12.296
3	-1	-1	-1	1	-1	13.31	13.232
4	0	0	0	0	-2.38	11.23	11.004
5	0	0	0	0	2.38	11.01	11.243
6	-1	1	-1	1	1	12.98	12.832
7	0	0	0	2.38	0	13.26	13.221
8	-1	-1	-1	1	1	13.08	12.972
9	1	-1	1	-1	1	12.92	12.916
10	2.38	0	0	0	0	12.97	12.788
11	-1	-1	1	1	1	11.21	11.286
12	0	0	0	0	0	14.65	14.701
13	0	0	0	-2.38	0	12.69	12.736
14	0	0	0	0	0	14.68	14.701
15	1	1	1	-1	-1	11.81	11.970
16	-1	1	1	1	-1	12.59	12.605
17	1	1	-1	1	-1	11.71	11.833
18	1	-1	1	1	1	12.92	12.780
19	1	1	-1	1	1	12.02	11.959

Run No	Coded Values					Amylase Activity (U/ml)	
	A	B	C	D	E	Exp.	Pred.
20	-1	1	1	-1	1	11.61	11.624
21	-1	-1	-1	-1	1	13.13	12.891
22	1	1	1	-1	1	12.64	12.430
23	-1	-1	1	-1	-1	11.25	11.134
24	1	1	-1	-1	-1	11.55	11.502
25	0	0	2.38	0	0	10.41	10.659
26	0	0	0	0	0	14.71	14.701
27	0	-2.38	0	0	0	11.58	11.763
28	0	0	-2.38	0	0	12.35	12.108
29	1	-1	-1	1	1	12.72	12.999
30	1	-1	-1	1	-1	12.28	12.356
31	0	0	0	0	0	14.71	14.701
32	-1	1	-1	1	-1	13.36	13.609
33	-1	1	1	1	1	12.01	11.818
34	-1	1	1	-1	-1	12.21	12.066
35	1	-1	1	-1	-1	11.81	11.938
36	-1	-1	1	1	-1	11.55	11.555
37	0	0	0	0	0	14.71	14.701
38	1	1	1	1	1	12.35	12.412
39	0	0	0	0	0	14.71	14.701
40	1	-1	-1	-1	-1	12.02	12.142
41	0	2.38	0	0	0	11.81	11.634
42	1	-1	1	1	-1	12.43	12.147
43	1	-1	-1	-1	1	13.31	13.130
44	-1	1	-1	-1	-1	13.15	13.065
45	-1	-1	1	-1	1	11.39	11.209
46	-1	-1	-1	-1	-1	12.61	12.805
47	-1	1	-1	-1	1	12.36	12.633
48	-2.38	0	0	0	0	12.68	12.870
49	0	0	0	0	0	14.71	14.701
50	0	0	0	0	0	14.71	14.701
51	0	0	0	0	0	14.71	14.701
52	1	1	-1	-1	1	11.78	11.972

Figure 5.1 3D Plot shows the interaction between the medium components $(\text{NH}_4)_2\text{SO}_4$ and KH_2PO_4 for *Aspergillus oryzae* using rice bran

Figure 5.2 3D Plot shows the interaction between the medium components MgSO_4 and KH_2PO_4 for *Aspergillus oryzae* using rice bran

Figure 5.3 3D Plot shows the interaction between the medium components CaCl_2 and KH_2PO_4 for *Aspergillus oryzae* using rice bran

Figure 5.4 3D Plot shows the interaction between the medium components FeSO_4 and KH_2PO_4 for *Aspergillus oryzae* using rice bran

Figure 5.5 3D Plot shows the interaction between the medium components $(\text{NH}_4)_2\text{SO}_4$ and MgSO_4 for *Aspergillus oryzae* using rice bran

Figure 5.6 3D Plot shows the interaction between the medium components $(\text{NH}_4)_2\text{SO}_4$ and CaCl_2 for *Aspergillus oryzae* using rice bran

Figure 5.7 3D Plot shows the interaction between the medium components $(\text{NH}_4)_2\text{SO}_4$ and FeSO_4 for *Aspergillus oryzae* using rice bran

Figure 5.8 3D Plot shows the interaction between the medium components MgSO_4 and CaCl_2 for *Aspergillus oryzae* using rice bran

Figure 5.9 3D Plot shows the interaction between the medium components FeSO_4 and MgSO_4 for *Aspergillus oryzae* using rice bran

Figure 5.10 3D Plot shows the interaction between the medium components CaCl_2 and FeSO_4 for *Aspergillus oryzae* using rice bran

optimum values of the variables were found from the equations derived by the differentiation of the obtained second-order polynomial equations. The optimum values were found to be KH_2PO_4 - 2.69(g/L); $(\text{NH}_4)_2\text{SO}_4$ - 4.95(g/L); MgSO_4 - 1.70(g/L); CaCl_2 - 0.53(g/L) and FeSO_4 - 0.50(g/L) for rice bran shown in Table 5. Table 6 shows the results of the regression analysis of the second-order polynomial model for media optimization of *A. oryzae* MTCC-8624 utilizing rice bran as a substrate. The ANOVA for the fitted polynomial model for media optimization of *A. oryzae* MTCC-8624 utilizing rice bran as the substrate is shown in Table 7. The optimum values were found to be KH_2PO_4 - 2.69(g/L); $(\text{NH}_4)_2\text{SO}_4$ - 4.95 (g/L); MgSO_4 - 1.70(g/L); CaCl_2 - 0.53(g/L) and FeSO_4 - 0.50(g/L) for rice bran and the same is reported in Table 8. Results of the study revealed that rice bran can be used as a suitable and cost-effective substrate for alpha-amylase production, these

results are in agreement with the findings of previous researchers such as Konsula & Liakopoulou-Kyriakides (2004), Shivaramakrishnam et al. (2006), Bharathiraja et al. (2016), Subash et al. (2017) and Asrat & Girma (2018)

Conclusion

This investigation concludes that the optimization of mineral supplements for the fermentative α -amylase production and its suitability for a large-scale production using cheap and easily available substrate rice. The fungal strain *A. oryzae* MTCC-8624 is used in this study to investigate the α -amylase production capability. The significant media components identified by Plackett-Burman design are KH_2PO_4 = 2.69 g/L; MgSO_4 = 1.70 g/L; CaCl_2 = 0.53 g/L; FeSO_4 = 0.5 g/L and $(\text{NH}_4)_2\text{SO}_4$ = 4.95 g/L for rice bran.

Table 6 Results of the regression analysis of second order polynomial model for media optimization of *A. oryzae* MTCC-8624 utilizing rice bran as substrate

Term constant	Regression coefficient	T-statistics	P-value
Intercept	14.7015	240.049	0.000
A	-0.0173	-0.585	0.000
B	-0.0271	-0.915	0.563
C	-0.3046	-10.288	0.367
D	0.1019	3.443	0.000
E	0.0503	1.697	0.002
A ²	-0.3311	-12.997	0.100
B ²	-0.5308	-20.84	0.000
C ²	-0.5865	-23.026	0.000
D ²	-0.3045	-11.956	0.000
E ²	-0.6325	-24.83	0.000
A.B	-0.225	-6.532	0.000
A.C	0.3669	10.651	0.000
A.D	-0.0531	-1.542	0.133
A.E	0.2256	6.55	0.000
B.C	0.1681	4.881	0.000
B.D	0.0294	0.853	0.400
B.E	-0.1294	-3.756	0.001
C.D	-0.0013	-0.036	0.971
C.E	-0.0025	-0.073	0.943
D.E	-0.0863	-2.504	0.018

$R-Sq = 98.33\%$ $R-Sq(pred) = 93.53\%$ $R-Sq(adj) = 97.25\%$

Table 7 ANOVA for the fitted polynomial model for media optimization of *A. oryzae* MTCC-8624 utilizing rice bran as substrate

Sources of variation	Sum of squares	Degrees of freedom (DF)	Mean square (MS)	F- value	P-value
Regression	69.3666	20	3.4683	91.34	0.000
Linear	4.6235	5	0.9247	24.35	0.000
Square	55.3906	5	11.0781	291.76	0.000
Interaction	9.3525	10	0.9352	24.63	0.000
Residual Error	1.1771	31	0.038	-	-
Lack-of-Fit	1.1734	22	0.0533	130.09	0.000
Pure Error	0.0037	9	0.0004	-	-
Total	70.5437	51	-	-	-

Table 8 Optimum values of the media components obtained from regression equation for *A. oryzae* MTCC-8624 utilizing rice bran as substrate

Independent variables	Optimum value (coded)	Optimum value (real) (g/L)
KH ₂ PO ₄ (g/L)	-0.31231	2.69
(NH ₄) ₂ SO ₄ (g/L)	-0.02402	4.95
MgSO ₄ (g/L)	0.40841	1.70
CaCl ₂ , (g/L)	0.16817	0.53
FeSO ₄ (g/L)	0.02402	0.50

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgements

The authors wish to express their sincere gratitude for the support extended by the authorities of Annamalai University, Annamalai Nagar, Tamil Nadu, India in carrying out the research work in Bioprocess Laboratory, Department of Chemical Engineering, Faculty of Engineering and Technology, Annamalai University.

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