Taguchi design: Optimization *α-amylase* Synthesis by *Bacillus velezensis sp.*

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Abstract

The Taguchi's design was used in this investigation to optimize the process parameters to maximize the α-amylase synthesis from Bacillus velezensis sp. Thirteen parameters, namely pH, temperature, agitation, inoculum size, aeration, and carbon source, nitrogen source, K_2PO_4, $MgSO_4$, NaCl, incubation duration, fructose, and $NaNO_3$, were chosen. The Taguchi design is based on an orthogonal array arrangement of L_{27} (3^{13}), at three levels for each factors. After validation of the obtained model equation at maximum, at the optimum conditions this model predicated enzyme activity of 1097.32 U/ mL at pH (5.0), temperature (34.0°C), agitation (125.0 RPM), aeration (2.0 LPM), carbon source (5%), nitrogen source (3%), K_2PO_4 (0.4%), $MgSO_4$ (0.2%), NaCl (0.3%), Fructose (2.0%), and $NaNO_3$ (0.7%) with an incubation time of 60 hours in the presence of 2.0% inoculum density. Under this optimal experimental circumstances, the maximal experimental α-amylase activity is 1090.12 U/mL reported, the maximum yield of α-amylase.

Keywords: *α-amylase, Bacillus velezensis sp., Optimum condition, Taguchi's methodology, Orthogonal array*

1. Introduction

α-amylase is a starch-degrading enzyme used in a variety of industries, including laundry, textiles, chocolate, detergent, and biofuel production. It is becoming increasingly important in the pharmaceutical industry, where it is used to make digestive aids, biodegradable polymers to regulate drug release rate, and cross-linked starches in tablets to govern drug release kinetics. The traditional optimization strategy uses single-factor variation while keeping the other parameters fixed. This approach is inadequate for multifactor optimization not only because it is time-consuming, but also because it is incapable of detecting the genuine optimum, owing to interactions between the components in particular. This article focuses on statistical experimental design methods to increase the yield of *α-amylase* using environmentally friendly, less expensive agro-solid substrates such as moong husk and soya bean cake. The Taguchi's technique, developed by Taguchi in the 1980s, uses a few tests to assess the impacts of various process variables and find those that have a significant impact on the process. The derived model with an orthogonal array layout of L_{27} (3^{13}) yield an equation was tested experimentally to maximize *α-amylase* yield. Thus in this present research, Taguchi's experimental design with L_{27} (3^{13})-orthogonal approach, thirteen factors were chosen at three levels: pH, temperature, agitation, inoculum size, areration, and carbon source (moong husk), nitrogen source (soya bean cake), K_2PO_4, $MgSO_4$, NaCl, Incubation time, Fructose, and $NaNO_3$. Thus, orthogonal array layout of the derived model equation was tested experimentally in order to maximize *α-amylase* yield. The resultant *α-amylase* can be purified further using different chromatographic techniques, and its molecular weight can be measured, for example, by SDS-PAGE.

2. Materials and Methods

2.1 Chemicals and microorganism

All chemicals used **(Table 1**.) in this study were purchased from HiMedia Labs, C40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane (West) - 400 604, Maharashtra, India. *Bacillus velezensis sp.* was obtained from School of Pharmaceutical Sciences, Lovely Professional University, Punjab, India. The chosen strain is purified using the streak plate technique and kept at 4^{o}C as slant cultures.

Table 1. Chemical media

Media	Composition	%
Luria-Bertani (LB) Starch Medium:	Tryptone	1.0
	Yeast extract	0.5
	Sodium chloride	1.0
	Starch	0.25
	Starch	0.5

Basal Medium:	Peptone	2.0
	$MgSO_4$	0.1
	K_2HPO_4	0.3
Seed Medium:	Starch	2.0
	NH_4NO_3	0.7
	K_2HPO_4	0.1
	$MgSO_4 .7H_2O$	0.01
	$FeCl_3$	0.005
	$CaCl_2$	0.002
Production Medium:	Moong Husk	3-5
	Fructose (Fru)	1.5
	Soybean Cake (Soy)	1-3
	$NaNO_3$	0.5
	KH_2PO_4	0.3
	$MgSO_4.7H_2O$	0.1
	NaCl	0.2

2.2 Submerged fermentation

In order to evaluate the growth pattern, *Bacillus velezensis sp.*, a potential *α-amylase*-producing bacterial strain, is cultured in seed media containing Luria-Bertani broth. A 2% inoculum of the 16-hour-grown seed medium is added to basal media containing (g/L) starch 5.0, peptone 20.0, $MgSO_4$ 1.0, and K_2HPO_4 3.0, and the incubation process is repeated. Every hour, turbidity at 600 nm and the 3,5-dinitro salicylic acid test (DNSA) are used to determine the rate of bacterial growth and the synthesis of the enzyme *α*-amylase. To get the crude extract, which serves as an enzyme source for the *α-amylase* test, the medium is centrifuged at 5000 RPM for 15 minutes. Each test is run three times, and the average results are reported. The bacterium strain with the highest α -amylase activity is then cultured in a seed medium that has been specially designed for it.

2.3 Enzyme assay

The 3, 5-dinitro salicylic acid test is used to measure α -amylase activity. At pH 5.5 and 55 °C, one mL of 1% starch is incubated with 0.05 mL of enzyme-containing supernatant for 8 minutes before adding 0.5 mL DNSA to the reaction mixture. After 10 minutes in a boiling water bath, the reaction mixture is cooled and 3.45 mL of distilled water is added. The released reduced sugar is measured at 540 nm by using Miller's method. Blank is prepared without the use of enzyme and the concentration of protein is estimated by Lowry's method.

2.4 Taguchi experimental design

In contrast to traditional experimental design, Taguchi invented the Taguchi's experimental design approach to reduce the number of tests necessary. This approach employs a unique design of orthogonal arrays to investigate the whole parameter space with a limited number of tests. Taguchi advocates analyzing the mean response for each run in the inner array, as well as analyzing variance using a signal-to-noise (S/N) ratio. The quadratic loss function yields several S/N ratios, three of which are standard and generally applicable.

The larger the better.

For each factor level combination, the signal-to-noise (S/N) ratio is determined. The formula for the larger-is-better principle:

$S/N = -10*\log (\Sigma (1/Y^2)/n)$, where Y = replies for the specified factor level combination and n = number of responses in the factor level combination.

Smaller is preferable.

For each factor level combination, the signal-to-noise (S/N) ratio is determined. The formula for the smaller-is-better principle:

$S/N = -10*\log (\Sigma (Y^2)/n)$, where Y = replies for the specified factor level combination and n = number of responses in the factor level combination.

The S/N ratio, regardless of the performance criteria, is the most important factor in determining the ideal level of process parameters. The Taguchi experimental design, consisting of a typical orthogonal array L_{27} (3^{13}), with 24 degrees of freedom, was used to investigate thirteen parameters at three levels. **Tables 2 and 3** indicate the levels of the components analyzed as well as the arrangement of the L_{27} Taguchi's orthogonal array. The data was statistically analyzed using analysis of variance (ANOVA) to investigate the contributions of the factors and interactions, as well as the impacts of each step on the observed value. Controlling variables were discovered, effect magnitudes were qualified, and statistically significant effects were found. The Design-Expert software version 13.0.5.0 packet program (Stat-Ease, Inc., USA) was used for all computations and statistical analyses.

Table 2. Taguchi's Experimental Design for *Bacillus velezensis sp.*'s Production of *a*-Amylase

Factor	Level 1	Level 2	Level 3
pH	4	5	6
Temp(°C)	32	34	36
Agitation(RPM)	100	125	150
Inoculum size (%)	1.5	2	2.5
Aeration (LPM)	1.5	2	2.5
Carbon Substrate (%)	3	4	5
Nitrogen Substrate (%)	1	2	3
$K2HPO_4$ (%)	0.2	0.3	0.4
$MgSO_4$ (%)	0.05	0.125	0.2
NaCl (%)	0.1	0.2	0.3

Incubation (hr)	40	60	80
Fructosse (%)	1	1.5	2
$NaNO_3$ (%)	0.3	0.5	0.7

Table 3. L_{27} (3^{13}) Orthogonal Array of Taguchi Experimental Design for *a*-Amylase by *Bacillus velezensis sp.*

Run	A	B	C	D	E	F	G	H	J	K	L	M	N	Enzyme activity Experiment	Enzyme activity predicted
		℃	RPM	%	LPM	%	%	%	%	%	Hours	%	%	U/mL	U/mL
1	6	36	125	1.5	2	3	3	0.2	0.2	0.2	80	1.5	0.3	998.83	998.33
2	4	34	125	2.0	2	4	2	0.4	0.2	0.3	40	1	0.3	1037.79	1036.82
3	5	36	100	2.0	1.5	4	3	0.4	0.05	0.2	60	2	0.3	1024.1	1024.51
4	5	36	100	2.0	2	5	1	0.2	0.125	0.3	80	1	0.5	1003.71	1003.31
5	4	36	150	2.5	2	4	2	0.2	0.05	0.1	80	2	0.7	966.786	966.46
6	5	34	150	1.5	2	5	1	0.4	0.05	0.2	40	1.5	0.7	1031.31	1031.18
7	6	32	150	2.0	2.5	4	1	0.4	0.125	0.1	80	1.5	0.3	995.238	995.05
8	6	32	150	2.0	2	3	3	0.3	0.05	0.3	60	1	0.7	1011.4	1010.80
9	6	36	125	1.5	1.5	5	2	0.4	0.125	0.1	60	1	0.7	1020.74	1021.05
10	4	34	125	2.0	2.5	5	3	0.2	0.05	0.1	60	1.5	0.5	953.659	953.10
11	4	32	100	1.5	1.5	3	1	0.2	0.05	0.1	40	1	0.3	920.1	920.98
12	6	32	150	2.0	1.5	5	2	0.2	0.2	0.2	40	2	0.5	1035.47	1035.68
13	5	32	125	2.5	2	5	1	0.3	0.2	0.1	60	2	0.3	979.991	979.29
14	5	34	150	1.5	2.5	3	2	0.2	0.125	0.3	60	2	0.3	1012.32	1012.60
15	6	34	100	2.5	2	3	3	0.4	0.125	0.1	40	2	0.5	1006.31	1006.18
16	4	32	100	1.5	2	4	2	0.3	0.125	0.2	60	1.5	0.5	1092.9	1092.97

17	4	34	125	2.0	1.5	3	1	0.3	0.125	0.2	80	2	0.7	1049.55	1049.39
18	5	34	150	1.5	1.5	4	3	0.3	0.2	0.1	80	1	0.5	1003.22	1003.90
19	5	32	125	2.5	2.5	3	2	0.4	0.05	0.2	80	1	0.5	1056.16	1055.87
20	6	36	125	1.5	2.5	4	1	0.3	0.05	0.3	40	2	0.5	1023.74	1023.65
21	4	36	150	2.5	2.5	5	3	0.3	0.125	0.2	40	1	0.3	1040.55	1040.63
22	4	36	150	2.5	1.5	3	1	0.4	0.2	0.3	60	1.5	0.5	1028.56	1029.04
23	6	34	100	2.5	1.5	5	2	0.3	0.05	0.3	80	1.5	0.3	1032.27	1032.95
24	5	36	100	2.0	2.5	3	2	0.3	0.2	0.1	40	1.5	0.7	1016.67	1016.68
25	4	32	100	1.5	2.5	5	3	0.4	0.2	0.3	80	2	0.7	1020.2	1020.68
26	5	32	125	2.5	1.5	4	3	0.2	0.125	0.3	40	1.5	0.7	1007.73	1007.84
27	6	34	100	2.5	2.5	4	1	0.2	0.2	0.2	60	1	0.7	1006.8	1007.08

3. Results

The Taguchi experimental design is an excellent choice for optimizing biotechnological processes for the synthesis of microbial enzymes. In this example, the effect of 13 variables on *α-amylase* production by *Bacillus velezensis sp.* was investigated in 27 runs using a Taguchi experimental design. The results showed that at optimal levels **(Fig. 1. and 2.)**, these substances greatly promote the synthesis of *α-amylase*. In run 11, pH (4), temperature (32ºC), agitation (100 RPM), aeration (1.5 LPM), carbon source (3%), nitrogen source (1%), K_2PO_4 (0.2%), $MgSO_4$ (0.05%), NaCl (0.1%), Fructose (1.0%), and NaNO3 (0.3%) concentrations with incubation time to 40 hours in the presence of 1.5% inoculum density, the lowest production of 920.1 U/ml was observed. The production of 1056.16 U/ml was observed in run 19 with a combination of pH (5), temperature (32ºC), agitation (125 RPM), aeration (2.5 LPM), carbon source (3%), nitrogen source (2%), K_2PO_4 (0.4%), $MgSO_4$ (0.05%), NaCl (0.2%), fructose (1.0%), and $NaNO_3$ (0.5%) concentrations with incubation time to 80 h in the presence of 2.5% inoculum density. The Taguchi technique was used to analyze the outcomes of orthogonal array tests and estimate how much variance each element contributed. The data was analyzed to determine important parameters on *α-amylase* production, and the findings are displayed in ANOVA in **Table 4.** The estimated ratios (F) indicate that the experimental design elements are statistically significant at the 95% confidence level. The model F value of 368.54 indicates that the model is significant. The multiple correlation coefficient of R^2 is 0.9998, indicating that the

model can explain 99.98% of the variance in the answer. The standard deviation, mean, coefficient of variation (CV), and projected residual sum of squares (PRESS) values for the model are 1.81, 1013.66, 0.178, and 1191.92, respectively. The anticipated activity for *α-amylase* under ideal circumstances was 1097.32 U/ml.

Table 4. ANOVA for *α*-Amylase Production by *Bacillus velezensis sp.*

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	28932.71	20	1446.64	1591.18	< 0.0001*	significant
A-pH	39.96	2	19.98	21.98	0.0017	
B-Temp	11.42	2	5.71	6.28	0.0338	
F-Carbon Substrate	198.77	2	99.39	109.32	< 0.0001*	
G-NItrogen Substrate	3579.65	2	1789.83	1968.66	< 0.0001*	
H-K2HPO4	8113.33	2	4056.67	4461.99	< 0.0001*	
J-MgSO4	2439.59	2	1219.80	1341.67	< 0.0001*	
K-NaCl	12883.52	2	6441.76	7085.39	< 0.0001*	
L-Incubation	6.54	2	3.27	3.60	0.0941	
M-Fructosse	186.54	2	93.27	102.59	< 0.0001*	
N-NaNO3	1473.38	2	736.69	810.30	< 0.0001*	
Residual	5.45	6	0.9092			
Cor Total	28938.17	26				

**Significant terms.*

4. Discussion

The Taguchi experimental technique was used to optimize the cultivation conditions for bacterial growth and maximize *α-amylase* production of *B. velezensis sp*. in shaken flask cultures. Critical culture factors such as pH, temperature, agitation, inoculum size, aeration, carbon supply, nitrogen source, K_2PO_4, $MgSO_4$, NaCl, incubation duration, fructose, and $NaNO_3$ were examined. The optimal circumstances for maximal enzyme synthesis vary depending on the organism. In this study, *α-amylase* production by *Bacillus velezensis sp*. The relative relevance of medium components and environmental conditions on *α-amylase* production was tested using the Taguchi's design. The optimal variables for *α-amylase* synthesis were determined to be carbon source ($p = 0.0319$), nitrogen source ($p = 0.0018$), K_2PO_4 ($p = 0.0008$), $MgSO_4$ ($p = 0.0027$), NaCl ($p = 0.0005$), fructose ($p = 0.0339$), and $NaNO_3$ ($p = 0.0044$). The best conditions for the medium were found to be pH (5), temperature (34°C), agitation (125 RPM), aeration (2.0 LPM), carbon source (4%), nitrogen source (2%), K_2PO_4 (0.3%), $MgSO_4$ (0.125%), NaCl (0.2%), Fructose (1.5%), and $NaNO_3$ (0.5%) concentrations with incubation time of 60 hours while 2% inoculum was present. Under ideal experimental circumstances, maximal α -amylase production of 1090.12 U/mg was shown by point of prediction. (**Fig. 3**)

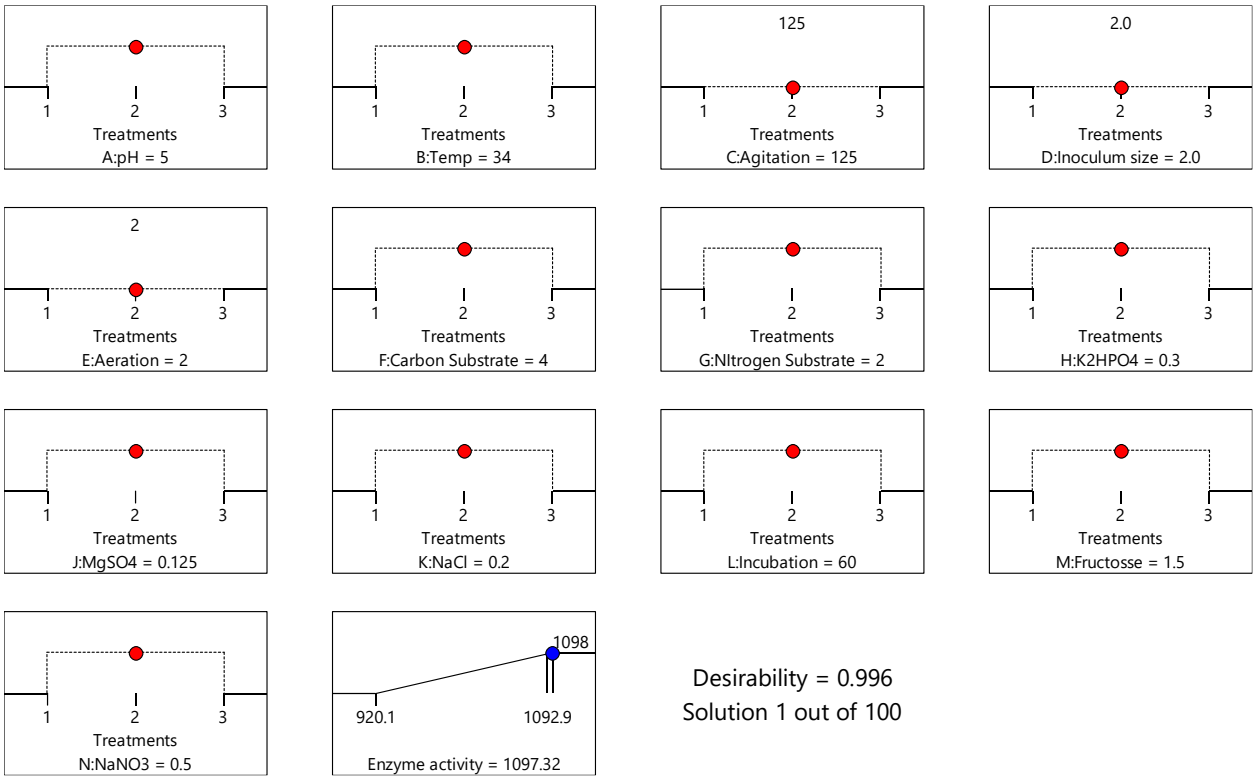

Figure 3. Ramp chart for statistically optimize factors for maximum *α-amylase* production by *Bacillus velegensis sp.* in Taguchi experimental design.

5. Conclusions

The Taguchi experimental design is an excellent choice for optimizing biotechnological processes for microbial enzyme synthesis. In this study, the effect of 13 variables on α-amylase production by *Bacillus velezensis sp.* was investigated in 27 runs using the Taguchi experimental design. The results showed that at optimal levels, these substances greatly promote the synthesis of *α-amylase*. The optimal conditions for the medium were pH (5), temperature (34°C), agitation (125 RPM), aeration (2.0 LPM), carbon source (4%), nitrogen source (2%), K_2PO_4 (0.3%), $MgSO_4$ (0.15%), NaCl (0.2%), Fructose (1.0%), and $NaNO_3$ (0.5%) concentrations with incubation time of 60 hours while 2% inoculum was present. Under ideal experimental circumstances, maximal α-amylase production of 1090.12 U/ml was shown by point of prediction. The Taguchi experimental design can be used to analyze the outcomes of orthogonal array tests and estimate the variance, each element contributes to α-amylase production.

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