CELLULASE PRODUCTION OPTIMIZATION BY *BACILLUS AERIUS* THROUGH RESPONSE SURFACE METHODOLOGY IN SUBMERGED FERMENTATION

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The objective of the present study was to get better production of FPase from *Bacillus aerius* (MG597041) by optimizing different process parameters under submerged fermentation through the statistical approach. Optimization of physical factors of culturing medium by one factor at a time (OFAT) revealed optimum incubation time of 24 h, inoculum size of 1%, pH 5.5, and substrate concentration of 4%. Plackett-Burman design (PBD) was performed to identify the significant nutritional influence of cellulase production. Among the nine parameters screened, peptone, yeast extract, $FeSO_4$ and K_2HPO_4 were found significant. CCD of significant parameters revealed maximum FPase activity (127.4 IU/mL/min) at the optimum concentration of yeast extract of 0.5 g/L, peptone of 0.5 g/L, $FeSO_4$ of 0.2 g/L, and K_2HPO_4 of 0.02 g/L. ANOVA was used to analyze these results. The analysis of the results showed an F-value of 8.74 and a p-value 0.00. Maximum hydrolysis of 10% of raw *Bombax ceiba* seed pods using this indigenous cellulase was obtained after 24 h. Also, the study explored the potential of the obtained cellulase to be applied in denim biostoning finishing. The findings demonstrated the efficient use of the obtained enzyme in saccharification of raw *Bombax ceiba* seed pods, which can be of interest for production of biofuel, and in biostoning treatment of denim fabrics.

Keywords: *Bacillus aerius*, cellulase, optimization, RSM, fermentation

INTRODUCTION

Cellulase is a complex of three enzymes, named exoglucanase, endoglucanase, and beta-glucosidase. The synergistic action of these three enzymes hydrolyzes cellulose into glucose units.[1-3] Due to the industrial importance of cellulase, its biosynthesis from microbes has been gaining interest.[4-6] The role of cellulase in the breakdown of cellulose marks them to be extremely handy in several industries, for example, for improvement of food texture, in acetate processing, cotton processing, biostoning, brewing, seeds fermentation, soybean hulls separation, deinking, paper and pulp industry, detergents production and animal feed, as well as for considerably increasing the production of bioethanol from lignocellulosic biomass.[7-11] Cellulase can be produced by many microbes, such as bacterial and fungal species of various ecological backgrounds.[12] Bacteria, with a higher growth rate than fungi, have much potential to be employed in the production of cellulase.[13] Conventionally, industrially important enzymes have been produced by submerged fermentation (SmF) due to the greater control of environmental factors, such as pH, temperature, and facility in handling.[14]

The yield of microbial cellulase can be enhanced by controlling nutritional and physical factors. The formation of economical media needs a suitable mixture of nitrogen, phosphorous,

potassium, carbon, and trace element sources. Different parameters, such as temperature, pH, incubation time, aeration, and growth nutrients, remarkably influence the yield of cellulases formed by various microorganisms.[15] Conventional or statistical methods can be used to manipulate nutritional parameters. The conventional method includes varying one independent factor at a time, while retaining the others at a constant level. However, the statistical approach presents various merits over others, being quick and authentic, putting up significant parameters, facilitating in comprehending the interactions between parameters at different concentrations, and lessens experiment number greatly, which results in saving glassware, manpower, time and chemicals.[13]

For building models, designing experiments, assessing the influence of various parameters, and discovering the optimum conditions for better results and decreasing the experiment number, response surface methodology (RSM) is now considered a standard statistical approach. In biological processes, *e.g.* in the production of enzymes, RSM has been employed for optimizing microorganisms' growth and enzyme production.[16,17] Keeping in mind a collection of potential medium constituents, we used initial screening of all constituents using Plackett–Burman design (PBD) for optimizing the medium components. This design discerns the constituents with a significant effect on response factors, *i.e.* cellulase production. Central composite design (CCD) was then employed to identify the optimal values of the significant parameters for improved cellulase production from *Bacillus* strain in submerged fermentation using seed pods of *Bombax ceiba* as cellulosic substrate. This substrate is cheap, easily and abundantly available, and has high polysaccharide content. To the authors' knowledge, there is no research reported so far on the use of this lignocellulosic substrate for cellulase production by *B. aerius*.

EXPERIMENTAL

Microorganism and inoculum preparation

A bacterial strain *Bacillus aerius* (MG597041) isolated from soil, identified by rRNA sequencing, was used for cellulase production.[18] Twenty milliliters of sterilized nutrient broth were inoculated with a loopful bacterial strain and kept for 24 h in an incubator at 37 °C, at a shaking speed of 120 rpm. The inoculum was freshly prepared each time.

Cellulosic substrate preparation

Seed pods of *B. ceiba* were picked locally, in district Sargodha, Punjab, Pakistan. Seed pods were washed, dried, and milled into powdered form for further use.[8]

Enzyme production

For enzyme production, twenty-five milliliters of the medium as per experimental design was taken in 100 mL conical flasks.[18] The media were sterilized at 121 °C, 15 psi for 15 min. After cooling, each flask was inoculated with the bacterial strain and incubated at 37 °C in a shaking incubator for four days. Then, samples were taken aseptically after every 24 h. The samples were centrifuged at 10000 rpm for 10 min to obtain the crude extract, which served as an enzyme source.

Optimization of physical and nutritional parameters

Cellulase production from the isolated strain was optimized using the one factor at a time (OFAT) approach, by changing fermentation variables, *i.e.* concentration of substrate (seed pods of *B. ceiba*) (0.5-5%), pH (4-8), incubation time (24-96 h), inoculum size (0.5-5%). Cellulase production was estimated by FPase activity. In PBD, nine variables, including $MgSO_4$, yeast extract, NaCl, peptone, $(NH_4)_2SO_4$, $FeSO_4$, $MnSO_4$, KH_2PO_4 and K_2HPO_4, were set at two levels: -1 and +1, for low and high levels, respectively (Table 1) in a 12 run experiment.[19]

After shortlisting significant parameters by PBD, CCD of RSM was performed to determine the optimum levels of growth medium components. CCD was conducted by an experiment with 31 runs, and enzyme activity was measured by FPase assay.

Enzyme assay

A mixture of exoglucanases and endoglucanases constitutes the Filter paper (FPase) activity, resulting from the degradation of a strip of Whatman filter paper No.1 (1.0 cm×6.0 cm in size).[20] One milliliter of a 50 mM sodium citrate buffer solution with a pH of 4.8, a filter paper strip, and 0.5 mL of the crude enzyme were added to the test tube containing the reaction assay. The samples were left in a water bath at 50 °C for 30 min, then, the reaction was stopped by adding 1.5 mL of DNS. The tubes were left in boiling water for 10 min before measuring absorbance at 540 nm. One-unit enzyme activity was defined as the amount of enzyme required to produce 1 micromole reducing sugar equivalent per minute under assay conditions.

Application of cellulase for saccharification

Biomass saccharification was performed by following the method described by Irfan *et al.*[21] Specifically, a substrate loading of 2% (raw *Bombax ceiba* seed pods) was hydrolyzed with 100 IU/mL of cellulase (produced by *Bacillus aerius* MG597041) in

a 250 mL Erlenmeyer flask. Saccharification was conducted at 50 °C for a total time period of 28 h. The material was centrifuged at 10,000 rpm for 10 min at the end of saccharification. The supernatant was collected for the analysis of sugar. Saccharification (%) was calculated using the following formula:[21]

$$\text{Saccharification (\%)} = \frac{\text{Reducing sugars released (mg/ml)}}{\text{Substrate used (mg/ml)}} \times 100 \quad (1)$$

Table 1
Range of parameters used for Plackett Burman design

Sr. No.	Parameters	Label	Codes	
			+1	-1
1	$MgSO_4$ (%)	X_1	0.2	0.01
2	Yeast extract (%)	X_2	0.5	0.1
3	NaCl (%)	X_3	0.5	0.1
4	Peptone (%)	X_4	0.5	0.1
5	$(NH_4)_2SO_4$ (%)	X_5	0.5	0.1
6	$FeSO_4$ (%)	X_6	0.2	0.1
7	$MnSO_4$ (%)	X_7	0.03	0.01
8	KH_2PO_4 (%)	X_8	0.05	0.02
9	K_2HPO_4 (%)	X_9	0.05	0.02

Application of cellulase as biostoning agent

The biostoning experiment was carried out by following the method reported by Gautam and Sharma.[22] A piece of fabric (denim) was prewashed for 10 min at 60 °C. Then, it was cut to pieces of 5 × 5 cm size. Cellulase treatment was done in 250 mL conical flasks containing 100 mL of crude cellulase and a magnetic pellet was added into the conical flask to help the color removal. A conical flask containing 100 mL of distilled water was used as control. The conical flasks were placed at 50 °C on a heated magnetic stirrer for 30 min at 50 rpm. After removing the fabrics from the conical flask, they were soaked for 10 min in 100 mL of 10 mM NaOH. The denim was rubbed and then washed gently with 10 mM NaOH for 2 min. Finally, it was rinsed with tap water. The denim piece was dried for 1 h at 105 °C and air dried at room temperature. The color of the fabric samples was observed and optical density at 390 nm was recorded.

Statistical analysis

ANOVA was applied to analyze results statistically.

RESULTS AND DISCUSSION

Cellulose is the major structural constituent of plants.[22] Microbial cellulolytic enzymes are required for the degradation of cellulosic materials, as it is a complex process.[23] In this study, we employed a soil isolated bacterium *B. aerius* (MG597041) in submerged fermentation for cellulase production using *B. ceiba* waste. Previously, Irfan *et al.* isolated cellulose-degrading bacteria *Bacillus subtilis* K-18 from soil and used potato peel as a carbon source in submerged fermentation for cellulase production.[24] *Paenibacillus terrae* was also found to be a potential producer of cellulase, isolated from soil in the subtropical region of China.[25] *Cellulomonas* sp. ASN2 was isolated from soil and showed maximum cellulase production.[26] Sharma *et al*. isolated *Bacillus tequilensis* from soil and used wheat bran as a carbon source in submerged fermentation for cellulase production.[27]

As for cellulase production, the environmental factors influence it significantly, thus the shaken flask fermentation method was used to optimize these factors.[28] We optimized four physical parameters, including substrate concentration, pH, inoculum size and incubation time, by OFAT for cellulase production using the selected strain (*B. aerius*) and the selected substrate (seed pods of *B. ceiba*). FPase activity was estimated to determine cellulase production. Enzyme activity increased with an increase in substrate concentration and maximum FPase activity of 116.58 IU/mL/min was observed at 4% concentration of the substrate. Further increase in substrate loading caused a drop in FPase activity (Fig. 1). The bars in the graphs indicate variation among triplicates. Sharma *et al*. found maximum cellulase production by *Bacillus tequilensis* S28, using 3% concentration of wheat bran.[27] *Bacillus aquimaris* isolated from the gut of *Labeo rohita* exhibited maximum cellulase yield at 2.5% sugarcane bagasse.[19]

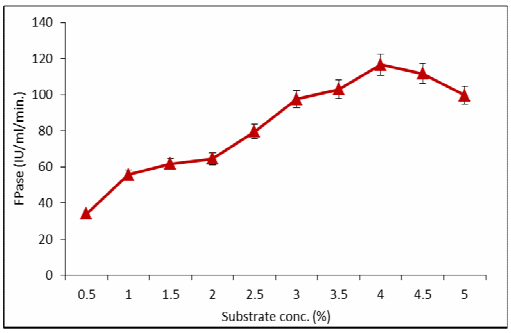

Figure 1: Optimization of substrate (%) for cellulase production through OFAT

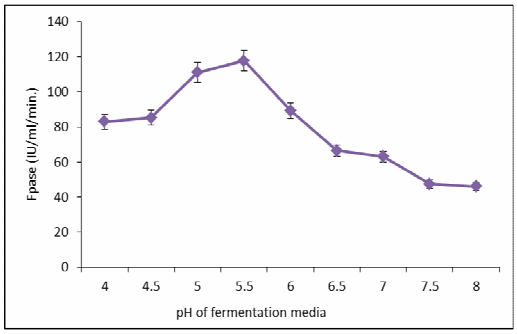

Figure 2: Optimization of pH for cellulase production through OFAT

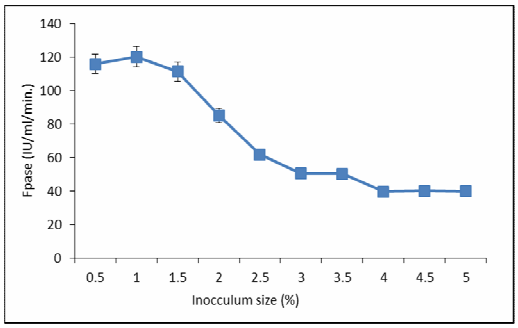

Figure 3: Optimization of inoculum size (%) for cellulase production through OFAT

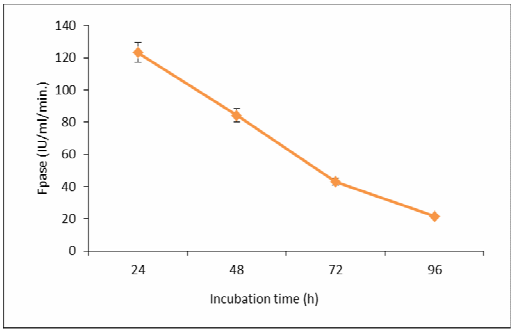

Figure 4: Optimization of incubation time (h) for cellulase production through OFAT

Maximum activity (117.8 IU/mL/min) in the case of pH optimization was observed at pH 5.5; a decline in activity was recorded as the pH increased towards neutrality and alkalinity (Fig. 2). Singh *et al*. found the highest production of cellulase at pH 5.65 by *Bacillus amyloliquefaciens* SS35.[29] Liang *et al.* recorded maximum cellulase activity at pH 5.5 by *Paenibacillus terrae*.[25] Irfan *et al.* studied *Bacillus subtilis* K-18 and reported optimum pH of 7 for maximum cellulase activity, which decreased as the pH increased towards alkalinity.[24] Nkohla *et al*. investigated optimum culture conditions for cellulase production from *Bacillus cereus* SAMRC-UFH1 under SmF. Cellulase was optimally produced (102.7 U/mL) after 84 h of cultivation at pH 6, at a temperature of 25 °C, and agitation speed of 150 rpm.[30]

Optimization of inoculum size resulted in the maximum activity of 120.3 IU/mL/min at 1% inoculum. Enzyme activity increased from an inoculum size of 0.5% to 1%, but it declined gradually as inoculum size increased (Fig. 3). The enzyme activity declined beyond the optimum inoculum concentration, because the increasing competition among microbes for space and nutrients reduced their growth. Another reason for the loss of enzyme activity was an accumulation of secondary metabolites and toxic products, which affected the duration of the stationary phase. Acharya and Chaudhary examined cellulase production by *Bacillus licheniformis* WBS1 and reported maximum production of cellulase at an inoculum concentration of 2% (v/v).[31] Shankar and Isaiarasu worked on *Bacillus pumilus* and found an optimal inoculum size of 2% (v/v) for cellulase production.[32]

As for time optimization, the best enzyme titer (FPase activity of 123.2 IU/mL/min) was attained after a fermentation period of 24 h. Enzyme activity was studied for four days. It was observed that enzyme activity decreased continuously after the first 24 h of incubation, as shown in Figure 4.

To obtain maximum cellulase titer, nine nutritional parameters ($MgSO_4$, yeast extract, NaCl, peptone, $(NH_4)_2SO_4$, $FeSO_4$, $MnSO_4$, KH_2PO_4, K_2HPO_4) were optimized by PBD. Among these nine parameters, yeast extract, peptone, $FeSO_4$ and K_2HPO_4 were found as significant parameters (Fig. 5), whereas the other six factors appeared insignificant for cellulase production. The observed values of FPase activity are given in Table 2. Contour plots for interactions of yeast extract, peptone, $FeSO_4$ and

K_2HPO_4 for cellulase production are displayed in Figure 6. The significant nutritional parameters influencing the production of cellulase were further employed in CCD for maximizing enzyme production. The optimum medium composition for maximum cellulase production (127.4 IU/mL/min) was 0.5 g/L yeast extract, 0.5 g/L peptone, 0.2 g/L $FeSO_4$ and 0.02 g/L K_2HPO_4 (Table 3). ANOVA was performed and resulted in an F-value of 8.74 and a p-value of 0.00 (Table 4). The regression equation depicts the significance of the results (Eq. 2).

Regression equation in uncoded units:

$$FPase = 104.9 + 42.0X_1 - 48.7X_2 - 132X_3 - 1709X_4 - 139.8X_1^2 + 75.1X_2^2 - 426X_3^2 + 20118X_4^2 + 220.8X_1*X_2 + 20X_1*X_3 + 305X_1*X_4 + 346X_2*X_3 1919X_2*X_4 + 4492X_3*X_4 \quad (2)$$

Sharma *et al*. also performed PBD, followed by CCD, to investigate significant nutritional parameters for cellulase production by *Bacillus tequilensis* and found ammonium chloride (4.99 g/L), peptone (4.94 g/L), Tween-20 (0.53 g/L), yeast extract (2.00 g/L), calcium chloride (0.20 g/L) and cobalt chloride (0.60 g/L) as effective parameters.[27] Anjum and coworkers obtained the highest cellulase production (1.3617 IU/mL/min) by *Bacillus subtilis* K-18 using acacia sawdust.[33] To optimize cellulase production by *Bacillus aquimaris*, Khalid *et al.* performed PBD, followed by BBD of RSM, and reported maximum yield of endoglucanase (437.3833 IU) achieved at 2.5% sugarcane bagasse, 0.01% $MgSO_4$ and 0.5% $(NH_4)_2SO_4$ after 24 h of fermentation.[19]

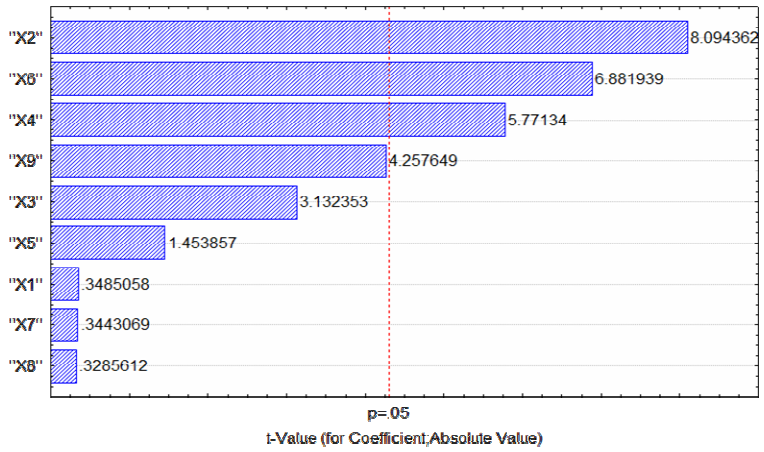

Figure 5: Pareto chart showing effects of independent variables on production of cellulase

Table 2
PBD for screening of nutritional parameters (g/L) for cellulase production by *B. aerius* in submerged fermentation

Run No.	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	Response (IU)
1	0.2	0.5	0.5	0.5	0.5	0.2	0.03	0.05	0.05	80.77
2	0.01	0.5	0.1	0.5	0.5	0.2	0.01	0.02	0.02	120.5
3	0.01	0.1	0.5	0.1	0.5	0.2	0.03	0.02	0.02	98.29
4	0.2	0.1	0.1	0.5	0.1	0.2	0.03	0.05	0.02	107.7
5	0.01	0.5	0.1	0.1	0.5	0.1	0.03	0.05	0.05	96.23
6	0.01	0.1	0.5	0.1	0.1	0.2	0.01	0.05	0.05	97.07
7	0.01	0.1	0.1	0.5	0.1	0.1	0.03	0.02	0.05	104.7
8	0.2	0.1	0.1	0.1	0.5	0.1	0.01	0.05	0.02	123.6
9	0.2	0.5	0.1	0.1	0.1	0.2	0.01	0.02	0.05	75.83
10	0.2	0.5	0.5	0.1	0.1	0.1	0.03	0.02	0.02	123.7
11	0.01	0.5	0.5	0.5	0.1	0.1	0.01	0.05	0.02	119.5
12	0.2	0.1	0.5	0.5	0.5	0.1	0.01	0.02	0.05	101.4

X_1 = $MgSO_4$, X_2 = yeast extract, X_3 = NaCl, X_4 = peptone, X_5 = $(NH_4)_2SO_4$, X_6 = $FeSO_4$, X_7 = $MnSO_4$, X_8 = KH_2PO_4, X_9 = K_2HPO_4

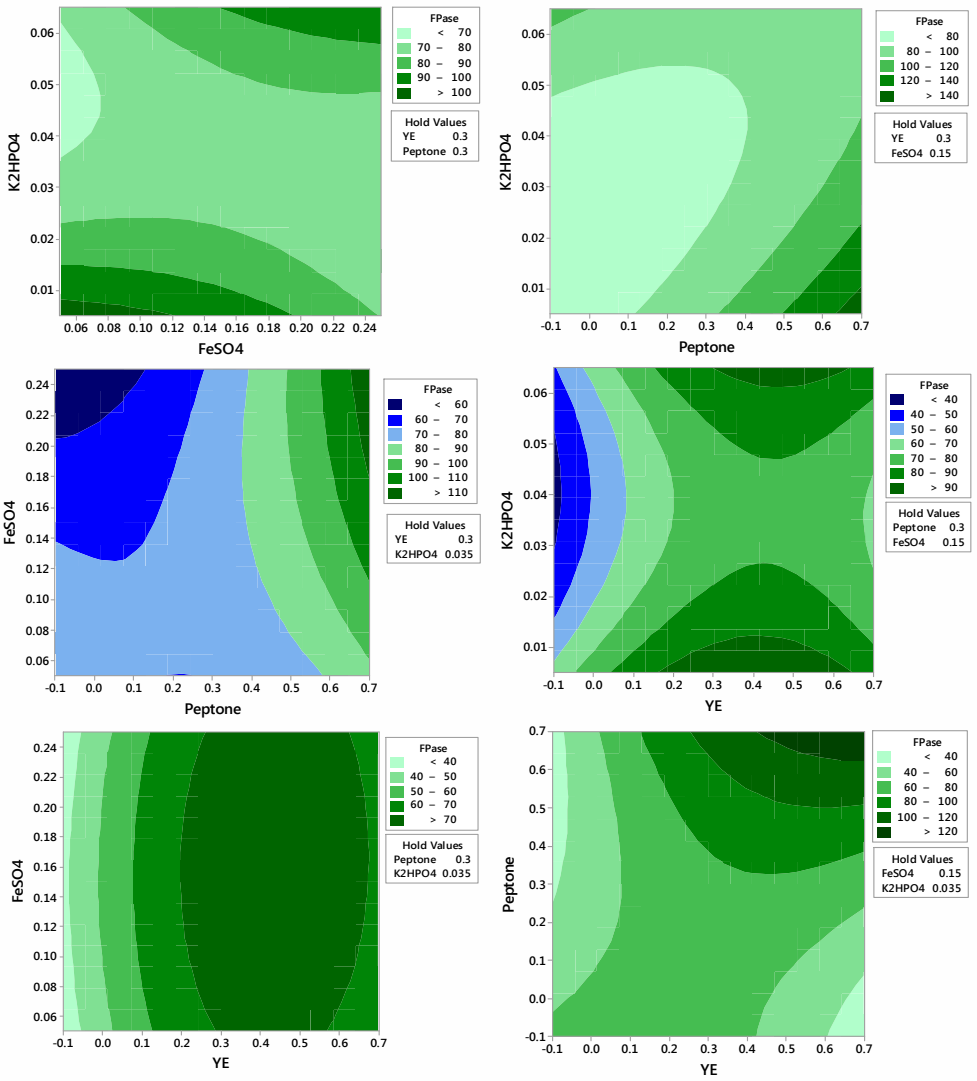

Figure 6: Contour plots of interactions of yeast extract, peptone, $FeSO_4$ and K_2HPO_4 for cellulase production

Table 3
CCD for optimizing significant nutritional parameters (g/L) for the production of cellulase by *B. aerius* in submerged fermentation

Run #	Yeast (X_1)	Peptone (X_2)	$FeSO_4$ (X_3)	K_2HPO_4 (X_4)	FPase (IU)		
					Observed	Predicted	Residual
1	0.5	0.5	0.1	0.05	89.01	88.2864	0.7236
2	0.3	0.3	0.15	0.005	99.61	96.8146	2.7854
3	0.5	0.1	0.2	0.02	51.82	54.2558	-2.4348
4	0.5	0.1	0.1	0.02	62.91	66.841	-3.931
5	0.1	0.5	0.1	0.05	53.94	53.9942	-0.0472
6	0.7	0.3	0.15	0.035	62.14	68.214	-6.074
7	0.3	0.3	0.15	0.035	74.26	75.3839	-1.1239
8	0.3	0.3	0.15	0.035	74.96	75.3839	-0.4239
9	0.1	0.5	0.1	0.02	86.34	77.4005	8.9395
10	0.3	0.3	0.15	0.035	74.19	75.3839	-1.1939
11	0.5	0.5	0.2	0.02	127.4	109.282	8.118
12	0.3	0.7	0.15	0.035	101.7	106.328	-4.628

13	0.5	0.5	0.2	0.05	103.2	103.0112	0.1888
14	0.1	0.5	0.2	0.02	60.99	77.8453	-16.8553
15	0.1	0.1	0.2	0.02	63.57	58.1499	5.4201
16	0.3	0.3	0.15	0.035	74.16	75.3839	-1.2239
17	0.5	0.5	0.1	0.02	109.5	108.0345	1.4855
18	0.5	0.1	0.2	0.05	68.21	71.0127	-2.8027
19	0.3	0.3	0.25	0.035	74.71	71.7903	2.9197
20	0.1	0.5	0.2	0.05	77.99	67.9162	10.0738
21	0.3	0.3	0.15	0.035	79.33	75.3839	3.9461
22	0.3	0.3	0.15	0.065	83.71	90.1651	-6.4551
23	-0.1	0.3	0.15	0.035	40.22	37.8158	2.4042
24	0.1	0.1	0.2	0.05	67.28	71.2485	-3.9685
25	0.5	0.1	0.1	0.05	84.48	70.1207	14.3593
26	0.3	0.3	0.05	0.035	63.88	70.4535	-6.5735
27	0.1	0.1	0.1	0.02	68.82	71.5378	-2.7178
28	0.3	0.3	0.15	0.035	76.14	75.3839	0.7561
29	0.3	-0.1	0.15	0.035	69.34	68.4668	0.8732
30	0.1	0.1	0.1	0.05	69.13	71.1592	-2.0292
31	0.3	0.3	0.15	0.035	74.62	75.3839	-0.7639

Table 4
Analysis of variance for FPase production

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	7750.24	553.59	8.74	0.000
Linear	4	3605.28	901.32	14.23	0.000
X_1	1	1386.07	1386.07	21.88	0.000
X_2	1	2150.20	2150.20	33.95	0.000
X_3	1	2.68	2.68	0.04	0.840
X_4	1	66.32	66.32	1.05	0.321
Square	4	1979.41	494.85	7.81	0.001
X_1^2	1	894.28	894.28	14.12	0.002
X_2^2	1	257.94	2257.94	4.07	0.061
X_3^2	1	32.46	32.46	0.51	0.484
X_4^2	1	585.91	585.91	9.25	0.008
2-way interaction	6	2165.55	360.92	5.70	0.002
X_1* X_2	1	1248.26	1248.26	19.71	0.000
X_1* X_3	1	0.64	0.64	0.01	0.921
X_1* X_4	1	13.38	13.38	0.21	0.652
X_2* X_3	1	191.34	191.34	3.02	0.101
X_2* X_4	1	530.28	530.28	8.37	0.011
X_3* X_4	1	181.64	181.64	2.87	0.110
Error	16	1013.44	63.34		
Lack-of-fit	10	992.34	99.23	28.211	0.000
Pure error	6	21.11	3.52		
Total	30	8763.68			

X_1 = yeast extract, X_2 = peptone, X_3 = $FeSO_4$, X_4 = K_2HPO_4

Thakkar and Saraf employed PBD followed by CCD to get the best medium for the production of cellulase enzyme from *Bacillus amyloliquefaciens* MBAA3, which revealed an optimal concentration of $MgSO_4$ (0.275 g), CMC (1.84 g), and pH (8.5) in media for highest enzyme production.[13] In a recent study, Tabssum *et al.* used CCD followed by BBD for optimizing the production of cellulase from *Bacillus cereus*. Their results indicated maximum production of cellulase at 2% poplar waste biomass, 0.09% $MgSO_4$, 0.04% peptone, 0.5% yeast extract, initial medium pH of 9.0, and inoculum size of 2% v/v at 37 °C for 24 h of SmF, with agitation speed of 120 rpm.[34]

This indigenously produced cellulase was used for hydrolysis of raw seed pods of *B. ceiba*. Maximum saccharification was observed after 24

h (Fig. 7). As hydrolysis time proceeded beyond 24 h, a decline in saccharification was observed. Our earlier report found maximum saccharification of 38% and 28.4% in KOH steam pretreated and KOH pretreated *B. ceiba* after 24 h using indigenous cellulase.[34] Tabssum and others found maximum hydrolysis percent (11.5%) of poplar biomass using indigenous cellulase produced by *Bacillus cereus*.[35]

The biostoning process aims at giving denim a more uniformly aged appearance. In fact, along with an external mechanical agitation, crude cellulase is mainly responsible for an effective indigo dye removal and soft texture of denim fabric.[36] The activity of crude enzyme was compared with that of commercial enzyme, while distilled water was taken as control. The enzyme activity was determined periodically after each hour, *i.e.* from 1st hour to 4th hour, as described in Table 5. A significant and clear change in texture, as well in colour, was noticed (Fig. 8). Thus, the cellulase produced in the present work can be successfully used for enzymatic biostoning of denim fabrics (black, indigo, blue), which is an eco-friendly and efficient approach to denim finishing.

The results of our present study are in agreement with those reported previously in the literature, where cellulase produced by *Bacillus subtilis subsp. inaquosorum* was used for denim biostoning. The authors' findings showed that crude CMCase significantly increased denim weight loss percent and indigo dye removal percent, as compared with the buffer only treatment. This confirms the use of cellulase as an efficient and environmentally friendly option to replace chemicals and stones in denim finishing.[37]

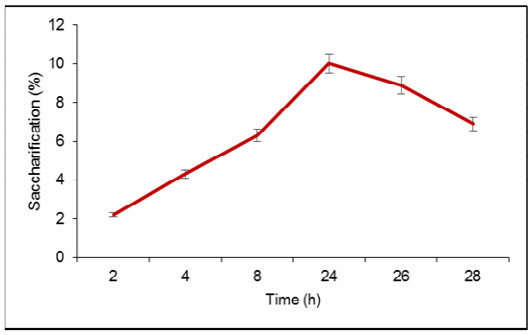

Figure 7: Saccharification of raw seed pods of *B. ceiba* using indigenous cellulase

Table 5
Effect of enzyme treatment on denim jeans (OD at 390 nm)

Sr. No	Colour	Control	Commercial	Indigenous
		1st Hour		
1	Black	1.125	1.112	1.147
2	Indigo	1.135	1.142	1.099
3	Blue	1.114	1.101	1.111
		2nd Hour		
1	Black	1.077	1.026	1.057
2	Indigo	1.100	1.027	1.027
3	Blue	1.090	1.056	1.061
		3rd Hour		
1	Black	1.083	1.064	1.077
2	Indigo	1.202	1.028	1.097
3	Blue	1.046	1.115	1.066
		4th Hour		
1	Black	1.121	1.106	1.110
2	Indigo	1.090	1.076	1.226
3	Blue	1.113	1.191	1.092

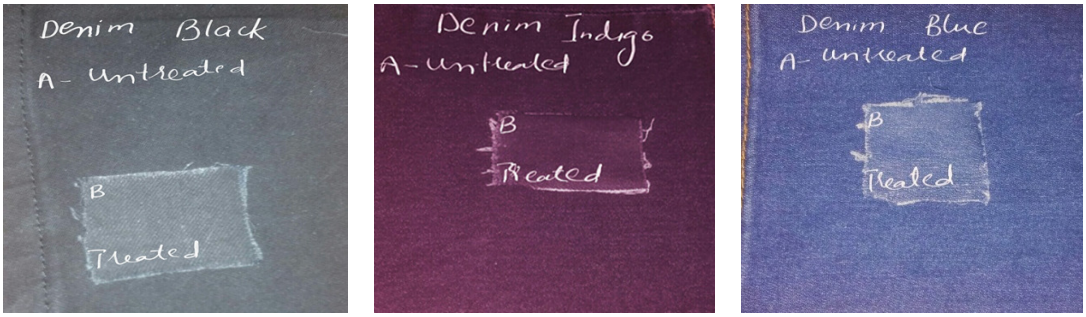

Figure 8: Biostoning of differently colored denim fabrics with indigenous cellulase

CONCLUSION

The present study used RSM to identify the optimum conditions for obtaining maximum cellulase production using *B. aerius*. The maximum cellulase production was found to be of 117.45 IU/mL/min under optimized conditions. The results achieved in this investigation demonstrated that *B. aerius* has cellulolytic potential and can easily be employed for industrial exploitation in saccharification of lignocellulose biomass for the production of biofuel. Also, the cellulase enzyme proved to be of interest in denim finishing treatments, specifically, in biostoning.

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