International Journal of Innovative Technology and Exploring Engineering (IJITEE)
ISSN: 2278-3075 (Online), Volume-9 Issue-7, May 2020

RSM based Process Modeling and Optimization for Amylase Enzyme Production and its Desizing Potential

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Abstract*: Enzyme technology is extensively touted as the way of the destiny for textile processing industry. Enzymes can be used safely in a wide selection of textile processes such as de-sizing, scouring, bleaching, dyeing, and finishing in textile processing. Recently, amylase enzyme is getting more important in textile industry for removal of starch. Amylase can be successfully applied at early pre-treatment stages, making a sturdy basis for the good finishing of fabrics. The present study was focused to isolate amylase producing bacteria and optimize the growth condition for maximizing amylase production using agro-industrial residues. In addition, immense important for RSM based optimization for the process parameters, pH, temperature, and agitation speed also given. Further, this study concentrated for determination of desizing potency of Denim by the amylase that produced at optimized condition.

***Keywords*: Agro-industrial residues, Amylase, Denim, Desizing potency.**

I. INTRODUCTION

In this day and age of design, denim possesses an extraordinary spot. The spread of denim culture, wherever all through the world, conveyed with it an example of rapidly developing designs. Denim pieces of clothing have likewise framed a remarkable piece of the attire send out the container from India. Denim is one of the world's most established textures, yet it remains unceasingly youthful. India has more than 10 remarkable denim fabricates and in excess of 300 denim article of clothing processors.

Revised Manuscript Received on May 30, 2020.
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Denim fabric is used in the different applications like furniture upholstery, curtains, and bedcovers. The denim fabric has become affordable, comfortable, and durable and offers a variety of style and color and gradually attains their way into our everyday lifestyle [1]. Pretreatments on textile processing are developing industry that customarily utilizes a great deal of water, vitality and brutal synthetic concoctions beginning from pesticides for cotton developing to the high quantity of wash waters that brings about waste streams inflicting environmental burdens.

The consciousness of natural effects of substance handling of materials, joined with progressively exacting enactment on modern emanating and buyer enthusiasm to utilize ecofriendly items has prompted the progressed, non-contaminating chemical process for textile materials, explicitly in pre Treatment Processing [2].

Present day society anticipates that biotechnology should be the response for some, overall issues like consumption of the ozone layer, a dangerous atmospheric deviation, narrow minded asset abuse, contamination of water and air, shortage of landfills and waste administration. Modern utilization of green technology is realizing new items and procedures at sustainable assets, just as the use of green advancements with low vitality utilization and earth solid practices. It is quickly picking up significance because of the different favorable circumstances it offers over ordinary contaminating chemical process. There is an increasing application of modern biotechnology in medicine and agriculture also the present pattern manages the capability of biotechnology in the material business. Textile industry being diversified in its activities is fast adapting to newer technological implementation review.

Application of biotechnology in textile wet processing opens up new horizon towards environmentally friendly technology [3]. It is rapidly gaining importance due to its eco-friendly nature and energy and water-saving potential. Technologies based on enzymes are broadly recommended for handling enterprises. This innovation is one approach to accomplish initiative in the conventional wet preparing region, by both securing nature and it lessens the necessities of vitality and damage synthetic concoctions. Enzymes can be utilized securely in a wide determination of pretreatment, for example de-sizing, scouring, Whitening, coloring, and finishing. It is proven that growths, microorganisms, ruminants, marine life forms and plants are wellsprings of catalysts.

Bacterial compounds are delivered by developing societies of certain bacterial microorganisms that have gotten one of the significant hotspots for the creation of mechanical size of chemicals. Various catalysts like amylase, cellulase, laccase, and catalase are utilized in pretreatment processing. Among those enzymes, amylases are getting more important in textile industry for removal of starch [4].

They can be effectively applied in the early stages of pretreatment, making a solid establishment for the effective completing of textures. Amylase specifically hydrolyzes starch to desize the texture with most extreme excellently.

In material preparing the materials are given wet treatment which is usually known as desizing that includes the essential expulsion of starch and fixings. It is by and large accepted that productive desizing is vital to the scouring, fading, and coloring. In desizing process, amylase enzyme has been utilized entrenched specialist for a long time since chemical desizing is eco-accommodating. The most extraordinary highlights of the catalyst desizing are the particular idea of compound activity, that is, it hydrolyzes starch yet doesn't delicate cellulose. Consequently, chemical desizing is more secure than traditional desizing [5]. At present, a more prominent consideration is being paid on the biotechnological capability of agro-mechanical buildups, for example, oil cakes, wheat grain, and rice wheat for their utilization as crude materials in the creation of significant worth included items, for example, chemicals, natural acids, and single cell protein.

Agro buildups endeavor to decrease the creation cost by advancing the utilization of agro deposits as source material and furthermore maintaining a strategic distance from or lessening exorbitant enhancement. Utilization of these agro-modern deposits in bioprocesses additionally takes care of contamination issues. Subsequently, the current examination was engaged to seclude amylase delivering microscopic organisms and enhance the development condition for boosting amylase creation utilizing agro-mechanical deposits.

Hence, the current examination was engaged to seclude amylase producing bacteria and optimize the growth condition for maximizing amylase production using agro-industrial residues. Further, this study concentrated for determination of desizing potency of Denim by the amylase that produced at optimized condition.

II. MATERIALS AND METHODS

A. Isolation of Amylolytic Bacteria

Aamylolytic microscopic organisms occur from various sources (plants, creatures, and microorganisms), they are principally delivered from the microorganisms, because of their better return and thermo-stable. Amylolytic microscopic organisms were secluded from rotted products of the soil vegetables tests that were gathered from nearby market and put away in a spotless polythene sack and kept at 4º C until disengagement method was completed. The foods grown from the ground were washed and ground with sterile water. 10 grams of each grounded test was weighed into 90 ml of sterile refined water independently. This weakening was made up to 10-8 weakening. 1ml of the example from every weakening was filled sanitized supplement agar medium (hamburger extricate 0.3g, NaCl-0.5g, peptone-0.5g, agar-2g, refined water-100ml) and hatched at 37 ºC for 24 hrs. To screen amylolytic microscopic organisms, very much developed states were picked and streaked on starch agar plates. The plates were hatched for 24 hrs. The zones shaped on starch agar plates were envisioned by flooding the plates with iodine arrangement. The segregates which demonstrated a zone of leeway more noteworthy than 3mm in distance across were thought to be amylolytic microscopic organisms and chosen for additional investigation [6].

B. Selection of Amylolytic Bacteria and Biochemical Characterization

The well gown amylolytic strains were further isolated and sub-cultured several times using nutrient broth to attain pure culture. Subcultures were carried out by inoculating (2% v/v) in the broth of nutrient for one day at 37 ºC in a shaker incubator. After attaining well grown culture, the culture was extracted at 5000 rpm for 20 minutes at 4 ºC in a refrigerated centrifuge. Amylase activity from each culture was determined in the cell-free supernatant obtained from the centrifuge. The isolate which delivered greatest movement was chosen and kept up on supplement agar incline at 4 ºC and sub-refined like clockwork interim. The chose microscopic organisms were described by various biochemical attributes including Gram's staining, motility and different tests.

C. Determination of Amylase Activity

Amylase activity was intended by the DNS method [9]. Enzyme assay was carried out by having 100µl of 1% starch solution and 20µl of enzyme with 80µl of 20 mM Tris-HCl of pH 8.0. The response was ended by including of 200µl 3, 5-dinitrosalicyclic corrosive arrangement and kept in the heated water shower for 5 minute .Then, the solution mixture was diluted by adding 2ml deionized water and finally absorbance was measured at 492 nm using colorimeter. As per the procedure adopted, According to the technique received, one unit of compound action is characterized as the measure of catalyst required for discharging 1 mg of decreasing sugar, comparable to maltose, per min under the ideal states of examine. To assess the particular movement of the catalyst, the given condition (1) was utilized. [7].

Specific activity =

[Total activity (U)] / [Total Protein (mg)] ……. (1)

D. Identification of Primary Impacts on Enzyme Production Medium

Impact of inoculum concentration on amylase production was identified by using different inoculum concentration ranging from 1% to 5% (v/v). Each concentration was inoculated in the nutrient broth and incubated at 37 ºC for 24 hrs. After the incubation time, the cell growth was examined and the culture medium was centrifuged in refrigerated centrifuge at 4 ºC. The cell-free supernatant was taken for the determination of amylase activity. To identify suitable carbon source on amylase production, carbon sources such as maltose, fructose, lactose, glucose, and starch were selected and examined for their individual influence on cell growth and amylase production.

For that, 1% (w/v) of each carbon source was supplemented with production medium. 2% (v/v) overnight grown culture from mother culture was inoculated in a nutrient medium containing different carbon sources. Further, the cultures were incubated at 37 ºC for 24 hrs.

From theses flasks, the samples were harvested and supernatants were used for enzyme assay after centrifugation at 5000 rpm at 4 ºC in a refrigerated centrifuge. Different nitrogen sources such as yeast extract, beef extract, peptone, ammonium sulfate, ammonium oxalate, casein, ammonium chloride, potassium nitrate, ammonium nitrate and urea were also examined for their effect on amylase production by replacing peptone (0.5 %) in the production medium. The culture medium was incubated at same growth condition. Then, free-cell supernatant from individual flask was taken for assessing the enzyme activity [8].

E. Response Surface Method

One of the widely used statistical analyses is RSM. That can build an equation of model for a process. In addition, using this model, the improved operating conditions can be identified using measurable statistics from the suitable investigations [9]. RSM can provide the functional polynomial relationship among the response (Y) and the selected variables. By solving this model, the best (maximum) value for the response can be projected. A model of polynomial equation that developed by RSM is given below:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \varepsilon$$

Here $X_1, X_2, \ldots, X_{ii}$ are the independent factors response; n refers number of independent variables, b_0 is the constant, b_{ii} and b_{ij} are the quadratic and interaction coefficients, respectively. ε denotes the random error. Here, the response, Y = amylase enzyme activity and the chosen independent parameters are initial pH, temperature, and agitation speed.

The Central Composite Design is the common experimental design that can be used for the RSM. The possibilities of investigations of the chosen factors are coded to fix at ±1 for the factorial levels, ±β for the axial levels, and 0 for the centre levels. The codes can be calculated from the elements of the scope of enthusiasm of each independent parameter (Table 1). To follow the CCD experiments, the central-levels of selected factors (temperature, pH, and agitation speed) were taken from the different literature observations. In this study, the central-level value of parameters was considered as 6 for the pH, 37 °C for the temperature, 180 RPM for the shaking speed.

Table I. The codes and actual value relationship

Code value	Actual value
+β	M_{max}
+1	$[(M_{max}+M_{min})/2]+[(M_{max}- M_{min})/(2\lambda)]$
0	$(M_{max}+M_{min})/2$
−1	$[(M_{max}+M_{min})/2]-[(M_{max}- M_{min})/(2\lambda)]$
−β	M_{min}

F. Optimization of the parameters for Enzyme production based on CCD

The current investigation was planned to determine the effect of process parameters, pH, temperature, and agitation speed on the production of maximum quantities of amylase enzyme at the appropriate medium. The medium had maltose as carbon source (1% w/v), peptone as nitrogen source (1% w/v), inoculum size (2% v/v), NaCl (1% w/v). 250 ml Erlenmeyer flasks (containing 100 ml of medium) was vaccinated with the overnight old culture, and incubated at selected temperatures (since the temperature as the optimization parameter). The pH of the medium was adjusted to different predetermined values using phosphate buffer. After 3 days, samples (10 ml) were collected.

The microbe cells were centrifuged (3000 rpm for 25 min). The cell-free supernatant was used for enzyme assay. The three important parameters that show a major influence the enzyme production have been chosen for this study which includes pH, temperature, and agitation speed. The studies were designed using CCD. The ranges for each selected parameters are given in Table II. The groupings of chosen parameters based on the CCD design of experiment are given in Table III. Experimental outcomes were made to a model equation for enzyme activity. ANOVA was used to analyze the confidence level of the acquired polynomial model. The Design-Expert 8.0.0 (Stat-Ease, Inc., Minneapolis, USA), a common well known software package, was used to express the surface and contour plots, and the regression analysis.

Table II. The values of experimental range using CCRD

Parameters	Units	-2	-1	0	+1	+2
pH		2	4	6	8	10
Temperature	°C	33	35	37	39	41
Agitation Speed	RPM	140	160	180	200	220

G. Evaluation Desizing Potential of Amylase Enzyme

- **Fabric selection**

Denim fabrics have undergone a massive facelift with distinguishable appearance and finish combined with casual look. Denim has made their way into our everyday life [10]. Today, millions of people still wear jeans to work, not only to the mines, but also into the board rooms. In denim garment processing, desizing is the first step which enables the garment to properly receive subsequent chemical and mechanical treatments by removing the previous applied warp size and finishes confirms. In this study denim was taken as fabric to evaluate the desizing potential of obtained enzyme. The fabric used this study was procured from KG Denim, Coimbatore, India.

H. Determination of ideal condition for biodesizing

Various parameters such as pH, temperature, concentration of enzyme, and treatment time were optimized for effective desizing of the fabric. Desizing of denim fabric with five different enzyme concentrations were carried out. The concentrations of crude enzyme are 6, 12, 18, 24, and 30 with material liquor ratio of 1:30 (v/v) to water. For biodesizing, a pH value - 6 and temperature - 37 ºC was maintained for 24 hours. After the desizing treatment, the samples were removed and, washed, further were tested for starch content by adopting the Iodine test. Each desized sample was cut into 2×2 cm pieces. 0.005 % (w/v) Iodine solution was taken in the burette, which was fixed with the burette stand. The sample to be tested was placed below the burette tip. Iodine solution was dropped on the fabric, and then the sample was placed onto the clean white surface. Based on the colour developed, starch presence (appearance of deep blue) or absence (appearance and yellow colour) could be detected. Similarly, the other samples were optimized for amylase concentration. Desizing of denim was carried out at optimized concentration with constant pH 6 and temperature 37 ºC, for different time intervals like 6, 12, 24, 36 and 48 hrs. After the chosen incubation period of treatment, the fabrics were washed and dried and then it was tested with iodine solution for starch content. Appropriate value of pH for desizing the denim was determined by keeping the sample in desizing bath which maintained at different pH values. The chosen values of pH are 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0. The results obtained optimized enzyme concentration, incubation time, and at constant temperature of 37 ºC were used for the desizing test. The pH at which maximum removal of the starch acquired was identified as the ideal pH for desizing. Additionally, the enzymatic treatment for biodesizing was carried out at different temperatures such as 27, 37, 47 and 57 ºC. These tests were taken at optimized enzyme concentration, incubation time and pH. The temperature at which the removal size attained at maximum was designated as ideal temperature.

I. Enzymatic Treatment for Desizing of Denim

The desizing bath was prepared by 1:30 material liquor ratio at 37 ºC with pH 6. The fabric was immersed in the desizing bath and gently agitated in longitudinal shaker for 24 hrs. After treatment, the temperature of the bath was raised to 100 ºC to deactivate the enzyme. Further, the samples were subjected to one hot wash at boiled water and one cold wash for 10–15 min. Washed fabrics were dried in room temperature over a night .

J. Desizing Using Commercial Enzyme and Conventional Method

The commercial enzyme Tinozyme-LL10 was purchased from Enzyme India, Bangalore. Commercial desizing of denim fabric A was carried out with material liquor ratio 1:8 at 55º C for 2 hrs with 2% enzyme on weight of the fabric. After treatment, the fabric was washed with hot and cold water. The temperature was raised to 100º C to deactivate the enzyme as per the procedure [11]. Similarly, the other samples, B and C were subjected to desize with commercial enzyme. Dilute acid attacks the polymer chain of starch and due to chain cleavage of starch molecules owing to short water soluble or dispersible chain segments are formed. One meter of sample A was cut and weighed. Material liquor ratio was maintained as 1:15. Soft water was used for better desizing and 1% acetic acid was added to the bath. Temperature was raised to 55 ºC sample A was wetted and dipped into desize bath and was incubated for 4 hours. After treatment, the fabric was washed thoroughly. Similarly the other samples B and C were also desized.

K. Evaluation of Desized Fabric

Testing techniques in textile process is a needed aid in the distribution, production, and consumption of textiles. The right progression of action may be taken that result of testing. Thus, it can be said that the testing is a means to get effective finishing of the product.

Table III. Groupings of variables based on the CCD

Run	Combinations of variables		
	pH	Temp	RPM
1	6.00	37	180
2	8	35	160
3	6	33.64	180
4	2.64	37	180
5	6	37	146.36
6	8	35	200
7	4	39	160
8	8	39	160
9	6	37	180
10	8	39	200
11	6	40.36	180
12	4	39	200
13	4	35	200
14	6	37	180
15	6	37	180
16	6	37	180
17	6	37	180
18	6	37	213
19	4	35	160
20	9.36	37	180

L. Iodine Test for Starch

A portion of the processed cloth is treated with a drop of 0.005 N of the prepared iodine solution and the colour developed after 30 seconds was noted.

With starch, iodine gives deep blue -black colour, which falls to blue and then to brown, brownish yellow, and yellow as the starch is broken down. A pale yellow brown indicates complete breakdown of starch. The test is extremely sensitive and it will be positive even for traces of starch residue. The desized samples A1, A2, A3, B1, B2, B3, C1, C2, and C3 were placed over clean white chart and a drop of 0.005 N solution was allowed on the fabric and the color after 30 seconds was naturally evaluated [12].

M.Weight Loss Test

The weight of the size accounts approximately to 10% of the fabric weight. Thus the measurement of weight loss is the quantitative assessment of the size for the fabric.

N. Wettability Test

The wettability includes drop test and sinking time tests were carried out as per the following procedure.

O. Drop Test

The capacity of a fiber to take up dampness is named as receptiveness. Wettability is the time taken in seconds for a drop of water to sink into the texture [13]. On the off chance that any texture takes over 200 seconds to ingest water the equivalent is considered as un-wettable. A burette loaded up with refined water was clasped in a stand. The example was mounted in a weaving outline and was set at the base of the stand. The separation between the example and burette spout was kept consistent. The spout of the burette was opened just to permit a drop of water to fall on the example. The stopwatch was begun all the while and it was halted when the drop of water completely sank into the material. The time taken for this was noted. A similar method was rehashed for multiple times for the first and treated example and the mean worth was determined and recorded.

P. Sinking Time Test

Wickability is characterized as the time in seconds for a drop of water to sink into the texture. The capacity of a texture to assimilate water particularly by a wicking or slender activity might be found by timing the rate at which clusters up a limited piece of texture suspended vertically with its lower and plunged into the water .A rectangular strip of sample A with 1cm width and 7 cm length was marked to the fabric and cut. Immersed 1 cm into the water by clamping 5 gms of weight observed the time taken from the water to travel for each cm and the time was recorded. Wickability was tested in both warp and weft direction.

III. RESULTS AND DISCUSSION

A. Screening and Isolation of Amylolytic Bacteria

Serial dilution technique was performed on nutrient agar plates to isolate bacteria from spoiled food waste. Fifty two well grown colonies were obtained. These bacterial colonies were plated on starch agar medium to isolate amylolytic bacteria. The zones of growth and hydrolysis of the medium was measured. Only 20 isolates were found to show amylolytic activity. Out of 20 isolates, nine isolates showed good hydrolysis ratio (>3.0), six isolates showed moderate (>2.0) and five showed poor hydrolysis ratio (Table-IV). Amylase activity was determined for the nine isolates and the result were presented. Out of the nine isolates S_4 was found to produce maximum amylase activity and hence S_4 isolate was selected for the present study.

Table IV - Growth and hydrolysis ratio of the isolated bacterial strains from spoiled food waste

S. No	Growth: Hydrolysis Ratio	Number of colonies
1	3.0 - 4.0	9
2	2.0 – 3.0	6
3	0.5 - 2.0	5

Table V - Amylase activity of the selected isolates after 24 hrs of incubation

S. NO	Colonies	Enzyme Activity (IU/ml/min)
1	S1	0.093
2	S2	0.128
3	S3	0.123
4	S4	1.456
5	S5	1.043
6	S6	0.096
7	S6	0.063
8	S7	0.124
9	S8	1.042

B. Characterization of Selected Bacteria

Based on the colony morphology, Gram staining and biochemical characteristics, the isolated bacterium were identified as *Bacillus* sp. Table – III depicts the biochemical characteristics of the selected strain. Figure illustrates the biochemical characteristics of the selected isolate.

Table VI - Morphological and biochemical characteristics of selected isolate

Parameters	Characteristics
a. Cellular Characteristics	
Morphology	Straight, Rod shape
Staining Characteristics	Gram positive
b. Cultural Characteristics	
Nutrient Agar Colonies	Finger like projections
c. Motility	Motile
d. Biochemical Characteristics	
Indole Production	Negative
Methyl Red	Negative
Voges Proskauer	Positive
Citrate utilization	Negative
Catalase	Positive
Urea Hydrolysis	Positive
Oxidase	Positive
Nitrate Reduction	Positive
Gelatin Hydrolysis	Positive
Starch Hydrolysis	Positive

C. Influence of Inoculum Concentration on Amylase Production

The effect of inoculum concentration on enzyme production by *Bacillus* sp. was detected by activity and specific activity of the enzyme. The same is given in Figure I. The maximum amylase production was observed at an inoculum concentration of 2 % (2.31 IU/ml/min). Based on the obtained results the optimum inoculum concentration was used for maximum production of amylase. A higher inoculum concentration decreases the enzyme activity.

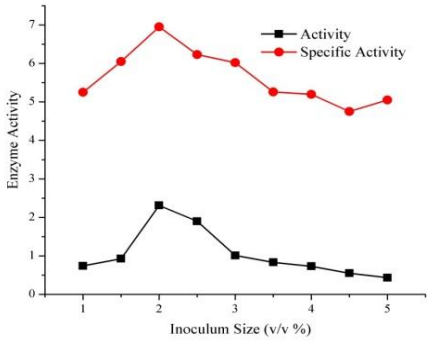

Fig. 1 Influence of inoculum concentration on amylase production

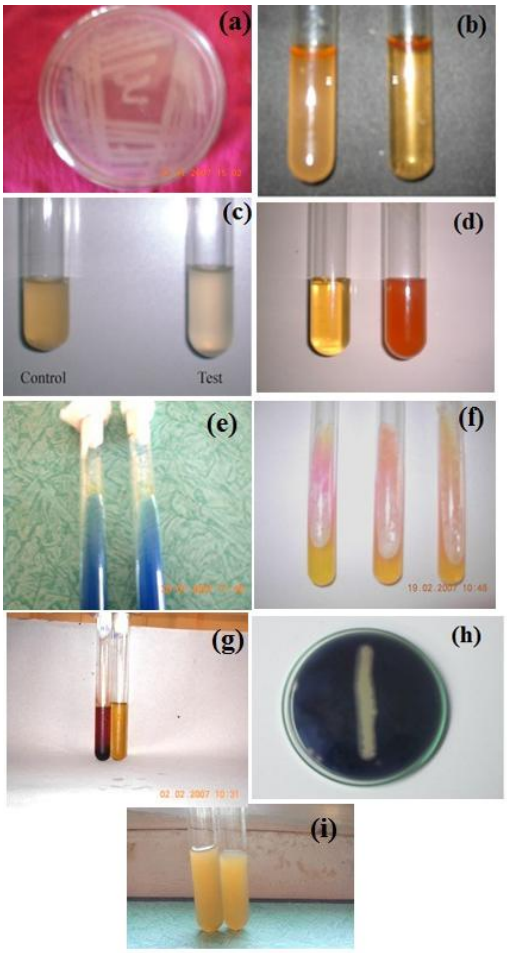

Fig 2. Biochemical characteristics of bacillus species

D. Influence of Carbon Sources on Amylase Production

An influence of carbon on enzyme production is quite significant. Generally, starch is the most widely utilized substrate. Various carbon sources such as soluble starch, lactose, glucose, sucrose and maltose were evaluated for their effect on amylase production in the production medium. The isolated strain showed high enzyme yield in maltose supplemented production media followed by starch and fructose. The results are shown in Figure III. The presence of concentration of the carbon source fermentation medium is essential for the effective growth and production of enzymes. Maltose served as a good substrate for enzyme synthesis [14,15].

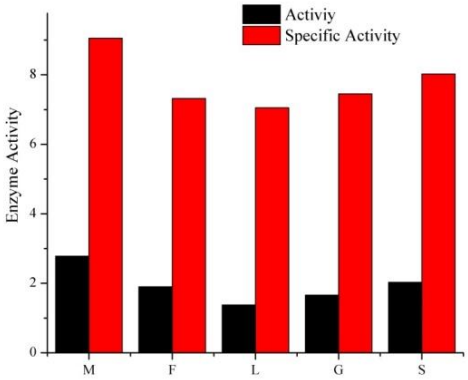

Fig 3. Influence of carbon sources on amylase production M-Maltose, F-Fructose, L-Lactose, G-Glucose, S-Starch

E. Influence of Supplemented Nitrogen Source for Amylase Production

The effect of different nitrogen sources registered at several reports. The relative concentration and nature of sources are also getting important for the production of amylase. Effect of different nitrogen source on amylase production was tested and summarized in Fig 4. From the results, it is obvious that the maximum amylase activity was observed in production medium which supplemented with peptone.

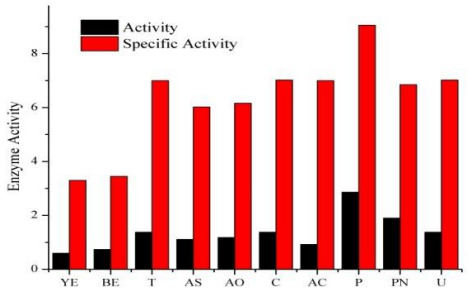

Fig 4. Influence of supplemented nitrogen source for amylase production

F. Model Construction

Table IX shows the results of the combination of selected parameters from the results of CCD experiments.

The outcomes were formed to a polynomial model of equation. This equation of model expressing the enzyme activity (E) was expressed in terms of pH (A), temperature (B), and rotational speed (C). That can be written as follows:

$$E= -18430.543+163.231A +768.398B+44.976C-656.25\times10^{-3}AB+0.040625AC-0.209375BC -12.177A^2-9.8793B^2-0.103655C^2 \ldots (2)$$

Table VII: Observed data on Lack of Fitness Test

Source	Sum of squares	df	Mean Square	F Value	p-Value Prob>F	
Linear	6.88	11	0.63	3352.49	< 0.0001	
2FI	6.82	8	0.85	4566.99	< 0.0001	
Quadratic	**4.064E-003**	**5**	**8.128E-004**	**4.35**	**0.0661**	**Suggested**
Cubic	1.723E-004	1	1.723E-004	0.92	0.3808	Aliased
Pure Error	9.333E-004	5	1.867E-004			

Table VIII: Summary of Statistical analysis for the Model

Source	Standard Deviation	R-Squared	Adjusted R-Squared	Predicted R-Squared	Press	
Linear	0.66	0.0163	-0.1681	-0.3222	9.25	
2FI	0.72	0.0255	-0.4243	-1.4926	17.45	
Quadratic	**0.022**	**0.9993**	**0.9986**	**0.9953**	**0.033**	**Suggested**
Cubic	0.014	0.9998	0.9995	0.9944	0.039	Aliased

The insignificance and significance properties of the Eq. 2 were examined by carrying out the analysis of Variance (Table X & XI). The value of coefficient of determination was found to be (R^2) 0.9993. That clears that the regression was observed to be significant. It reveals that the model for the enzyme activity is statistically accurate.

The high value of the R_{adj}^2 (0.9986) shows that the model is significant. In the polynomial model, linear terms coefficient (except pH and agitation speed), interaction terms coefficients (except pH-temperature and pH-agitation speed), and the square terms are observed to be good significant. Coefficient of Variation (CV) is determined to be 1.32% (<10%). Thus, the observed results from experiments were accurate (From table VII & VIII).

The value of "lack of fit" (>0.05) indicated as insignificant. It implies that the equation is statistically good for further applications [16,17].

I. Response Surface Plots and Contour Plots

The interactive effect between the chosen parameters with enzyme activity was optimized through RSM. Investigations were carried out using surface (3D) and contour (2D) plots. The influence of the selected variables, pH (A) and temperature (B) in the range of 4 - 8 and 35-39 ºC respectively, on the enzyme activity in the 3D surface plot is given in Fig 5(a) and contour plot in Fig. 5(b).

It is remarkably apparent that there is a combined effect of pH (A) and temperature (B) at constant agitation speed of 180 RPM. Under this condition, the maximum enzyme activity of 259 U/ml was occurred. The elliptical plot informs that interaction was significant.

The maximum yield of enzyme activity at optimum pH specifies about the pH sensitiveness of enzyme. The influence of variables pH (A) and agitation speed (C) in the range of 4-8 and 160-200 on enzyme activity is shown in Fig. 6. As per these observations, enzyme activity increased when the medium pH is increased.

The maximized activity (257.89 U/ml) occurred at the optimum levels at the pH of 6.27 and agitation speed of 181.23 RPM. Reduced enzyme activity was found beyond the observed optimum levels.

Table IX. Amylase enzyme activity results from CCD

Run No	Experimental Results (U/ml)	Predicted Value (U/ml)
1	257	257.36
2	127	126.01
3	158	160.04
4	121	119.14
5	134	134.81
6	154	152.29
7	126	128.29
8	121	120.33
9	255	257.36
10	111	113.10
11	134	131.13
12	113	114.57
13	142	143.26
14	257	257.36
15	258	257.36
16	259	257.36
17	258	257.36
18	147	145.36
19	125	123.48
20	119	119.14

Circular projection of the contour plot revealed that the interaction between the pH and agitation speed was provided notable influence in enzyme production. The interactive impact from the surface plot (3D) between agitation speed (C) and temperature (B) on enzyme activity at a fixed initial pH (value 6) is given in Fig. 7. The elliptical figure of the contour plot revealed that mutual interaction between agitation speed (C) and temperature (B) was quite good.

Table X: Analysis of variance for the acquired polynomial model

Source	Sum of Squares	df	Mean Square	F Value	p-Value Pron>F	
Model	6.99	9	0.78	1555.05	<0.0001	Significant
A (pH)	9.683E-005	1	9.683E-005	0.19	0.6692	
B (T)	0.10	1	0.10	201.82	<0.0001	
C (RPM)	0.013	1	0.013	26.92	0.0004	
AB	5.513E-003	1	5.513E-003	11.03	0.0077	
AC	2.112E-003	1	2.112E-003	4.23	0.0668	
BC	0.056	1	0.056	112.28	<0.0001	
A^2	3.42	1	3.42	6842.07	<0.0001	
B^2	2.25	1	2.25	4503.30	<0.0001	
C^2	2.48	1	2.48	4957.40	<0.0001	
Residual	4.997E-003	10	4.997E-004			
Lack of Fit	4.064E-003	5	8.128E-004	4.35	0.0661	Not significant
Pure Error	9.333E-004	5	1.867E-004			
Cor Total	7.00	19				

Table XI: Regression values of the model

Standard Deviation	0.022	R^2	0.9993
Mean	1.69	Adj R^2	0.9986
C.V %	**1.32**	Pred R^2	0.9953
PRESS	0.033	Adeq. Precision	91.257

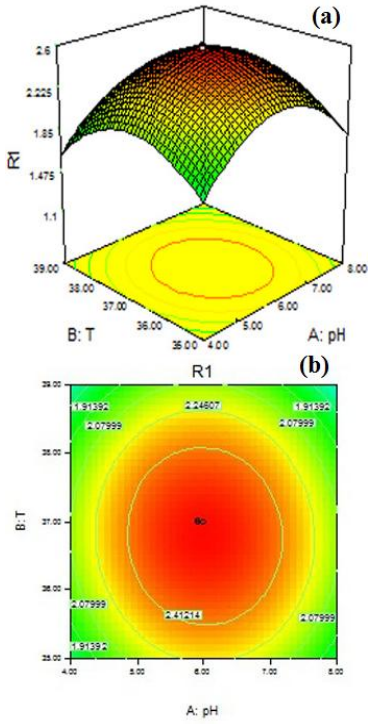

Fig. 5

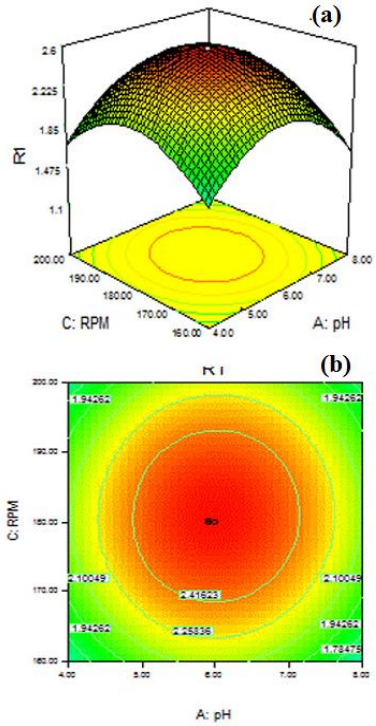

Fig. 6

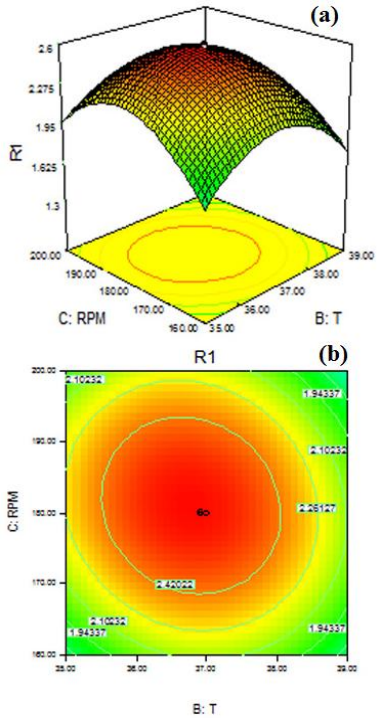

Fig. 7

Fig 5-The Interactive effect of temperature and pH, Fig.6-The Interactive effect of agitation speed and pH, and Fig.7- The Interactive effect of agitation speed and temperature on enzyme production

J. Desizing using crude enzyme

The enzyme desizing was very effective method. The desizing bath was prepared by 1:30 material liquor ratio at 37º C and pH 6. The fabric was immersed in the desizing bath. After 24 hr, the temperature of the bath was raised to 100 ºC to deactivate the enzyme. Finally, the samples were given one hot wash at boil for 10 – 15 min, one cold wash and dried [17].

Table XII-Enzymatic desizing

Sample	Fabric wt (g)	Desize bath (ml)	Material: Liquor ratio	Crude enzyme concentration (%)	Vol. of enzyme (ml)	Vol. of distilled water (ml)
A_0	125	3750	1:30	60	2250	1500
B_0	142	3408	1:24	60	2088	1320
C_0	102	3060	1:30	100	3060	-

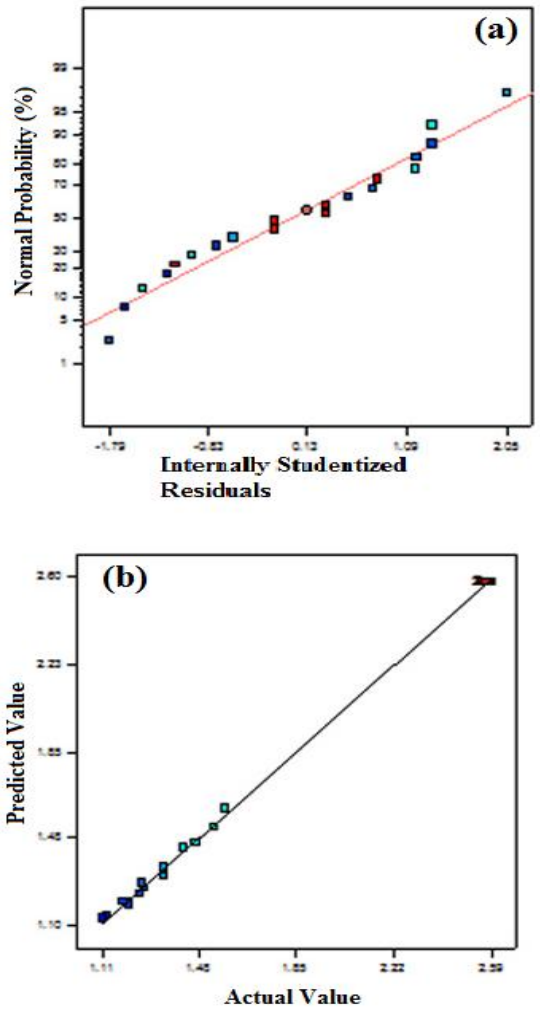

Fig 8: Normal probability vs internally studentized residuals (b) Predicted vs actual values

K. Fabric Evaluation

- **Iodine test of starch**

Iodine test was carried out on the treated samples. The absence of blue colour indicates efficient desizing. The treated samples did not show blue colour which directly indicates efficient desizing process.

L. Wettability Test

- **Drop test**

From the Table - IV it is clear that the absorbency time is less in all treated samples over their originals. Maximum difference in absorbency time is noticed in sample A1, B1 and B2. Where, minimum difference is seen in samples C2 and C3. From the results it could be concluded that the treatment has effectively reduced the absorbency time of fabric and particularly crude enzyme treated samples have significant difference in wickability of denim fabrics.

From Table VII it is obvious that absorbency time is less in all treated samples over their originals. Maximum difference in absorbency time is noticed in the sample A3, B1, B2, C1 and C3. Where, minimum difference is seen in sample A2. From the results it could be concluded that the treatments has reduced the absorbency time of fabric. Hence it is proved that the enzyme treatment particularly crude enzyme has great significant difference in Wickability of denim fabrics.

Table XIII – Observations from drop test

S. No	Sample	Mean Absorbency (in sec)	Gain / loss over original	% Gain loss over original
1	A0	150	-	-
2	A1	100	50	33.3
3	A2	109	41	27.3
4	A3	102	48	32
5	B0	165	-	-
6	B1	122	43	26.06
7	B2	130	35	38.6
8	B3	132	33	20
9	C0	120	-	-
10	C1	105	15	12.5
11	C2	109	11	8.8
12	C3	108	12	10

IV. CONCLUSION

Textile industry being diversified in its activities is fast to adapt to newer technological implementations. Application of biotechnology in textile industry is rapidly intensifying. With the advent of neoteric technological implementation into the textile processing industries, it is making inroads into the fashion industry at a vast place. Bio-preparation process decreases both effluent load and water usage to the extent that the new technology becomes an economically viable alternative.

Hence this study, which was based upon the use of enzymes for desizing has thrown light on reduction of cost, time and energy. Use of enzymatic methodology in the modern part not just decreases load on the profluent by keeping away from concoction utilization yet in addition improves quality separated from giving a sheltered working environment. It is normal that utilization of chemicals will increment in not so distant future since it can limit negative ecological impact which is the need of hour. The results of the study prove the potentiality of enzymes in producing green label products, and to solve environmental issues.

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