Optimization Of Xylanase Production By *Bacillus Substilis* Using Response Surface Methodology

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Abstract
Improved production of enzymes by microorganisms is dependent on various factors including nutritional, physiological parameters besides the biochemical nature of the microbial strain involved. This part of the study emphasizes on optimization of various parameters which could influence the productivity and yield of xylanase enzyme from *Bacillus subtilis*. An attempt was made to optimize both physical and nutritional parameters which play a major role in enhancing the productivity of the enzyme. Both cultural and medium parameters were optimized to yield highest xylanase production. The variables having the most relevant effect on xylanase yield were identified using a 2-level Plackett-Burman design and their levels were further optimized for enhanced xylanase production by employing a Box-Behnken design. Multiple trials were conducted as suggested by the model and all retrieved data was analysed for variance by ANOVA. The experiment was carried out in triplicate to estimate the experimental errors and to test for lack- of - fit of the data using the second-degree polynomial model. The results of statistical analysis shown that the 95% confidence levels of Xylose, Yeast extract, MgSO4, K2HPO4, KH2PO4, temperature, rpm, incubation time and pH were 5.85, 6.19, 4.61, 0.3819, 0.4212, -5.01, 0.4507, 6.23 and 6.05 respectively, which are considered to significantly influence xylanase production. The Xylanase activity by using all the optimized parameters was 141.23 U/mL.

Keywords: Optimization, xylanase, *Bacillus subtilis,* Plackett-Burman design, Box-Behnken design, ANOVA

INTRODUCTION

Xylan is a complex heteropolysaccharide consisting of different monosaccharides such as L-arabinose, D-galactose, D-mannoses and organic acids such as acetic acid, ferulic acid, glucuronic acid interwoven together with help of glycosidic and ester bonds. The breakdown of xylan is restricted due to its heterogeneous nature and it can be overcome by xylanases which are capable of cleaving the heterogeneous β-1,4-glycoside linkage. Xylanases are abundantly present in nature (e.g., molluscs, insects and microorganisms) and several microorganisms such as bacteria, fungi, yeast, and algae are used extensively for its production.

The genus *Bacillus* has been studied more extensively among bacterial xylanases. *Bacillus* species are industrially important bacteria due to their rapid growth rate and for their capacity to secrete important extracellular enzymes and proteins in the medium. Currently, cellulase-free xylanases are playing the most important role in paper and pulp industries (prebleaching of kraft pulp) in order to reduce the use of toxic chlorine chemicals and the enzymes are also found to be effective in saccharification process.

The commercial application of cellulase-free xylanase in various industrial processes is very limited due to partial hydrolysis of substrate, thermal and pH instability of enzyme, and time dependent enzyme production. The maximum production of xylanase with cost effective way in less time period can be achieved by employing alternate strategies of culture medium optimization. Fermentation medium optimization plays a critical role in enhancing the production yields of the industrially important enzyme. Optimization for enhanced production of enzyme depends upon medium components like carbon source, nitrogen sources, pH, temperature, and agitation and incubation time.

Response surface methodology (RSM) is a statistical strategy to improve enzyme yield by designing minimum number of experiments for large number of factors. RSM explains the combined effects of all the independent variables in a fermentation process and explores an approximate interaction between a response variable and a set of design independent variables.

The Plackett-Burman design is a widely used statistical technique for screening and selection of most important culture variables from a multivariable medium. This design is a tool for preliminary optimization and evaluation of the importance of different medium components.

Analysis of Variance (ANOVA) is a statistical formula used to compare variances across the means (or average) of different groups. A range of scenarios use it to determine if there is any difference between the means of different groups. The basic principle of ANOVA is to test for differences among the means of the populations by examining the amount of variation within each of these samples, relative to the amount of variation between the samples Improved

production of enzymes by microorganisms is dependent on various factors including nutritional, physiological parameters besides the biochemical nature of the microbial strain involved. This part of the study emphasizes on optimization of various parameters which could influence the productivity and yield of xylanase enzyme from *Bacillus subtilis*. An attempt was made to optimize both physical and nutritional parameters which play a major role in enhancing the productivity of the enzyme. Both cultural and medium parameters were optimized to yield highest xylanase production.

MATERIALS AND METHODS

Optimisation studies by Response Surface Methodology

The selected isolates were cultured in liquid xylan media with the same composition as birchwood xylan medium, except agar is not added. The fermentation media consists of (g/L): Birchwood xylan 2.5g, Yeast extract 5g, Peptone extract 5g, $MgSO_4$ 0.2g, and K_2HPO_4 1g. The enzyme production was carried out in 100 mL Erlenmeyer flasks containing 20 mL liquid Birchwood xylan media and incubated overnight in a shaking incubator for 24h at 37°C at 150 rpm. To determine the xylanase activity, it is important to obtain the cell-free extract. The xylanase extraction was done to determine the activity of the enzyme. About 2 mL of sample from the culture media was taken and centrifuged at 10000 g for 10 minutes at 4 °C (Cooling centrifuge) then, the supernatant was collected and the pellet was discarded. The collected supernatant was then used for extracellular enzyme assay for testing xylanase activity.

Optimisation by Plackett- Burman Design

For screening purposes, various medium components and culture parameters have been evaluated. Based on the Plackett–Burman factorial design, each factor was examined in two levels:-1 for a low level and +1 for a high level. Plackett–Burman experimental design is based on the first order model: $Y = \beta_0 + \Sigma\beta_i x_i$

Where, Y is the response (enzyme activity), β_0 is the model intercept and β_1 is the linear coefficient, and x_i is the level of the independent variable. This model does not describe interaction among factors and it is used to screen and evaluate the important factors that influence the response. In the present work, nine assigned variables and one dummy variable were screened in twelve experimental designs.

The inoculated culture flasks were incubated at different operational conditions and the extent of xylanase action was evaluated as described above. Optimization of medium constituents to improve xylanase activity was carried out initially using Plackett-Burman design considering nine variables viz, xylose (1-10 g/L); $MgSO_4$ (0.01-0.1g/L); K_2HPO_4 (0.1-0.5 g/L); KH_2PO4 (0.1-0.5g/L); yeast extract (0.1-1g/L); pH (5-10); incubation time (24-120 hours), rpm (100-200) and temperature (25-50°C) as depicted in table 2.1.

The variables having the most relevant effect on xylanase yield were identified using a 2-level Plackett-Burman design and their levels were further optimized for enhanced xylanase production by employing a Box-Behnken design. Multiple trials were conducted as suggested by the model and all retrieved data was analysed for variance by ANOVA. The experiment was carried out in triplicate to estimate the experimental errors and to test for lack- of - fit of the data using the second-degree polynomial model.

Optimization of cultural parameters

Effect of incubation period

The effect of incubation period on production of xylanase was studied by growing the isolates in basal production medium and incubating for varying intervals; 24 and 120 hours. Growth profiles of the isolates were studied simultaneously by determining the absorbance of the culture filtrate in order to determine the stages of growth. The samples were processed for crude enzyme preparation and used for determination of xylanase activities and total protein content in order to study if any relationship existed between their growth patterns and enzyme production.

Effect of pH

The effect of initial medium pH on production of xylanase was studied by growing the isolates in basal production medium whose initial pH was adjusted after autoclaving. The study was undertaken in the pH of 5 and 10 with an increment of 1.0 pH unit. Following completion of appropriate incubation periods, the crude enzyme was used for estimating xylanase activity and total protein

Effect of temperature

Xylanase production at varying temperatures was evaluated to determine the optimum temperature. 20 ml of sterile basal medium after inoculation was incubated at different temperatures of 25ºC and 50ºC. Crude enzyme was obtained from each of the flasks after incubation and thereafter subjected to analysis of xylanase activity and total protein.

Effect of shaking conditions

The study on the effect of static or shaking conditions on production of xylanase from the selected bacterial isolates was performed by incubating 20 ml of basal production medium after inoculation in an orbital shaking incubator (Rovitek, Mumbai) at varying agitation speeds of 100 rpm and 200 rpm. All the other cultural parameters were maintained at their optimized levels. Control flasks were set up at static conditions keeping all the remaining parameters the same. Crude enzyme extracts were prepared and subjected to xylanase assay and also the total protein was evaluated. The effect of

shaking on enzyme production was assessed by comparison against activity obtained from static conditions.

Optimization of nutritional parameters

Effect of carbon source

Basal medium containing birch wood xylan as the carbon source was replaced by a variety of other carbon sources in order to evaluate the potential of each individual carbon source to produce optimum levels of xylanase. The carbon sources investigated in the study included simple monosaccharide sugar xylose. Basal medium containing 1% xylan was considered as the control. All flasks after inoculation were incubated and thereafter subjected to crude enzyme preparation which was used for xylanase assay and protein determination. All the other physical factors were kept at their optimum levels.

Effect of nitrogen source

The impact of various nitrogen sources consisting of both inorganic and complex organic compounds were evaluated on xylanase production from the bacterial isolates. The organic nitrogen source tested was yeast extract. The media used for investigating the most optimum nitrogen source contained optimum carbon source and equimolar concentration of each of the nitrogen sources tested which replaced peptone and yeast extract of the basal medium. All the remaining cultural conditions were maintained at their respective optimum levels. Enzyme activities were evaluated from the crude enzyme enabling selection of the most suitable nitrogen source with the ability to induce maximum xylanase production.

Effect of metal salts

Metal salts are important micronutrients for growth and production of enzymes by microorganisms. In order to determine if any metal salt was effective in inducing maximum xylanase production from the selected bacterial isolates, a study was done to evaluate the effect of several metal salts which included MgSO4, KH2PO4 and K2HPO4. 0.1 % of each metal salt was assessed individually in 20 ml medium for their ability to induce maximum xylanase production, keeping the remaining factors at their optimum followed by determination of xylanase activity and total protein. A control without any metal salt served as control.

Optimisation by Box–Behnken Design

The variables selected by using the Plackett–Burman experimental design were further fine-tuned by using Box-Behnken design of Response Surface Methodology (RSM)**.**

Xylanase Assay

Xylanase production was carried out using the optimized parameters and the xylanase concentration was determined. Xylanase assay was done to determine the activity of the enzyme xylanase. The xylanase enzyme activity was done by measuring the reducing sugar released by the reaction on the birch wood xylan. Thus, the xylanase assay was done according to 3, 5 – dinitro salicylic acid (DNS) method. The amount of enzyme produced by each isolate in liquid xylan medium was found. 1.0% of the birch wood xylan was dissolved in 50 mM Glycine- NaOH buffer (pH – 9.2) and this was used as substrate. 0.5 mL of the buffered substrate (1.0% birch wood xylan and 50mM Glycine NaOH buffer) was reacted with 0.5 mL of crude xylanase enzyme at a temperature of 55℃. The reaction was stopped after 10 minutes by adding 3 mL DNS reagent and then kept in a boiling water bath for 5 minutes. After cooling for a few minutes, the released xylose was quantified at 540 nm against a reagent blank. A reagent blank was made in the same manner except the crude enzyme was not added and 0.5 mL buffer was added. An enzyme blank was also made in which the reagent was added before the addition of enzyme so that only the reducing sugar is estimated. A standard of xylose (reducing sugar) was prepared using stock concentration 1 mg/mL. One unit of xylanase enzyme activity is defined as 1 µmole of xylose liberated per minute per mL of enzyme preparation under standard assay conditions.

*Standard Curve:*ΔA540nm Std = A540nm Std - A540nm Std Blank

Prepare a standard curve by plotting the ΔA540nm Standard vs the µmoles of Xylose.

Sample Concentration Determination:

ΔA540nm Sample = A540nm Test - A540nm Blank

Determine the µmoles of xylose using the Standard Curve (µmoles of xylose liberated) x (df)

$$\text{U/mL} = \frac{(\mu moles\ of\ xylose\ liberated) * (df)}{(10) * (0.5)}$$

df = Dilutionfactor, 10 =Time of assay (in minutes) as per Unit Definition

0.5 = Volume (in millilitres) of enzyme used units

RESULTS

Optimisation studies by Response Surface Methodology

Optimisation by Plackett- Burman Design

As shown in **Table1 and 2** shows the maximum and minimum levels of variables chosen for trials in Plackett-Burman experimental design represent a big variation in nutrient sources. The design for 64 trials with two levels of concentrations for each variable with the resultant enzyme activities.

Table: 1 **Variables chosen for analysis by Plackett-Burman design and their values at high and low levels**

Sl. No	Nutrients code	Nutrient	Low value coded as (-1) (g/L)	High value coded as (1)(g/L)
1	A	Yeast extract	0.1	1
2	B	MgSO4	0.01	0.1
3	C	K2HPO4	0.1	0.5
4	D	KH2PO4	0.1	0.5
5	E	xylose	1	10
6	F	temperature	25	50
7	G	rpm	100	200
8	H	Incubation time	24	120
9	I	pH	5	10

Table.2: The yield of xylanase (U/mL) at various runs of Plackett-Burman design

	Xylose g/L		**Yeast extract g/L**		**MgSO4 g/L**		**K2HPO4 g/L**		**K2HPO4 g/L**		**Temperature Â°C**		**RPM rpm**		**pH**		**Incubation timehrs**		**Xylanase U/mL**
	1	10	0.1	1	0.01	0.1	0.1	0.5	0.1	0.5	25	50	100	200	5	10	24	120	range
1-64	4, 9, 13, 15, 18, 21, 23, 26, 30, 32, 34, 37, 39, 40, 43, 44, 46-56, 60	1,3, 5,8, 14,16, 17,19, 20,22, 24,25, 27,29 ,31,33, 35,36, 38,41, 45,57-59,62-64	4, 5, 7, 8, 10, 11 ,19, 21 26 ,29, 31 ,32, 34-36 ,40, 42-46 ,53, 54 ,57, 58 ,60, 61 ,64	1. 3, 6, 9, 12 ,18, 20 ,27, 28 ,30, 33 ,373 9, 41 ,4752, 55 ,56, 59 ,62, 63	3,5,7 ,9,11 , 12, 15, 16, 19-24, 28, 33, 35, 37, 40, 42, 44, 50, 52-55, 59-62	1, 2, 4, 6, 10, 13, 14, 17, 18, 25-27, 29-32, 34, 36, 41, 43, 45-49, 51, 56-58, 61, 63, 64	1, 3, 4, 6, 7, 9, 17-20, 22, 23, 28-30, 33-36, 39-41, 44, 46, 48, 50, 54, 55, 57, 58, 61	2, 8, 10-16, 21, 24-27, 31, 32, 37, 38, 42, 43, 45, 49, 51-53, 56, 59, 60, 62-64	5. 7, 9, 12 ,14-16 ,18, 22-24, 28, 29, 31-34, 36, 38, 41, 45, 48, 51, 54-56, 60, 61, 63	1, 4, 8, 13 ,17, 19-21, 25-27, 30, 35, 37, 39, 40, 42-44, 46, 47, 49, 50, 52, 53, 57-59, 62, 64	2, 3, 6, 7, 9, 13 ,15-19, 24-26, 30, 32-34, 36, 37, 40, 42, 45, 46, 53, 54, 56, 58, 60, 62, 63	1, 4, 5, 8, 10-12, 14, 20-23, 27-29, 31, 35, 38, 41, 43, 44, 47-52, 55, 57, 59, 61, 64	1,4,6 ,7,9, 11,13,16, 17, 19, 21, 22, 25, 31, 34, 35, 38, 39, 41, 45, 46, 49-51, 53, 55, 56, 59-62, 64	2,3,5 ,8,10 ,12,14,15, 18,20,23, 24,26 30, 32,33, 36,37,40, 42-44,47,48, 52,54,57, 58,63	2, 4, 7, 8, 11 ,12, 14 ,16-18 ,20, 23-25 ,29, 31-35 ,37, 39-41 ,43, 47 , 49 ,53, 55 , 56 ,58, 59	1, 3, 5, 6, 9, 10 ,13, 15 , 19 , 21 , 22 , 26-28 , 30 , 36 , 38 , 42 , 44-46 , 48 , 50-52 , 54 , 57 , 60-64	1-4, 6, 8, 10 ,13-16 , 18 , 19 , 21-26 , 28 , 31 , 34 , 36 , 39 , 40 , 47 , 51 , 52 , 55 , 57 , 59 , 60	5,7,9 ,11,12,17, 20, 27, 29, 30, 32, 33, 35, 37, 38, 41-46, 48-50, 53, 54, 56, 58, 61-64	**21-30** (23) **31-40** (4,8,11,21,40, 55) **41-50** (10,12,22,24, 31,34,35,39,43,44,47,52,53 ,59,60) **51-60** (5,7,14,-16,18-20,25,26,28,29,32,37,49-51,57,61) **61-70** (1,2,13,36,41, 46,48,54,56,58,64) **71-80** (3,6,9,17,27,30,33,38,42,45) **81-90** (62) **91-100** (63)

The variables represent 9 different substrates, organic and inorganic nitrogen sources, trace elements, and other affecting factors. **Table 3and 4** shows the results of statistical analysis shown that the 95% confidence levels of Xylose, Yeast extract, MgSO4, K2HPO4, KH2PO4, temperature, rpm, incubation time and pH were 5.85, 6.19, 4.61, 0.3819, 0.4212, -5.01, 0.4507, 6.23 and 6.05 respectively, which are considered to significantly influence xylanase production. Other independent variables with confidence levels below 95% were generally considered insignificant. After the first optimization, the nutrient sources were reduced to five major variables by the Plackett-Burman experimental design, suggesting that Plackett- Burman design is a powerful tool for screening fermentation factors. The exact optimal values of the individual factors were still unknown but could be determined by the subsequent Box-Behnken design.

Table: 3. Minimum and Maximum activity of Xylanase under 63 experimental run in Plackett-Burman Design

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Xylose	g/L	Numeric	1.0000	10.00	-1 ↔ 1.00	+1 ↔ 10.00	5.50	4.54
B	Yeast extract	g/L	Numeric	0.1000	1.0000	-1 ↔ 0.10	+1 ↔ 1.00	0.5500	0.4536
C	MgSO4	g/L	Numeric	0.0100	0.1000	-1 ↔ 0.01	+1 ↔ 0.10	0.0550	0.0454
D	K2HPO4	g/L	Numeric	0.1000	0.5000	-1 ↔ 0.10	+1 ↔ 0.50	0.3000	0.2016
E	KH2PO4	g/L	Numeric	0.1000	0.5000	-1 ↔ 0.10	+1 ↔ 0.50	0.3000	0.2016
F	Temperature	°C	Numeric	25.00	50.00	-1 ↔ 25.00	+1 ↔ 50.00	37.50	12.60
G	RPM	rpm	Numeric	100.00	200.00	-1 ↔ 100.00	+1 ↔ 200.00	150.00	50.40
H	pH		Numeric	5.00	10.00	-1 ↔ 5.00	+1 ↔ 10.00	7.50	2.52
I	Incubation time	hrs	Numeric	24.00	120.00	-1 ↔ 24.00	+1 ↔ 120.00	72.00	48.38

Table 4: Minimum and Maximum activity of Xylanase under 63 experimental run in Plackett-Burman Design

Response	Name	Units	Observ ations	Analysis	Mini mum	Maxi mum	Mean	Std. Dev.	Ratio	Transform	Model
R1	Xylanase	U/mL	64	Factorial	26.25	91.25	58.69	13.34	3.48	None	Reduced 3FI

Table 5 and 6 shows the coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multicollinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable. **Table 7 and 8** shows the Nine variables of Xylose, Yeast extract, MgSO4, K2HPO4, KH2PO4, Temperature, rpm, pH and incubation time show 64 experimental runs with different combinations of the nine variables along with experimental responses. **Table 9 and 10** shows the considerable variation in the xylanase production was found, depending on the levels of the nine variables in the medium. The maximum xylanase production was found to be 91.25 U/mL in run number 63 and the minimum 26.25 U/mL in run number 23.

Table 5: Effect estimates for xylanase production from the results of Plackett-Burman Design

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	58.69	1	0.0358	58.24	59.15	
A-Xylose	5.40	1	0.0358	4.94	5.85	1.0000
B-Yeast extract	5.74	1	0.0358	5.28	6.19	1.0000
C-MgSO4	4.15	1	0.0358	3.70	4.61	1.0000
D-K2HPO4	-0.0735	1	0.0358	-0.5290	0.3819	1.0000
E-KH2PO4	-0.0343	1	0.0358	-0.4897	0.4212	1.0000
F-Temperature	-5.46	1	0.0358	-5.92	-5.01	1.0000
G-RPM	-0.0048	1	0.0358	-0.4602	0.4507	1.0000
H-pH	5.60	1	0.0358	5.14	6.05	1.0000
J-Incubation time	5.77	1	0.0358	5.31	6.23	1.0000

Table 6: Effect estimates for xylanase production from the results of Plackett-Burman Design

Factor		Coefficient Estimate		df	Standard Error	95% CI Low (-)	95% CI High(+)	VIF
(-)	(+)	(-)	(+)	1	0.0358	0.5132,0.5225,0.5305,0.5368,0.4837,0.5680,0.6071,0,6319,0.6399,0.6397,0.6462,0.6553,0.4819,0.5071,0.4288,0.4132,0.4116,0.4587,0.3883,0.3727,0.3196,0.3180,0.3024,0.2803,0.2413,0.2087,0.1616,0.1399,0.1383,0.3883	0.3977,0.3883,0.3803,0.3741,0.4272,0.3428,0.3038,0.2790,0.2710,0.2712,0.2647,0.2555,0.4290,0.4038,0.4821,0.4977,0.4993,0.4522,0.5225,0.5382,0.5913,0.5928,0.6085,0,6305,0,6696,0.7022,0.7493,0.7710,0.7725	1.0000
BH,EF,DF,**FG,FH**,AC,BJ,EJ,AH,CG,AG,FJ,BD,DE	BG,BC,DG,BE,EG,AB,EH,CJ,CF,**AF,AJ,** AE,GJ,CD,AD,CE,BF	0.0577,0.2530,0.0751,0.**081 3**,0.1126,0.1517,0.1765,0.1845,0.1843,0.1907,0.1999,0.0265,0.05176	0.0267,0.0423,0.0438,0.0671,0.0827,0.1358,0.1374,0.1530,**0.1751**,0.2142,0.2468,0.2938,0.3155,0.3171					
ABE,ABF,ADE,ADF,AEJ,AFJ,AGH,AGJ,BCE,BCF,BDF,BFH	ABH,ABJ,ACJ,AEG,AFG,BDE,CEG,CFG	0.1767,0.1532,0.2688,0.2142,0.0343,0.733,0.1140,0.0829,0.1202,0.1593,0.0437,0.0124	0.2688.0.0187,0.1358,0.0499,0.2608,0.0423,0.0907,0.2079	1	0.0358	0.6321,0.6087,0.7243,0.6696,0.4897,0.5288,0.5694,0.5383,0.5757,0.6147,0.4991,0.4678,0.1866,0.4368,0.3196,0.4055,0.1946,0.4132,0.3647,0.2475	0.2788,0.3022,0.1866,0.2413,0.4212,0.3821,0.3725,0.3352,0.2962,0.4118,0.4430,0.7243,0.4741,0.5913,0.5053,0.7163,0.4977,0.5462,0.6633	1.0000

Table 7: Analysis of variance (ANOVA) of Plackett-Burman design

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	11203.49	62	180.70	2197.63	0.0170
A-Xylose	1863.07	1	1863.07	22657.99	0.0042
B-Yeast extract	2106.74	1	2106.74	25621.49	0.0040
C-MgSO4	1103.02	1	1103.02	13414.57	0.0055
D-K2HPO4	0.3460	1	0.3460	4.21	0.2888
E-KH2PO4	0.0752	1	0.0752	0.9147	0.5142
F-Temperature	1910.72	1	1910.72	23237.51	0.0042
G-RPM	0.0015	1	0.0015	0.0178	0.9156
H-pH	2005.85	1	2005.85	24394.52	0.0041
J-Incubation time	2131.05	1	2131.05	25917.07	0.0040

Table 8: Analysis of variance (ANOVA) of Plackett-Burman design

Source	Sum of Squares	df	Mean Square	F-value	p-value
AB,AC,AD,AE,AF,AG,AH,AJ,BC,BD,BE,BF,BG,BH,BJ,CD,CE,CF,CG,CH,CJ,DE,DF,DG,EF,EG,EH,EJ,FG,FH,FJ,GH,GJ	0.4379, 0.8114, 6.37, 2.94, 1.96, 2.33, 2.18, 1.96, 0.1144, 0.0448, 0.0007, 0.2881, 0.0455, 0.2132, 1.47, 5.53, 6.44, 1.50, 2.17, 4.10, 1.21, 0.1708, 0.3609, 0.1230, 0.2881, 1.18, 1.99, 0.4235, 0.0510, 2.56, 0.0006, 3.90,	**1**	0.4379, 0.8114, 6.37, 2.94, 1.96, 2.33, 2.18, 1.96, 0.1144, 0.0448, 0.0007, 0.2881, 0.0455, 0.2132, 1.47, 5.53, 6.44, 1.50, 2.17, 4.10, 1.21, 0.1708, 0.3609, 0.1230, 0.2881, 1.18, 1.99, 0.4235, 0.0510, 2.56, 0.0006, 3.90,	5.33, 9.87, 77.49, 35.70, 23.86, 28.31, 26.49, 23.86, 1.39, 0.5453, 0.0081, 3.50, 0.5531, 2.59, 17.90, 67.21, 78.26, 18.23, 26.43, 49.83, 14.70, 2.08, 4.39, 1.50, 3.50, 14.36, 24.24, 5.15, 0.6198, 31.10, 0.0072, 47.40,	0.2603, 0.1962, 0.0720, 0.1056, 0.1285, 0.1183, 0.1222, 0.1285, 0.4477, 0.5951, 0.9430, 0.3124, 0.5929, 0.3538, 0.1478, 0.0773, 0.0717, 0.1465, 0.1223, 0.0896, 0.1624, 0.3862, 0.2835, 0.4363, 0.3124, 0.1642, 0.1276, 0.2642, 0.5754, 0.1129, 0.9463, 0.0918,
ABE,ABF,ABH,ABJ,ACJ,ADE,ADF,AEG,AEJ,AFG,AFJ,AGH,AGJ,BCF,BDE,BDF,BFH,CEG,CFG	2.00, 1.50, 4.63, 0.0223, 1.18, 4.63, 2.94, 0.1594, 0.0752, 4.35, 0.3443, 0.8313, 0.4399, 0.9250, 1.62, 0.1144, 0.1220, 0.0099, 0.5267, 2.77,	**1**	2.00,1.50, 4.63, 0.0223, 1.18, 4.63, 2.94, 0.1594, 0.0752, 4.35, 0.3443, 0.8313, 0.4399, 0.9250, 1.62, 0.1144, 0.1220, 0.0099, 0.5267, 2.77,	24.29, 18.27, 56.26, 0.2709, 14.36, 56.26, 35.70, 1.94, 0.9147, 52.96, 4.19, 10.11, 5.35, 11.25, 19.75, 1.39, 1.48, 0.1198, 6.41, 33.64,	0.1274, 0.1463, 0.0844, 0.6945, 0.1642, 0.0844, 0.1056, 0.3965, 0.5142, 0.0869, 0.2894, 0.1940, 0.2598, 0.1845, 0.1409, 0.4477, 0.4376, 0.7879, 0.2395, 0.1087,
	Residual=0.0822	**63**	**Core Total**=11203.57		

Table 9: Observed Xylanase activity versus the predicted xylanase activity under the optimum fermentation conditions

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Cook's Distance	Externally Studentized Residuals and	Standard Order
1-32	68.75,68.00,71.25,35.00,60.00,78.00,60.00,37.00,71.25,45.75,37.00,48.75,68.75,57.50,60.00,60.00,80.00,57.70,60.00,60.25,37.50,48.75,26.25,48.75,57.50,57.75,80.00,60.75,57.50,80.25,46.50,57.50	68.79,67.96,71.29,35.04,60.04,77.96,59.96,37.04,71.21,45.79,36.96,48.79,68.79,57.54,59.96,60.04,79.96,57.74,59.96,60.21,37.46,48.79,26.21,48.71,57.54,57.71,80.04,60.71,57.46,80.29,46.46,57.46	.0358	0.984	±1.000	1.000	0.000	56,32,20,53,42,8,2,58,3,45,41,43,31,48,11,12,24,7,18,52,57,34,33,10,30,29,64,36,38,23,46,13
33-64	71.25,46.50,48.76,61.75,60.00,71.25,48.75,37.50,68.75,71.25,46.25,48.75,80.00,68.75,46.75,68.75,57.75,60.00,57.50,48.75,46.25,65.00,40.00,68.25,57.25,68.50,48.50,48.76,57.75,82.50,91.25,68.75	71.29,46.46,48.79,61.74,59.96,71.71,48.71,377.54,68.79,71.21,46.29,48.71,80.04,68.71,46.71,68.71,57.71,60.04,57.46,48.79,46.29,65.04,0.04,68.29,57.21,68.54,48.46,48.79,57.79,82.54,91.21,68.71	±0.0358	0.984	±1.000	1.000	0.000	4,5,50,6,27,44,19,17,40,26,61,49,14,21,55,39,63,51,47,59,25,1,35,15,54,22,60,9,37,28,16.62

Table . 10. Optimization by Box-Behnken Design

Parameters	Units	Level of variable (g/L) coded as		
		(-1)	(0)	(+1)
Xylose	(g/L)	1	5.5	10
yeast extract	(g/L)	0.1	0.55	1
MgSO4,	(g/L)	0.01	0.055	0.1
pH	-	5	7.5	10
Temperature	°C	25	37.5	50
Incubation time	hrs	24	72	120

Factor coding is Coded and Sum of squares is Type III – Partial

The Model F-value of 2197.63 implies the model is significant. There is only a 1.70% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, F, H, J are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model **Table 11.**

Xylanase(U/ml)
58.69+5.40A+5.74B+4.15C-0.0735D-0.0343E-5.46F+0.0048G+5.60H+5.77J+0.0827AB
0.1126AC+0.3155AD+0.2142AE+0.1751AF-0.1907AG-0.1845AH+0.1751AJ+0.0423BC-0.0265BD-
0.0032BE+0.0671BF+0.0267BG-0.0577BH0.1517BJ+0.2938CD+0.3171CE+0.1530CF-0.1843CG-
0.2530CH+0.1374CJ-0.0517DE-0.0751DF+0.0438DG-0.0671EF+0.0671EG+0.1358EH-0.1765EJ-0.0813FG-
0.0282FH-0.1999FJ-0.0030GH+0.2468GJ-0.1767ABE0.1532ABF+0.2688ABH+0.0187ABJ+0.1358ACJ-
0.2688ADE-0.2142ADF+0.0499AEG-
0.0343AEJ+0.2608AFG-0.0733AFJ-0.1140AGH-0.0829AGJ-0.1202BCE0.1593BCF+0.0423BDE-0.0437BDF-
0.0124BFH+0.0907CEG+0.2079CFG

Table: 11 Variables chosen for analysis by Box-Behnken design

	Name	Units	Type	Mini mum	Maxi mum	Coded Low	Coded High	Mean	Std. Dev.
A	Xylose	g/L	Numeric	1.0000	10.00	-1 ↔ 1.00	+1 ↔ 10.00	5.50	3.03
B	Yeast extract	g/L	Numeric	0.1000	1.0000	-1 ↔ 0.10	+1 ↔ 1.00	0.5500	0.3028
C	MgSO4	g/L	Numeric	0.0100	0.1000	-1 ↔ 0.01	+1 ↔ 0.10	0.0550	0.0303
D	Temperature	°C	Numeric	25.00	50.00	-1 ↔ 25.00	+1 ↔ 50.00	37.50	8.41
E	pH		Numeric	5.00	10.00	-1 ↔ 5.00	+1 ↔ 10.00	7.50	1.68
F	Incubation time	hrs	Numeric	24.00	120.00	-1 ↔ 24.00	+1 ↔ 120.00	72.00	32.30

Final Equation in Terms of Coded Factors:

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors:

Xylanase(U/ml)=+29.95822+1.01244Xylose+10.81207Yeastextract+126.48310MgSO4-9.87707K2HPO4-
5.75533KH2PO40.360469Temparature+0.030884RPM+2.41273pH+0.110269Incubationtime
0.013410Xylose*YeastExtract1.56227Xylose*MgSO4+1.51252Xylose*K2HPO4+0.816709Xylose*KH2PO4+0.000
197Xylose*Temperature-0.002586Xylose*RPM
0.015213Xylose*pH+0.002345Xylose*IncubationTime+34.59028Yeastextract*MgSO4+0.4
56424Yeastextract*K2HPO4+3.29118Yeastextract*KH2PO4+0.098087Yeastextract*Temperature+0.001185Yeastex
tract*RPM-0.310299YeastExtract*pH
0.008077Yeastextract*IncubationTime+32.64931MgSO4*K2HPO4+21.31906MgSO4*KH2PO4-
0.490685MgSO4*Temparature-0.419590MgSO4*RPM-2.24917MgSO4*pH-
0.013252MgSO4*IncubationTime+5.63134K2HPO4*KH2PO4+0.096004K2HPO4*
Temperature+0.004384K2HPO4*RPM-0.026837KH2PO4*Temperature-
0.010478KH2PO4*RPM+0.271687KH2PO4*pH-0.014018KH2PO4*IncubationTime-
0.001047Temperature*RPM-0.000418Temperature*pH-
0.000184Temparature*IncubationTime+0.001090RPM*pH+0.000145RPM*IncubationTime-
0.436188Xylose*YeastExtract*KH2PO40.006053Xylose*YeastExtract*Temperature+0.053105Xylose*YeastExtract
*pH+0.000192Xylose*YeastExtract*IncubationTime+0.013976Xylose*MgSO4*IncubationTime-
1.49358Xylose*K2HPO4*KH2PO4-
0.019036Xylose*K2HPO4*Temperature+0.001109Xylose*KH2PO4*RPM-
0.000794Xylose*KH2PO4*IncubationTime+0.000093Xylose*Temperature*RPM-
0.000027Xylose*Temperature*IncubationTime-0.000203Xylose*RPM*pH-
7.67650E06Xylose*RPM*IncubationTime-29.68364YeastExtract*MgSO4*KH2PO4-
0.629259YeastExtract*MgSO4*Temperature+2.34896Yeastextract*K2HPO4*KH2PO4-
0.038806YeastExtract*K2HPO4*Temperature-
0.000882YeastExtract*Temperature*pH+0.201597MgSO4*KH2PO4*RPM+0.007392MgSO 4*Temperature*RPM

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

The statistical optimal values of variables are obtained when moving along the major and minor axis of the contour and the response at the centre point yields the maximum xylanase production. These observations were also verified from canonical analysis of the response surface. Canonical analysis revealed a minimum region for the model. The stationary point presenting maximum xylanase had the following critical values (g/L): xylose, 10g/L; yeast extract, 1g/L; MgSO4, 0.1g/L; K2HPO4, 0.5g/L KH2PO4, 0.5g/L; temperature, 50°C; rpm, 200; pH, 10; and incubation time 120hrs. The predicted xylanase activity for these conditions was 91.21 U/mL. A repeat fermentation of xylanase by *Bacillus subtilis* under optimal conditions was carried out to verify the optimization. The maximum xylanase level obtained was 91.25 U/mL, which was close to the predicted value

Six variables of Xylose, Yeast extract, MgSO4, Temperature, pH and incubation time were chosen for further study based on Box-Behnken design. **Table 12** shows the 54 experimental runs with different combinations of the six

variables along with experimental responses. As shown in **Table 13,14** a considerable variation in the xylanase production was found, depending on the levels of the six variables in the medium. The maximum xylanase production was found to be 141.75 U/mL in run number 47 and the minimum 72.75 U/mL in run number 5.

Table: 12 Box-Behnken Design matrix showing the xylanase production using *Bacillus subtilis*

RUN	A:Xylose	B:Yeast extract	C:MgSO4	D:Temperature	E:pH	F:Incubation time	Xylanase
	g/L	g/L	g/L	Â°C		hrs	U/mL
1-27	5.5, 10, 10,5.5,1,5.5,5.5, 1,1,10,10,5.5,5. 5,5.5,10,1,1,10, 5.5,1,5.5,5.5,10, 5.5,10,5.5,5.5,	0.1, 1,0.55,0.55,0.55,0.55 ,1,0.1,0.55,0.1,0.1,0. 1,0.55,0.55,0.55,0.55 ,1,0.55,0.1,0.55,1,0.5 5,1,0.55,0.55,0.1,0.1	0.1, 0.055,0.055,0.1,0.05 5,0.1,1,0.1,0.55,0.05 5,0.055,0.055,0.01,0. 1,0.055,0.1,0.055,0.0 55,0.1,0.1,0.01,0.055 ,0.055,0.055,0.055,0. 055,0.01	37.5, 25,50,50,50,25,37.5,50,50 ,25,50,37.5,50,50,25,37.5, 25,25,37.5,37.5,37.5,37.5, 50,37.5,50,37.5,37.5	10, 7.5,10,7.5,5,7.5,10,7. 5,10,7.5,7.5,10,7.5,7. 5,5,7.5,7.5,10,5,7.5,5 ,7.5,7.5,7.5,5,5,10,	72, 72,72,120,72,24.24 ,72,72,72,72,120,1 20,24,72,120,72,72 ,72,24,72,72,72,72, 72,24,72	105.85, 125.5,108.25,107,72.75,1 06.75,98.5,73.98,86.25,10 2,89.05,106.37,101.25,90. 25,103.85,105.8,106.62,1 22.75,89.56,90.37,101.55, 141.37,106.12,141.5,85.1 2,74.75,100.25
28-54	5.5,1,5.5,5.5,5.5 ,10,10,1,5.5,5.5, 1,1,5.5,5.5,5.5,5 .5,5.5,10,10,5.5, 5.5,5.5,5.5,5.5,1 ,1,5.5	0.55,0.55,1,1,1,0.55, 0.55,0.55,0.55,0.55,0 .55,0.1,1,1,1,1,0.55,0 .55,0.55,0.55,0.55,0. 55,0.55,0.1,0.1,1,0.5 5,0.55	0.055,0.055,0.055,0. 01,0.1,0.01,0.1,0.01, 0.01,m0.01,0.01,0.05 5,0.055,0.01,0.1,0.05 5,0.055,0.01,0.1,0.05 5,0.1,0.01,0.055,0.05 5,0.055,0.055,0.055	37.5,25,37.5, 37.5, 37.5, 37.5, 37.5, 37.5, 37.5,50,25, 37.5,,25, 37.5, 37.5, 37.5, 37.5, 37.5, 37.5, 37.5, 37.5,25,25, 37.5, 37.5,50,25, 37.5,	7.5,5,5,10,10, 7.5, 7.5, 7.5,7.5,7.5,7.5, 7.5,10,5,5,5, 7.5, 7.5, 7.5, 7.5, 7.5, 7.5, 5,10, 7.5,10, 7.5,	72, 72, 24, 72, 72,120,24,120,24,2 4,24, 72,120, 72, 72, 120,72,24,120,72,1 20,120,120,24,72,7 2,72	141.5,91.25,91.37,117.62. 125,117.5,106.62,100.65, 85,101.37,85.12,88.15,12 5,85,106.75,97.5,141.62,1 01.62,123,141.75,123.12, 117.62,80,80,85.75,101.0 1,141.25

Table: 13 Minimum and Maximum activity of Xylanase under 54 experimental run in Box- Behnken Design

Response	Name	Units	Observa tions	Analysis	Mini mum	Maxi mum	Mean	Std. Dev.	Ratio	Trans form	Model
R1	Xylanase	U/mL	54	Polynomial	72.75	141.75	104.55	18.91	1.95	None	Quadratic

Table: 14 Observed Xylanase activity versus the predicted xylanase activity under the optimum fermentation conditions

Run Order	Actual Value	Predicted Value	Residual	Lever age	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1-27	105.85, 125.50, 108.25, 107.00, 72.75, 106.75, 98.50, 73.98, 86.25, 102.00, 89.05, 106.37, 101.25, 90.25, 103.85, 105.80, 106.62, 122.75, 89.56, 90.37, 101.55, 141.37, 106.12, 141.50, 85.12, 74.75, 100.25	105.81, 125.54, 108.33,106.64, 72.57, 107.00, 98.27, 74.28, 86.32, 101.99, 89.01, 106.31, 101.02, 90.34, 103.44,106.01, 106.32,122.60, 89.26, 90.12, 101.58,141.50, 106.00,141.50, 85.19, 74.75, 100.15	0.0415, -0.0375, -0.0825, 0.3583, 0.1842, -0.2533, 0.2277, -0.2975, -0.0742, 0.0125, 0.0392, 0.0640, 0.2258, -0.0867, 0.4092, -0.2083, 0.2958, 0.1508, 0.3035, 0.2467, -0.0252, -0.1283, 0.1242, 0.0017, -0.0692, -0.0040, 0.1015	0.563,0.563, 0.562,0.563, 0.563,0.563, 0.563,0.562, 0.563,0.563, 0.563,0.563, 0.563,0.563, 0.563,0.563, 0.562,0.563, 0.563,0.167, 0.562,0.563, 0.563,	0.219, -0.198, -0.437, 1.896, 0.975, -1.341, 1.205, -1.575, -0.393, 0.066. 0.207, 0.339, 1.195, -0.459, 2.166, -1.103, 1.566, 0.798, 1.607, 1.305, -0.133, -0.492, 0.657, 0.006, -0.366, -0.021, 0.537,	0.215, -0.195, -0.430,2.003, 0.974, -1.363, 1.216, -1.623, -0.386, 0.065, 0.203, 0.333, 1.206, -0.452, 2.346, -1.107, 1.613, 0.793, 1.660, 1.324, -0.131, -0.485, 0.650, 0.006, -0.360, -0.021, 0.529,	0.002,0.002, 0.009,0.165, 0.044, 0.083, 0.067, 0.114, 0.007, 0.000, 0.002, 0.005, .066, 0.010, 0.215, 0.056, 2.660, 0.113, 0.029, 0.119, 0.078, 0.001, 0.002, 0.020, 0.000, 0.006, 0.000, 0.013,	0.244, -0.221, -0.487, 2.272, 1.104, -1.545, 1.379, -1.841, -0.438, 0.074, 0.231, 0.377, 1.367, -0.512, 2.660, -1.256, 1.829, 0.899, 1.882, 1.502, -0.148, -0.217, 0.737, 0.003, -0.408, -0.023, 0.600,	15,4,32, 24,27,18,36, 5,31,2,6,39, 23, 20,26,473,3 0,11,43,10,5 3,8,52,28,33 ,13.
28-54	141.50, 91.25, 91.37, 117.62, 125.00, 117.50, 106.62, 100.65, 85.00, 101.37, 85.12, 88.15, 125.00, 85.00, 106.75, 97.50, 141.62, 101.62, 123.00, 141.75, 123.12, 117.62, 80.00, 80.10, 85.75, 101.01, 141.25	141.50, 91.50, 91.45, 117.94, 124.81, 117.72, 106.80, 100.45, 84.97, 101.70, 84.82, 87.94, 124.98, 85.21, 106.84, 97.46, 141.50, 101.44, 123.33, 141.50, 123.12, 117.56, 80.21, 80.15, 86.10, 101.28, 141.50	0.0017, -0.2525, -0.0802, -0.3198, 0.1902, -0.2192, -0.1767, 0.2042, 0.0258, -0.3308, 0.3042, 0.2108, 0.0202, -0.2065, -0.0852, 0.0373, 0.1217, 0.1808, -0.3317, 0.2517, 0.0017, 0.0592, -0.2115, -0.0535, -0.3475, -0.2658, -0.2483	0.167, 0.563, 0.563, 0.562, 0.563, 0.563, 0.563, 0.563, 0.563, 0.563, 0.563, 0.562, 0.563, 0.563, 0.167, 0.563, 0.563, 0.563, 0.563, 0.563, 0.563, 0.563, 0.562, 0.563, 0.167,	0.006, -1.336, -0.425, -1.693, 1.007, -1.160, -0.935, 1.081, 0.137, -1.751, 1.610, 1.116, 0.107, -1.093. -0.451, 0.197, 0.467, 0.957, -1.755, 0.965, 0.009, 0.313, -1.119, -0.283, -1.839, -1.407, -0.952	0.006, -1.358, -0.418, -1.759, 1.007, -1.168, -0.933, 1.084, 0.134, -1.828, 1.664, 1.121, 0.105, -1.097, -0.444, 0.194, 0.459, 0.955, -1.833, 0.964, 0.009, 0.308, -1.125, -0.278, -1.934, -1.435, -0.951	0.000, 0.082, 0.008, 0.132, 0.047, 0.062, 0.040, 0.054, 0.001, 0.141, 0.119, 0.057, 0.001, 0.055, 0.009, 0.002, 0.002, 0.042, 0.141, 0.007, 0.000, 0.005, 0.058, 0.004, 0.155, 0.091, 0.006	0.003, -1.540, -0.474, -1.995, 1.142, -1.324, -1.058, 1.229, 0.152, -2.073, 1.886, 1.271, 0.119, -1.244, -0.503, 0.220, 0.205, 1.083, -2.079, 0.431, 0.010, 0.349, -1.275, -0.316, -2.192, -1.628, -0.425	**49,** 25,34,14,16, 46,44,45,19, 17,41,1,40,9 ,12,38,50,42 ,48,54,22,21 ,37,35,7,29, 51

The statistical optimal values of variables are obtained when moving along the major and minor axis of the contour and the response at the centre point yields the maximum xylanase production. These observations were also verified from canonical analysis of the response surface. Canonical analysis revealed a minimum region for the model. The stationary point presenting maximum xylanase had the following critical values (g/L): xylose, 10g/L; yeast extract, 1g/L; MgSO4, 0.1g/L; temperature, 50°C; pH, 10 and incubation time, 120hrs. The predicted xylanase activity for these conditions was 141.50 U/mL. The observed xylanase activity versus the predicted xylanase activity under optimum fermentation conditions is shown in **Figure1**. A repeat fermentation of xylanase by *Bacillus subtilis* under optimal conditions was carried out to verify the optimization. The maximum xylanase level obtained was 141.75 U/mL, which was close to the predicted value.

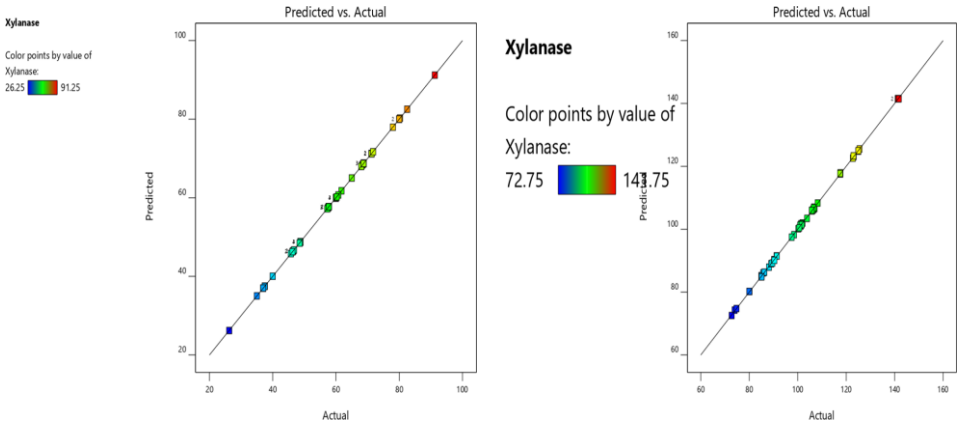

Fig: 1. Observed Xylanase activity versus the predicted xylanase activity under the optimum fermentation conditions

Three-dimensional response plots and their corresponding contour plots were drawn on the basis of the model equation, to investigate the interaction among the variables and to determine the optimum concentration of each factor for maximum xylanase production by *Bacillus subtilis*. The contour plots affirm that the objective function is unimodal in nature which shows an optimum at the boundaries. The effect of varying the concentration of Xylose and one of the other variables is shown in below **Figures 2,3,4,5 and 6**. It can be seen from Figure that xylanase production tends to increase while gradually increasing the value of xylose concentration. The Xylanase activity by using all the optimized parameters was 141.23 U/mL.

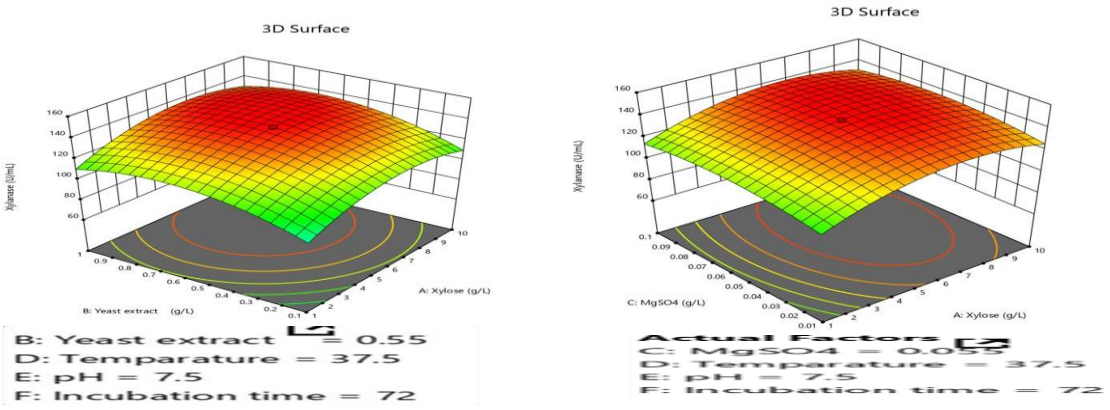

Fig: 2 & 3. Response surface plot and contour plot of the combined effects of xylose and yeast extract, MgSO4 on xylanase production by *Bacillus subtilis*

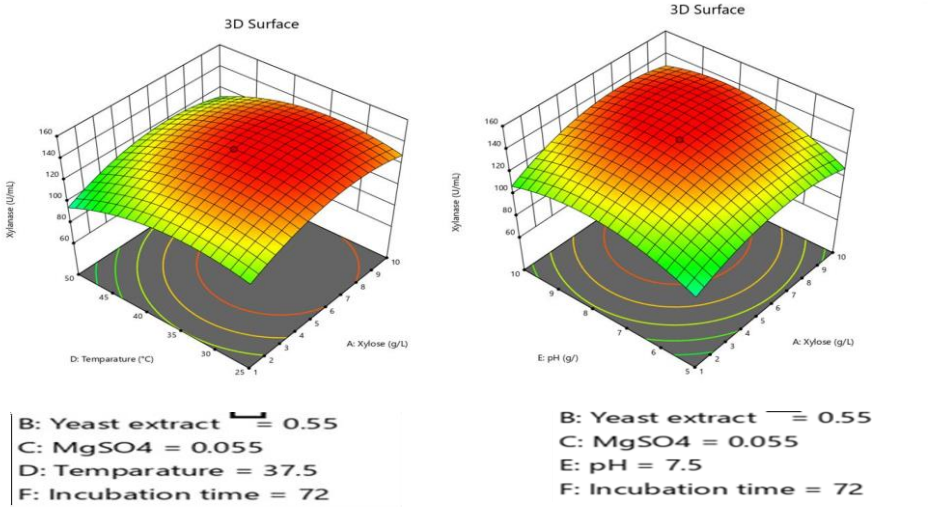

Fig.4 &5. Response surface plot and contour plot of the combined effects of xylose and Temperature, pH on xylanase production by *Bacillus subtilis*

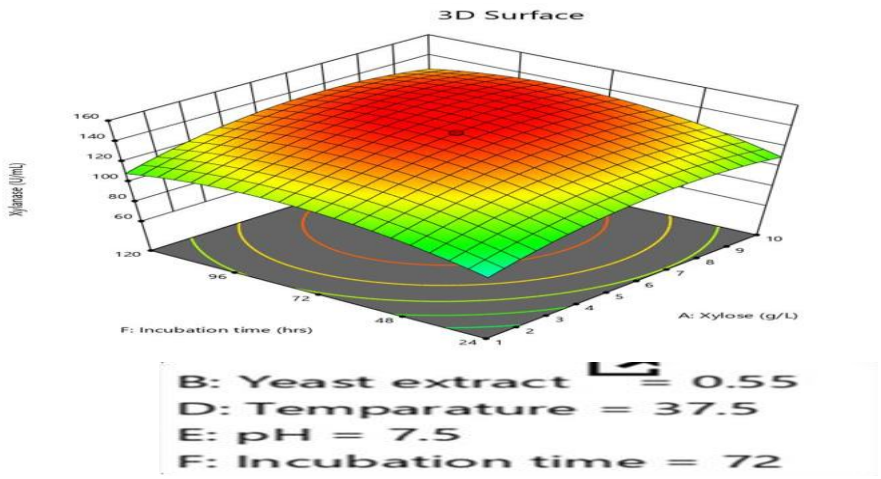

Fig.6. Response surface plot of the and contour plot combined effects of xylose and Incubation Time on xylanase production by *Bacillus subtilis.*

CONCLUSION

From the study. the first optimization by Placket-Burman experimental design ,shows that it's a powerful tool for screening fermentation factors. The maximum xylanase production was found to be 91.25 U/mL in run number 63 and the minimum 26.25 U/mL in run number 23.From the coefficient estimate, it shows that the Variance Inflation Factor (VIFs) are in a tolerable range. In analysis of variance The Model F-value of 2197.63 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, F, H, J are significant model terms. The predicted xylanase activity for the conditions xylose, 10g/L; yeast extract, 1g/L; MgSO4, 0.1g/L; K2HPO4, 0.5g/L KH2PO4, 0.5g/L; temperature, 50°C; rpm, 200; pH, 10; and incubation time 120hrs was 91.21 U/mL.. The maximum xylanase level obtained was 91.25 U/mL, which was close to the predicted value. By Box-Behnken Design, A considerable variation in the xylanase production was found, depending on the levels of the six variables- Xylose, Yeast extract, MgSO4, Temperature, pH and incubation time in the medium. The maximum xylanase production was found to be 141.75 U/mL in run number 47 and the minimum 72.75 U/mL in run number 5. Xylanase production tends to increase while gradually increasing the value of xylose concentration. The xylanase activity by using all the optimized parameters was 141.23 U/mL

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