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MODEL-BASED OPTIMIZATION OF PECTINASE PRODUCTION USING BOX–BEHNKEN EXPERIMENTAL DESIGN

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ABSTRACT

Pectinase is one of the important industrial enzymes that is used in food processing and biotechnology. The purpose of this study was to optimize the production of pectinase using agro-industrial wastes and using statistical experimental designs. The potato peel, orange peel, ammonium sulfate and magnesium sulfate were found to be influential medium components by factorial screening. "Box–Behnken Design (BBD) was used with Response Surface Methodology (RSM) to optimize the fermentation parameters". The quadratic model was significant ($F = 512.00$, $p < 0.0001$), and had a good predictive ability. The optimum pH, temperature and agitation speed for maximum pectinase activity were 72 h, pH 5, and 32.5°C, respectively, with 93.62 IU/mL activity.

KEYWORDS: *Pectinase, Fermentation, Magnesium sulphate, Response Surface Methodology.*

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1. INTRODUCTION

Pectinase is an enzyme of industrial significance and is commonly applied in food processing, clarification of juices, and different biotechnological applications. The production efficiency is influenced by the composition of the media and the fermentation processes, so optimization of the fermentation is necessary for an enzyme production. Statistic experimental designs are a good method to assess the process variables and interaction of the variables with minimal experimental effort. Response Surface Methodology (RSM), especially Box–Behnken Design (BBD), is suitable to develop quadratic models for predicting responses and finding optimum operating conditions with limited number experiments when compared to conventional factorial approach (Satapathy et al., 2021). RSM can then be used to study the effect of the media components (temperature, pH, fermentation time, and agitation rate) on pectinase activity after performing factorial screening on the media components. Analysis of variance (ANOVA) and response surface plots can be used to evaluate model significance, model interaction, and model predictive accuracy. Thus, the objective of this study was to optimize the fermentation conditions by Box–Behnken Design (BBD) approach based on RSM, to maximize pectinase production and to find appropriate conditions for effective enzyme synthesis.

2. MATERIALS AND METHODS

2.1 OPTIMISATION OF MEDIA COMPONENTS USING RSM FOR PRODUCTION OF PECTINASE

Four medium components found in a factorial screening test, potato peel, orange peel, ammonium sulfate and magnesium sulphate were selected and optimized by using "Box–Behnken Design (BBD) of Response Surface Methodology (RSM)." The BBD was chosen as a design method over CCD because it requires less number of experimental trials and allows the quadratic model to be developed (Satapathy et al., 2021). Twenty-nine experiments were designed at three levels (–1, 0 and +1) and each experiment was repeated thrice. The pectinase expression and the autonomous variables' quadratic, deterministic linear, creative, and creative interaction impacts were included into a quadratic formula. A, B, C, and D were the labels given to the factors. In order to evaluate the element influences and find the ideal circumstances, the design-professional 13 program was used to create the cubic model, evaluation of a variance (ANOVA), graphical response area plots, graphical contour diagrams, and primary impact plots.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{44} D^2 + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD + \beta_{23} BC + \beta_{24} BD + \beta_{34} CD \quad (3.2)$$

Table 1: The media component levels utilised in RSM BBD

Media components	Levels		
	-1	0	+1
Potato peel (g/l)	2	6	10
Orange peel (g/l)	10	20	30
$(NH_4)_2SO_4$ (g/l)	1	2	3
$MgSO_4.7H_2O$ (g/l)	0.05	0.275	0.5

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3. RESULTS AND DISCUSSION

3.1 OPTIMISATION OF PROCESS PARAMETERS FOR PECTINASE SYNTHESIS UTILISING BOX BEHNKEN DESIGN

Table 2: Design of Experiment data and experimental and predicted responses for optimisation of process variables

		Factor 1	Factor 2	Factor 3	Factor 4	Response 1	
Std	Run	A: Temperature	B: pH	C: Fermentation Time	D: Agitation Rate	Pectinase Activity (Experimental)	Pectinase Activity (Predicted)
		°C		Hrs	RPM	IU/mL	IU/mL
13	1	32.5	3	24	175	29.53	30.63
17	2	25	5	24	175	36.09	35.98
22	3	32.5	7	72	50	55.05	54.68
12	4	40	5	72	300	47.56	49.44
10	5	40	5	72	50	51.47	52.06
23	6	32.5	3	72	300	41.94	42.00
14	7	32.5	7	24	175	48.98	50.45
3	8	25	7	72	175	44.91	44.70
21	9	32.5	3	72	50	37.05	37.81
1	10	25	3	72	175	39.58	38.59
7	11	32.5	5	24	300	40.12	39.47
28	12	32.5	5	72	175	92.2	92.33
6	13	32.5	5	120	50	68.16	67.95
2	14	40	3	72	175	45.27	44.63
18	15	40	5	24	175	47.65	45.90
19	16	25	5	120	175	60.34	61.77
16	17	32.5	7	120	175	67.32	67.39
5	18	32.5	5	24	50	39.07	39.00
9	19	25	5	72	50	41.94	41.23
29	20	32.5	5	72	175	91.42	92.33
25	21	32.5	5	72	175	91.58	92.33
11	22	25	5	72	300	38.87	39.45
27	23	32.5	5	72	175	93.62	92.33
26	24	32.5	5	72	175	92.84	92.33
24	25	32.5	7	72	300	47.17	46.09
20	26	40	5	120	175	72.88	72.67
15	27	32.5	3	120	175	66.54	66.25
4	28	40	7	72	175	59.35	59.48
8	29	32.5	5	120	300	63.86	63.07

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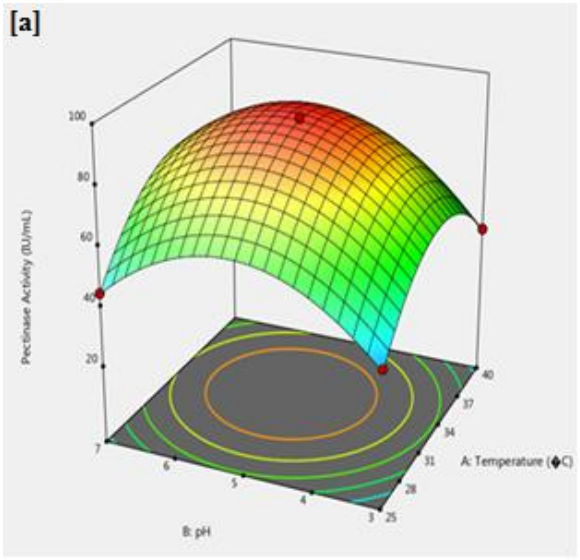
The experimental and predicted pectinase activities obtained from Box–Behnken Design (BBD) on four variables, which are temperature, pH, fermentation time and agitation rate, are given in table 2. The near-accuracy of the model and experimental results shows the performance of the model is good. Maximum activity (93.62 IU/mL) was achieved at 32.5°C, pH 5, 72 h, and 175 rpm. For the most part, extreme parameter levels led to decreased enzyme production, supporting the need for optimum fermentation conditions.

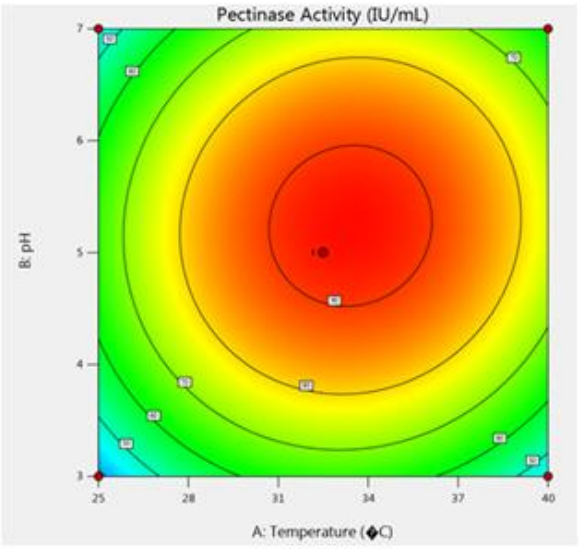
Table 3: ANOVA parameters for the quadratic model for optimisation of process parameters

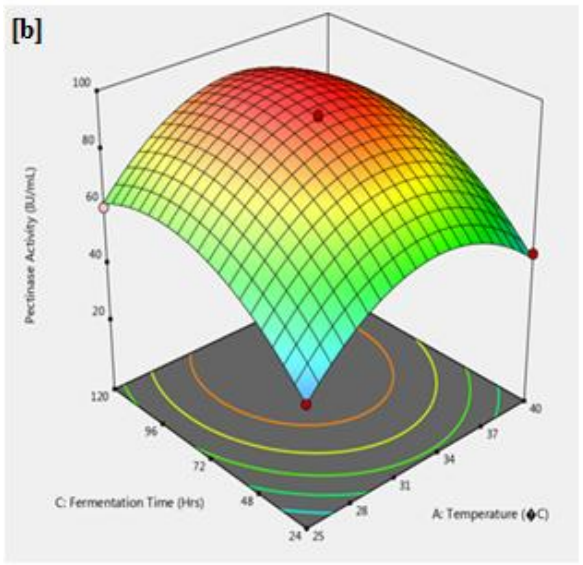
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	10799.59	14	771.40	512.00	< 0.0001	significant
A-Temperature	325.00	1	325.00	215.71	< 0.0001	
B-pH	329.39	1	329.39	218.62	< 0.0001	
C-Fermentation Time	2071.39	1	2071.39	1374.83	< 0.0001	
D-Agitation Rate	14.56	1	14.56	9.67	0.0077	
AB	19.14	1	19.14	12.70	0.0031	
AC	0.2401	1	0.2401	0.1594	0.6958	
AD	0.1764	1	0.1764	0.1171	0.7373	
BC	87.14	1	87.14	57.84	< 0.0001	
BD	40.77	1	40.77	27.06	0.0001	
CD	7.16	1	7.16	4.75	0.0469	
A^2	3295.40	1	3295.40	2187.24	< 0.0001	
B^2	3414.14	1	3414.14	2266.05	< 0.0001	
C^2	1601.10	1	1601.10	1062.69	< 0.0001	
D^2	3813.20	1	3813.20	2530.92	< 0.0001	
Residual	21.09	14	1.51			
“Lack of Fit”	17.76	10	1.78	2.13	0.2423	not significant
“Pure Error”	3.33	4	0.8329			
Cor Total	10820.68	28				

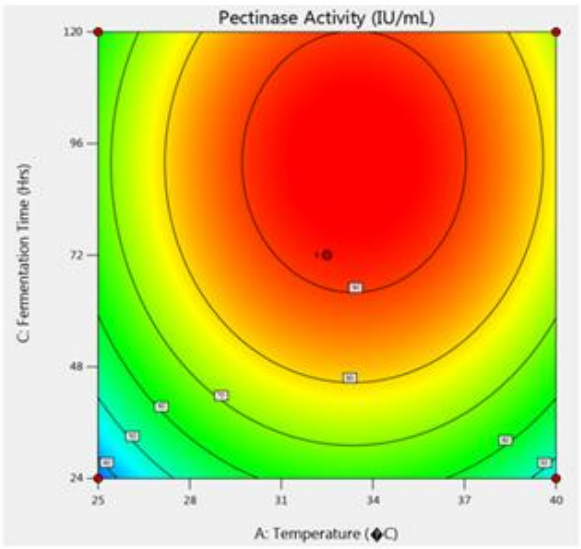
“Table 3 shows that the quadratic model was significant ($F = 512.00$, $p < 0.0001$)”, indicating its suitability for explaining variations in pectinase activity. Fermentation time, pH, temperature, and agitation rate significantly influenced enzyme production. All four quadratic terms were significant ($p < 0.0001$), demonstrating nonlinear effects. The lack of fit was insignificant ($p = 0.2423$), confirming the model’s adequacy.

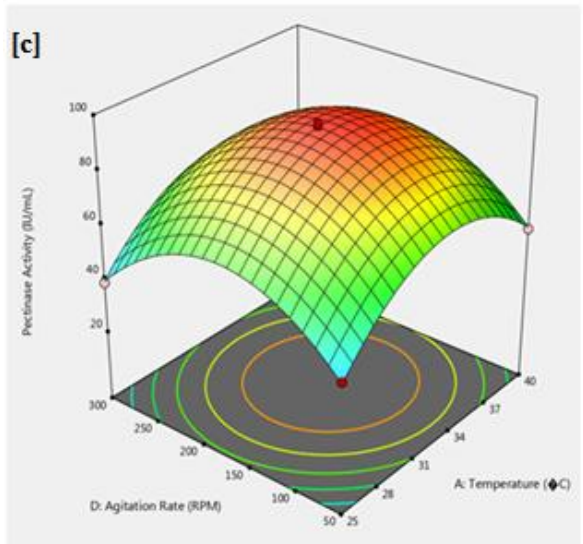
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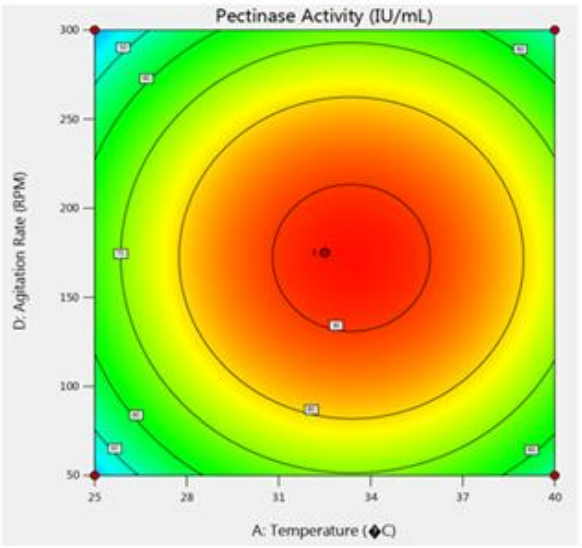












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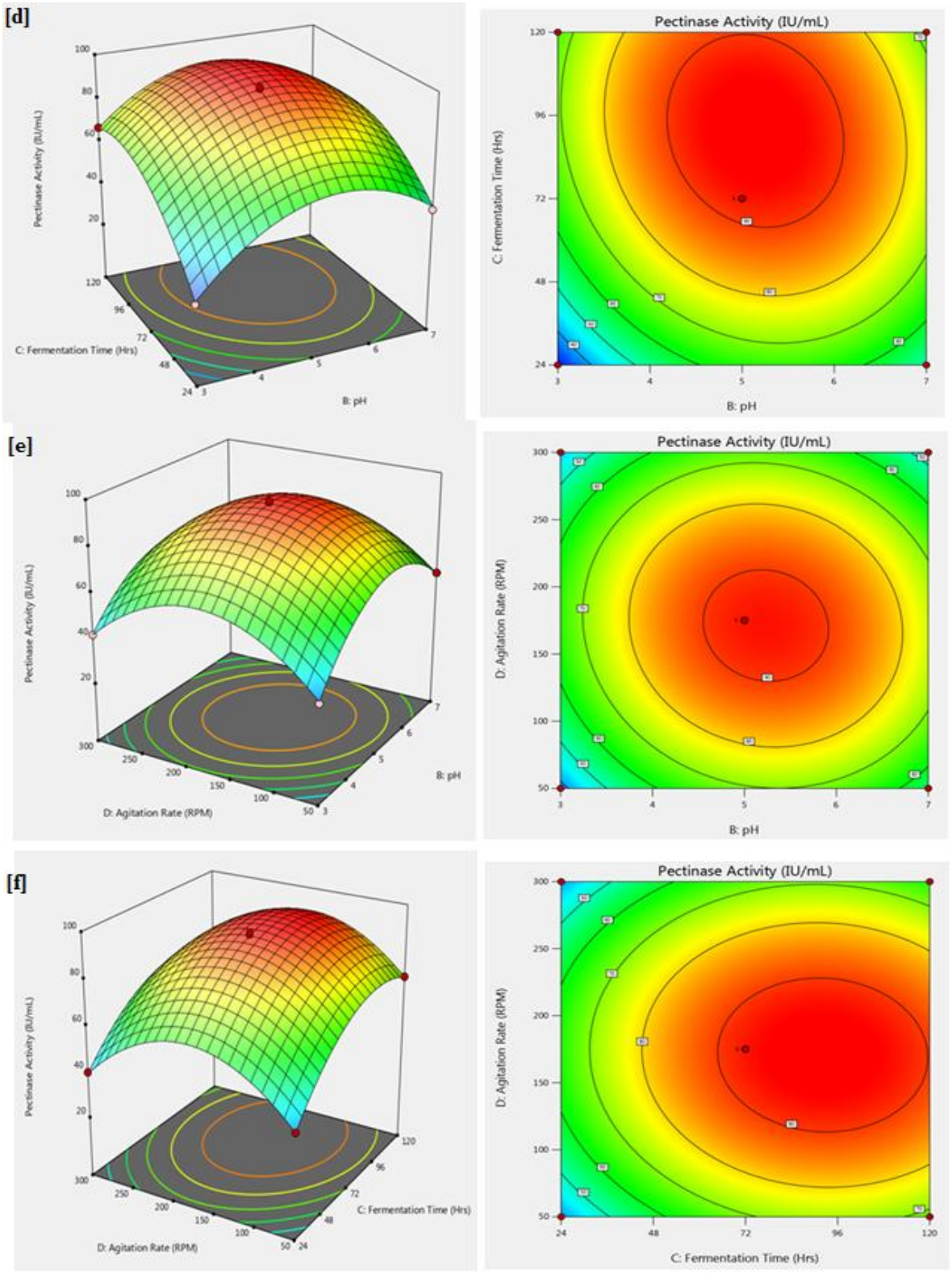


Figure 4 [a-f]: "The surface plot(3D) and contour plot(2D) representing the effect of process variables for pectinase activity. **[a]** Temperature and pH **[b]** Temperature and fermentation time **[c]** Temperature and Agitation rate **[d]** pH and Fermentation time **[e]** pH and Agitation rate **[f]** Fermentation time and Agitation rate"

The interactions between the process variables were reflected on the pectinase activity in 3D response surface plot and 2D contour plot as shown in figure 4. The best activity was obtained at moderate temperatures (32–34°C), at pH 5, at intermediate agitation (175 rpm) and at longer fermentation periods. Production of enzymes was usually decreased under deviations from these conditions. The nearly circular contour patterns indicate that the interaction effects were relatively weak, and the significant quadratic terms suggest that the effects of individual variables and of squaring the variables have strong effects on the pectinase activity, similarly as the results of ANOVA.

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4. CONCLUSION

The Box–Behnken Design combined with "Response Surface Methodology was used to optimize the fermentation conditions in the production of pectinase." The quadratic framework is appropriate for optimizing processes since it was of statistical significance ($F = 512.00$, $p < 0.0001$, $p = 0.2423$ with regard to negligible lack of fit). At 32.5°C, pH 5, 72 hours of enzymatic fermentation, and 175 rpm of movement, the production of pectinase enzyme activity was found to be the highest (93.62 IU/mL). An important factor in enzyme synthesis was the interplay between fermentation duration, pH, and temperature. Fermentation duration, pH, thermal conditions, and nonlinear effects all had significant impacts on enzyme yield. The results clearly showed the potential of statistical optimization on yield of pectinase and could be used as a starting point for further investigation and scale-up studies.

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