INDUSTRIAL ENZYMOLOGY

OPTIMIZATION OF PECTINASE PRODUCTION BY THE FILAMENTOUS FUNGUS PA2S4T USING DCCR

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ABSTRACT

Biotechnology harnesses cellular systems to develop and refine processes and products. Fungi secrete enzymes that are efficient and valuable for industry applications exemplified by citric acid production facilitated by *Aspergillus niger*. The diversity of fungal enzyme capable of degrading complex polymers is pivotal for biotechnology, advancements, notably invertase, which catalyzes sucrose hydrolysis and is industrially utilized to clarify high-quality syrups. The objective was to optimize the production of invertase from filamentous fungus PA2S4T under the conditions of Central Composite Rotatable Design (DCCR) for temperature, cultivation time, and carbon source concentration. In this study the fungus PA2S4T collected in a fragment of Atlantic Forest was cultured on Potato Dextrose Agar (PDS) and incubated at 40 ºC for seven days. Spore suspensions were prepared in 0.8% NaCl and 0.05% Tween and inoculated into Khanna medium under varying conditions. Enzyme production was optimized using DCCR and its conditions previous described. The highest pectinase production (38.8 U/mL) was achieved with 4.2% orange peel concentration. Statistical analysis indicated that orange peel concentration exerted the most significant positive impact on pectinase production. These findings are pivotal for refining pectinase production process for industrial applications.

Keywords: Mycology. Enzyme. Biotechnology. Optimization. Industrial.

1 INTRODUCTION

Biotechnology employs cellular systems to foster the development of processes and products. Fungi are highly valuable for their enzymatic efficiency in industrial applications, particularly within the food and textile sectors, as well as for mitigating industrial waste[1]. Microbiological fermentation employed since the early 20th century, has found industrial applications, exemplified by the utilization of citric acid produced by the fungus *Aspergillus niger*[2]. Fungi are well-suited for biotechnological endeavours owing to their diversity and capacity to degrade complex polymers. Fungal enzymes are indispensable and extensively utilized across pharmaceutical, chemical, and food industries owing to their efficacy and versatility[3].

Pectinase is an enzyme that targets pectin, a polysaccharide abundant in sugars, galacturonic acid and methanol, found in vegetables, cereals, fruits, and fibres. It forms the primary cell wall of plants and the middle lamella. Pectinases, including pectin methyl esterase, pectin lyase, and polygalacturonases, can alter pectin during fruit ripening. Industrially, the use of fungal-derived pectinases are favored due to their pH compatibility with fruit juices and their broad biodiversity[4].

Thus, the filamentous fungus PA2S4T is little known and studied, despite having already demonstrated favourable characteristics for industrial application. Therefore, the objective of this work was to optimize the production of pectinase by DCCR, by determining the best concentration of carbon source, cultivation time and incubation temperature.

2 MATERIAL & METHODS

The experiments utilized the filamentous fungus PA2S4T. Microorganism was collected in a fragment of Atlantic Forest on municipality of Nova Aurora, located in the state of Paraná, between 24° 32' 00'' South and 53° 15' 10'' West, at an altitude of 520 meters above sea level, and it is available at the Microorganism Biochemistry Laboratory of the Western Paraná State University. The fungus was maintained on Potato Dextrose Agar (PDA) and incubated at 40 ºC for seven days. Spore suspensions were prepared in 0,8% NaCl and 0,05% Tween and inoculated into Khanna culture media, at different temperatures, cultivation times, and varying concentrations of orange peel. The submerged cultures were filtered to obtain the crude extract, which was used to measure enzymatic activity and quantify proteins. Enzymatic activity was determined by the DNS method and spectrophotometry[5].

Enzyme production was optimized using the Central Composite Rotation Design[6], testing variables such as temperature, cultivation time, and orange peel concentration[7] according to Table 1. Statistical analyses were performed with Statistica software.

3 RESULTS & DISCUSSION

The results of the experimental design for the effect of three variables – temperature, cultivation time, and carbon source concentration – on enzyme production are summarized in Table 1. In experimental trial 4, pectinase exhibited the highest production of 38.8 U/mL, coinciding with the highest concentration of orange peel (4.2%). Conversely, the lowest concentration of orange peel (1.8%) resulted in the lowest production values in trial 7 (14.2 U/mL). This correlation is evident in the Surface Plot graph depicted Figure 1.

Table 1 Matrix of cultivation conditions and generated responses.

	Variables			Response
Trials	Temperature (ºC)	Cultivation time (hours)	Orange peel (%)	Pectinase(U/mL)
1	-1 (28.0)	-1 (60.0)	-1 (1.8)	20.8 ± 0,21
2	-1 (28.0)	-1 (60.0)	1 (4.2)	38.4 ± 0,19
3	-1 (28.0)	1 (132.0)	-1 (1.8)	33.1 ± 0,17
4	-1 (28.0)	1 (132.0)	1 (4.2)	38.7 ± 0,15
5	1 (48.0)	-1 (60.0)	-1 (1.8)	15.0 ± 0,15
6	1 (48.0)	-1 (60.0)	1 (4.2)	22.9 ± 0,08
7	1 (48.0)	1 (132.0)	-1 (1.8)	14.2 ± 0,17
8	1 (48.0)	1 (132.0)	1 (4.2)	36.3 ± 0,19
9	-1.68 (21.2)	0 (96.0)	0 (3.0)	16.5 ± 0,13
10	+1.68 (54.8)	0 (96.0)	0 (3.0)	18.7 ± 0,10
11	0 (38.0)	-1.68 (35.5)	0 (3.0)	27.2 ± 0,15
12	0 (38.0)	+1.68 (156.5)	0 (3.0)	30.2 ± 0,17
13	0 (38.0)	0 (96.0)	-1.68 (1.0)	21.5 ± 0,17
14	0 (38.0)	0 (96.0)	+1.68 (4.1)	32.7 ± 0,17
15	0 (38.0)	0 (96.0)	0 (3.0)	33.2 ± 0,19
16	0 (38.0)	0 (96.0)	0 (3.0)	32.8 ± 0,21
17	0 (38.0)	0 (96.0)	0 (3.0)	32.9 ± 0,17

In Figure 1a, it is evident that the optimal condition for pectinase enzymatic activity was observed at the lowest temperature (28 ºC), which remained consistent throughout the range with a slight increase at 132 hours. Figure 1b illustrates the relationship between carbon source and temperature, revealing optimal conditions at the highest carbon source concentration and lowest temperature (4.2% at 28 ºC). Figure 1c depicts the relationship between carbon source and time, showing that the highest activity occurs at the highest carbon source concentration and longest duration. Consistently observed in the three graphs is the significant positive effect of carbon source concentration indicating that higher concentrations result in increased pectinase production.
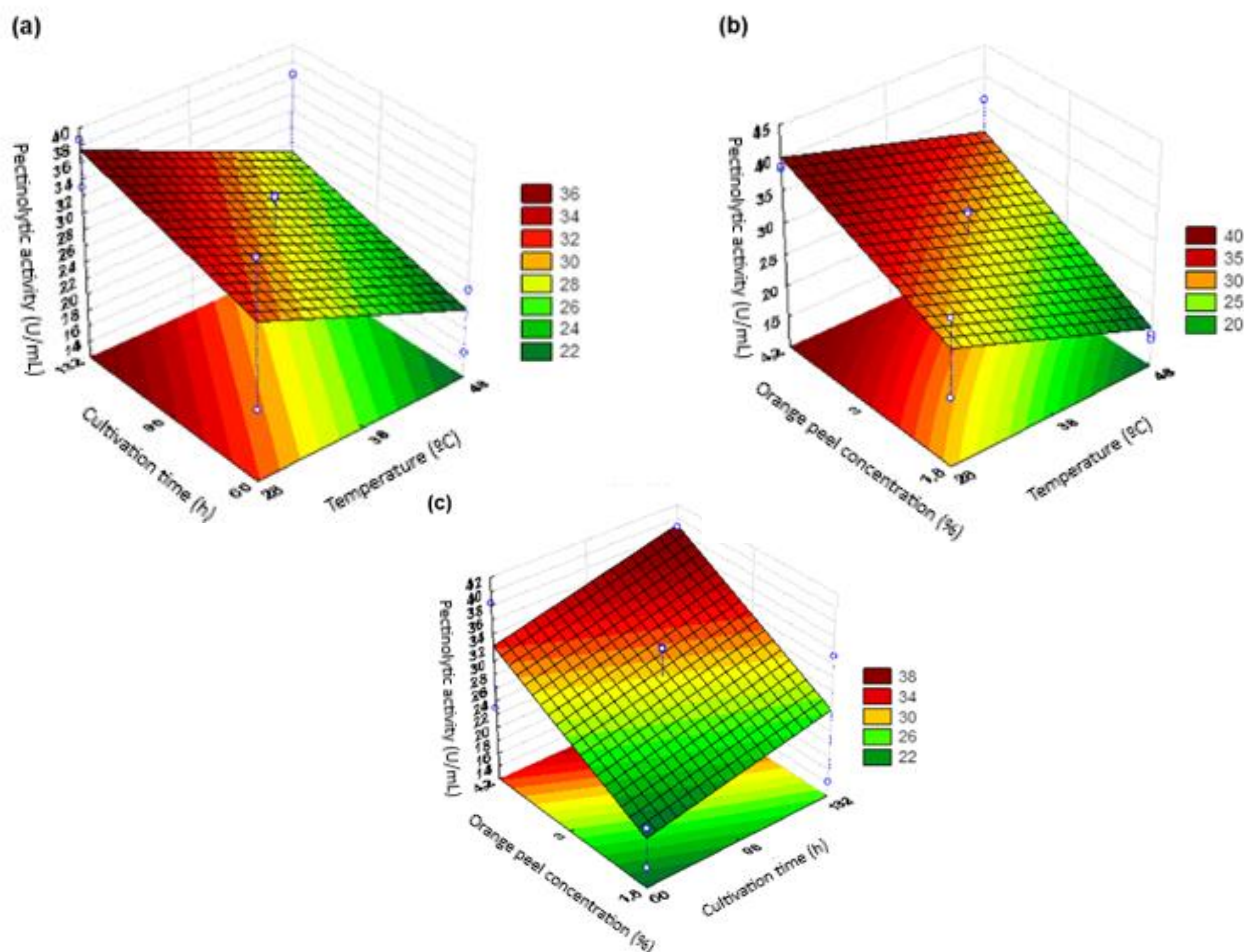

Figure 1 Response Surface Plot graphs, (a) Interaction of Temperature x Cultivation time. (b) Interaction of Orange peel concentration x Temperature. (C) Interaction of Orange peel concentration x Cultivation time.

From ANOVA Table (Table2), it can be observed that there was significance and predictability in the regression, as the *Fcal* (8.35) was greater than the critical F value (*Ftab*) of 3.26, specifically 2.56 times greater. This ensures, statistically, that the results are reliable according to the methodology. The lack of fit *Fcal* (534.75775) was significantly larger than *Ftab* (19.40), indicating that the quadratic model fit the experimentally obtained data. The coefficient of determination (R^2) shows that the quadratic model explains 73.58% of the variability in the experiments, meaning it provides 73.58% confidence in the findings.

Table 2 ANOVA Table of the model for pectinase production.

Sources of variation	Sum of squares (SS)	Degrees of freedom (d.f)	Mean sum of squares (MS)	*F*-test		R²
				Fcal	*Ftab*	
Regression	827.505	4	206.876	8.35626	3.26	73.58
Residual	297.084	12	24.757			
Lack of fit	296.973	10	29.697	534.75775	19.40	
Pure error	0.111	2	0.056			
Total	1124.590	16	70.287			

4 CONCLUSION

The variable that had the most significant positive impact on pectinase production by the filamentous fungus PA2S4T collected in a fragment of Atlantic Forest was the concentration of orange peel, as analyzed among the variables of cultivation time and temperature. Consequently, these data contribute to the optimization of pectinase production by PA2S4T for its application in industrial processes.

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