OPTIMIZATION BY DCCR OF INVERTASE PRODUCTION BY THE FILAMENTOUS FUNGUS PA2S4T

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ABSTRACT

Biotechnology utilizes cellular systems to create and optimize processes and products. Fungi possess enzymes that are efficient and valuable for industry, with notable applications such as citric acid production by *Aspergillus niger*. The diversity of fungal enzyme that degrade complex polymers is essential for biotechnology, particularly invertase, which hydrolyses sucrose and is used industrially to clarify high-quality syrups. The objective of this work was to optimize the production of invertase from filamentous fungus PA2S4T. This study used filamentous fungus PA2S4T on Potato Dextrose Agar (PDS), 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 different conditions. Enzyme production was optimized using Central Composite Rotatable Design (DCCR) for temperature, cultivation time, and carbon source concentration. The highest invertase production (76.7 U/mL) occurred with a 4.2% orange peel concentration. Statistical analysis showed that orange peel concentration had the greatest positive impact on invertase production. These data are crucial for optimizing invertase production by the fungus for food industry applications.

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

1 INTRODUCTION

Biotechnology utilizes cellular systems to develop process and products. Fungi are valuable for their efficient enzymes in industrial processes, especially in food and textile industries, and for reducing industrial waste[1]. Microbiological fermentation, used for centuries, was industrially applied in the early 20th century, exemplified by the use of citric acid production by the fungus *Arpergillus niger*[2]. Fungi are ideal for biotechnology due to their diversity and ability to degrade complex polymers. Fungal enzymes are essential and widely used in pharmaceutical, chemical, and food industries for their efficiency and adaptability[3].

Invertase, β-fructofuranosidade, is the enzyme that catalyses the hydrolysis of sucrose, forming two sugars called inverted sugars: glucose, and fructose, which are reducing sugars. Industrially, it is used in the food sector for clarifying colourless syrups, known for their high quality[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 invertase by DCCR, by determining the best carbon source concentration, cultivation time and incubation temperature.

2 MATERIAL & METHODS

The experiments used 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. Submerged cultures were filtered to obtain the crude extract, which was used to measure enzymatic activity, and quantify proteins. Enzymatic activity was determined by DNS method and spectrophotometry[5].

Enzyme production was optimized using Central Composite Rotatable Design[6], testing variables such as temperature, cultivation time, and orange peel[7] concentration as shown in Table 1. Statistical analyses were conducted using 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 trial 9, invertase production was highest at 76.7 U/mL. The highest enzymatic production was observed in trials with higher concentrations of orange peel (2, 6, 8, and 14) at 4.2% orange peel. Conversely, trials with longer incubation periods resulted in lower invertase production, as seen in trials 3, 4, 7, 8, and 12, with production as low as 4.7 U/mL (trial 13). The positive influence of orange peel concentration is illustrated in Figure 1 through the Response Surface Plot graphs.

Table 1 Matrix of cultivation conditions and generated responses.

Trials	Variables			Response
	Temperature (ºC)	Cultivation time (hours)	Orange peel (%)	Invertase (U/mL)
1	-1 (28.0)	-1 (60.0)	-1 (1.8)	24.1 ± 0.18
2	-1 (28.0)	-1 (60.0)	1 (4.2)	73.9 ± 0.32
3	-1 (28.0)	1 (132.0)	-1 (1.8)	22.7 ± 0.22
4	-1 (28.0)	1 (132.0)	1 (4.2)	28.6 ± 0.14
5	1 (48.0)	-1 (60.0)	-1 (1.8)	24.2 ± 0.10
6	1 (48.0)	-1 (60.0)	1 (4.2)	56.9 ± 0.37
7	1 (48.0)	1 (132.0)	-1 (1.8)	20.2 ± 0.18
8	1 (48.0)	1 (132.0)	1 (4.2)	59.1 ± 0.16
9	-1.68 (21.2)	0 (96.0)	0 (3.0)	76.7 ± 0.39
10	+1.68 (54.8)	0 (96.0)	0 (3.0)	63.2 ± 0.30
11	0 (38.0)	-1.68 (35.5)	0 (3.0)	65.0 ± 016
12	0 (38.0)	+1.68 (156.5)	0 (3.0)	19.5 ± 0.32
13	0 (38.0)	0 (96.0)	-1.68 (1.0)	4.7 ± 0.16
14	0 (38.0)	0 (96.0)	+1.68 (4.1)	61.5 ± 0.39
15	0 (38.0)	0 (96.0)	0 (3.0)	45.8 ± 0.30
16	0 (38.0)	0 (96.0)	0 (3.0)	38.6 ± 0.28
17	0 (38.0)	0 (96.0)	0 (3.0)	44.7 ± 0.39

In Figure 1a, analysing the response surface for invertase, it is noted that the best conditions for enzymatic activity were found at the shortest cultivation time, at both lower and higher temperatures (21.2ºC), as evidenced by the quadratic shape of the graph. In Figure 1b, the relationship between carbon source and temperature shows that the best conditions were observed at the highest carbon source concentration across the temperature range, peaking at 48ºC, and at both lower and higher temperature values. Figure 1c shows the relationship between carbon source and time, with higher production occurring at the highest carbon source concentration and the shortest time. However, the variable that showed the most significant impact on invertase production was the carbon source concentration.

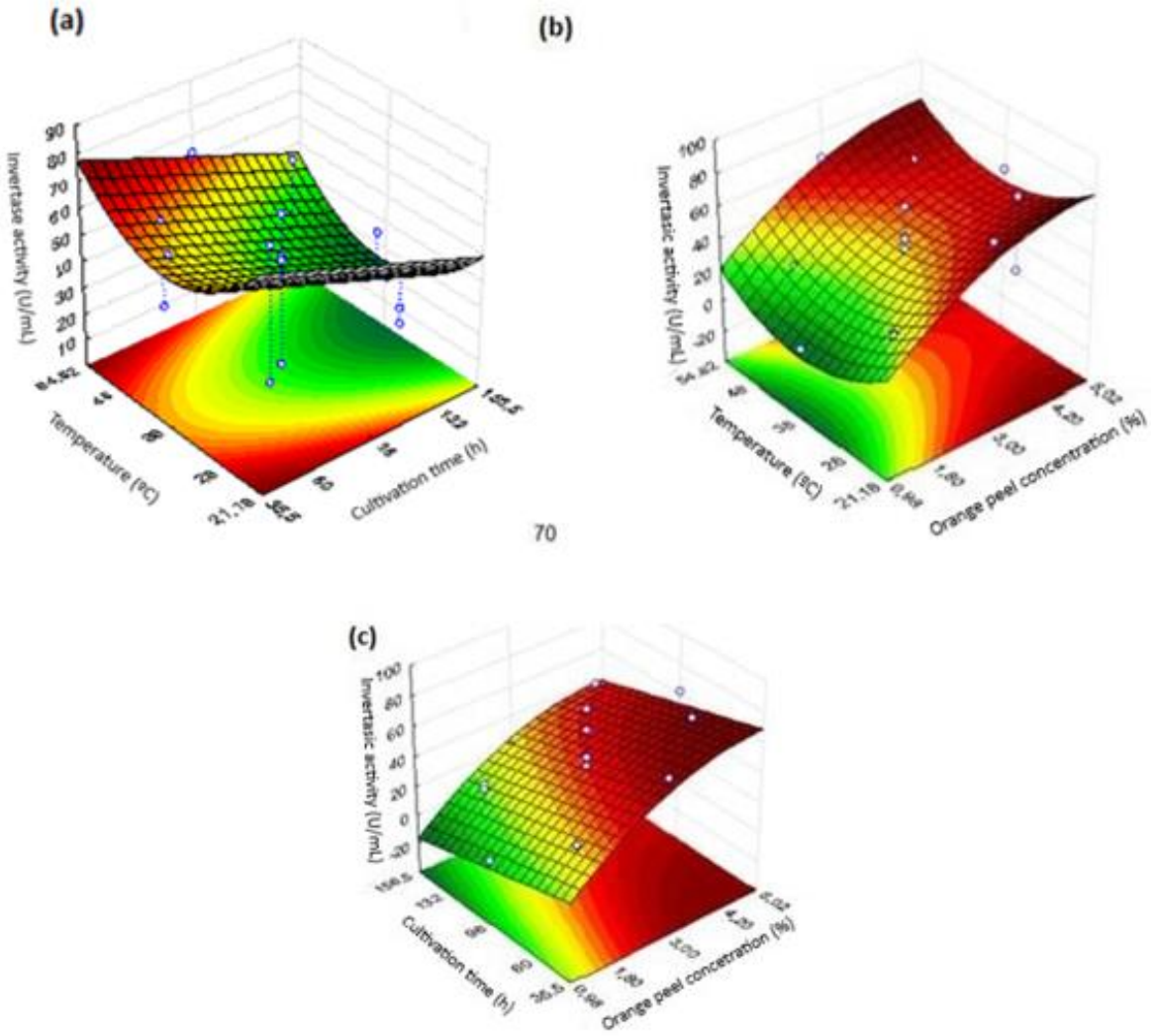

Figure 1 Response Surface Plot graphs for Invertase. (a) Interaction of Temperature x Time. (b) Interaction of Carbon Source x Temperature. (c) Interaction of Carbon Source x Time.

4 CONCLUSION

The Variable that most positively impacted invertase production by the filamentous fungus PA2S4T was the concentration of orange peel, as analysed among the variables of cultivation time and temperature. Thus, these data contribute to the optimization of invertase production by PA2S4T aimed at its application in the food industry.

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