2-PHENYLETHANOL INHIBITION OF GROWTH OF Saccharomyces cerevisiae: EFFECT OF TEMPERATURE

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ABSTRACT

The 2-phenylethanol (2-PE) is an aromatic alcohol that has a characteristic rose odor and can be produced biotechnologically using microorganisms. 2-PE is known to have a strong inhibitory effect on microorganisms, which is considered a limiting factor in its large-scale biotechnological production. In the present study, the inhibition effect of different concentrations of 2-PE and temperatures on the yeast *Saccharomyces cerevisiae* was evaluated. The results indicated that at 28 °C favored yeast tolerance to 2-PE, while at 34 °C made yeast more sensitive to the toxicity of this aromatic alcohol. It was also observed that increasing the concentration of 2-PE provides greater values of the degree of cellular inhibition (GX, %). This fact was observed in the experiment at 34 ºC in concentrations of 4 and 5 g.L-1, the results of which indicated an inhibition of 94.09 and 98.18%, respectively. These findings suggest that adjusting cultivation temperature may be a strategy to optimize bioaromas production via biotechnology.

Keywords: Inhibition. 2-Phenylethanol. *Saccharomyces cerevisiae*.

1 INTRODUCTION

Aromatic compounds attribute sensory characteristics to various products in the food, pharmaceutical, cosmetic and chemical industries [1]. In addition to improving organoleptic characteristics, aromas can also be useful in applications as emollients, surfactants and antioxidants [1]. Aromatic compounds can be obtained from plant extracts, but the great potential production is on microbial sources, such as fungi, yeasts and bacteria [2]. 2-Phenylethanol (2-PE) is an aromatic alcohol that has a characteristic rose odor and can be produced biotechnologically using microorganisms [3]. The 2-PE has been increasingly used by industry and its market is estimated to exceed USD 370 million by 2028 [4]. Among the microorganisms that produce this alcohol, *Saccharomyces cerevisiae* yeasts have great potential [5]. During production, some process variables such as temperature, pH, carbon source and initial amount of inoculum affect the production of this aroma, which may result in different values yield [6]. Furthermore, 2-PE has an inhibitory effect on the microorganism's cells, which contributes to a decrease in product concentration at the end of the process [7]. The final concentration of 2-PE is a critical factor in the production process. The study of 2-phenylethanol inhibition is very important since this aromatic alcohol has great economic interest. Therefore, the present work aimed to evaluate the effect of temperature on the inhibition of the growth of S. cerevisiae yeasts by different concentrations of 2-phenylethanol.

2 MATERIAL & METHODS

This work was carried out at the Biotechnological Processes Laboratory of the Department of Antibiotics at the Federal University of Pernambuco. All inhibition experiments were performed with commercial lyophilized *Saccharomyces cerevisiae* (Fleischmann, AB Brazil) with an initial concentration of 5 $g.L^{-1}$ (in dry basis). The composition of the culture medium was ($g.L^{-1}$): sucrose (100.0), KH_2PO_4 (5.6), $MgSO_4.7H_2O$ (1.4), yeast extract (6.8), and urea (5.32). The experiments were conducted in 500 mL erlenmeyer flasks and maintaining in an orbital shaker (Model C25KC, Brand New Brunswick Scientific) for 24 h at 200 rpm and 34 our 28 °C.

In order to evaluate the inhibitory effect of 2-PE on the yeast *S. cerevisiae*, the microorganism was inoculated in the erlenmeyer flask with the culture medium. Subsequently, the bottles were supplemented with different concentrations of 2-Phenylethanol (Sigma), being: 0 (control), 1, 2, 3, 4 and 5 $g.L^{-1}$. The inhibition experiments were performed in duplicate for 24 hours. Samples (2 mL) were collected at determined times (0h, 2h, 4h, 6h, 8h, 10h, 12h, 24h) to monitor cell growth.

Cell concentration was measured by dry weight method, the medium samples were centrifuged at 13,400 rpm for 5 minutes, and the cell pellet was washed with distilled water and placed in an oven at 80 ºC for 24 h for drying.

The degree of cellular inhibition (GX, %) was estimated by the ratio between the growth of yeast in the medium with inhibitors and the growth of yeast in the control medium. Equation 1 was used to determine the degree of inhibition.

$$GX\ (\%) = \left(1 - \frac{\Delta XE}{\Delta XC}\right) \times 100 \quad (1)$$

where ΔX_E is the biomass variation in each inhibition assay and ΔX_C is the biomass variation in the control test.

3 RESULTS & DISCUSSION

According to several authors, the growth of the yeast *S. cerevisiae* is affected by the presence of 2-Phenylethanol due to its cytotoxicity to microbial cells, being this the limiting factor for its large-scale production [8, 9, 11].

In present study, the effect of inhibition by different concentrations of 2-PE at two temperatures was evaluated. In the sequence, the results for inhibition test performed at 34 °C and 28 °C (Figure 1(a) and (b) are shown, respectively. We can observe that in the absence of 2-PE (control), the *S. cerevisiae* cell biomass was maximum for both temperatures. However, at a temperature of 34 °C (Figure 1a) and 2-PE concentrations greater than 2 $g.L^{-1}$ the inhibitory effect was significantly greater, when compared to tests at 28° C, in same concentrations of 2-PE (Figure 1b). These results indicated that yeast showed higher tolerance to 2-PE at low temperature. This result was due to a strong effect of 2-PE inhibition which is more intense at high temperature, resulting in a decrease in the cell growth for this assay. This effect observed for high concentrations of 2-PE at 34 °C may also be added to the effect of ethanol accumulated in the fermentation broth [5,6,7]. Since alcohol accumulates quickly in the medium resulting in reduced yeast growth which is intensified with increasing fermentation temperature [2, 3]. This occurs due to alcohol ability to insert itself into the hydrophobic part of the phospholipid bilayer of the yeast cell, altering the fluidity of the cell membrane and, consequently, causing the accumulation of toxic substances inside the cell. Thus, a reduction in the maximum rate of glucose uptake will occur in these affected cells[10].

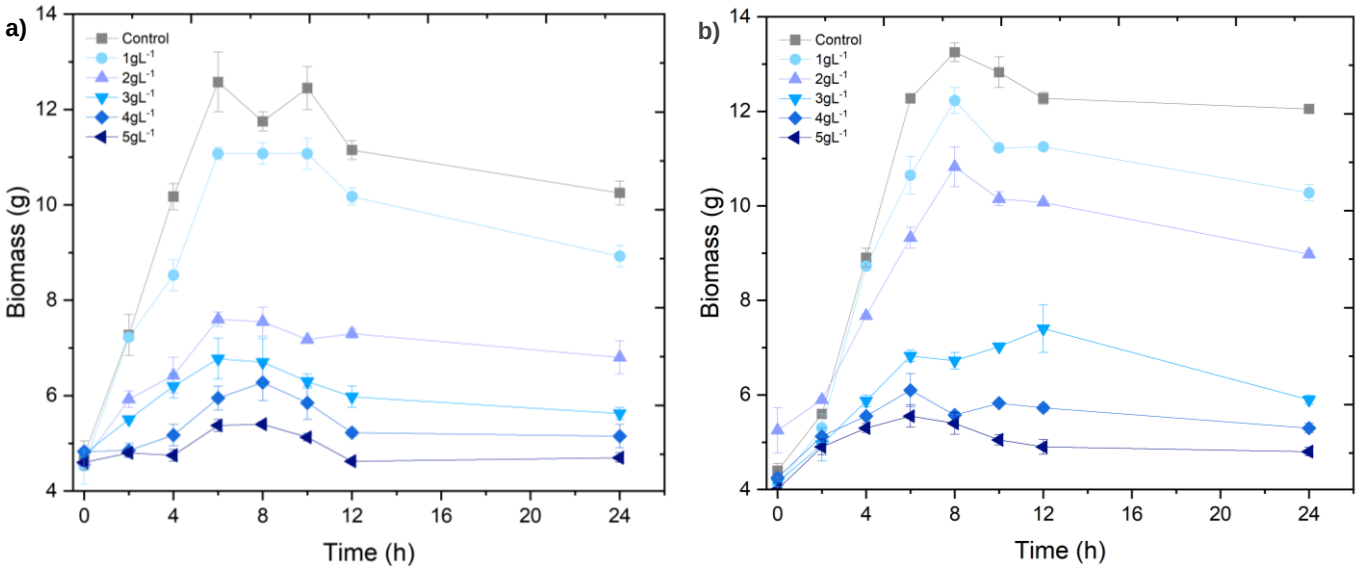

Figure 1. Comparative plots of inhibition test at different concentrations of 2-PE: (a) experiment at 34 °C and (b) experiment at 28 °C.

The degree of cellular inhibition (GX, %) was calculated (Equation 1), the values obtained are presented in Table 1, where it is observed that 2-PE inhibited the growth of the yeast *S. cerevisiae* progressively with increasing concentration of the compound. From Table 1, it can be seen that at a temperature of 34 °C there was practically total inhibition for concentrations of 4 and 5 $g.L^{-1}$ at the end of 24 hours of experiment.

Table 1 Values of degree of cellular inhibition at different temperatures

2 – PE ($g.L^{-1}$)	Degree of cellular inhibition (GX, %)	
	Temperature - 28 ºC	Temperature - 34 ºC
1	19.28	20.00
2	51.31	60.45
3	77.12	84.55
4	86.27	94.09
5	89.54	98.18

The yeast *S. cerevisiae* has limited tolerance in the presence of 2-PE, in the maximum concentration at which growth occurs is around 4 $g.L^{-1}$ [8]. For the results obtained in the present study, the test carried out at a temperature of 34 °C made yeast cells more sensitive to the inhibitory effects of 2-PE. These findings suggest that decreasing temperature is a factor that positively affects the tolerance of the yeast *Saccharomyces cerevisiae* to 2-Phenylethanol. Alternatively, other species with better tolerance and the

study of variables, such as carbon source and initial amount of inoculum, can be used to better intensify the biotechnological production of 2-phenylethanol.

4 CONCLUSION

The study of inhibition test from of the essay under different conditions of concentration of 2-PE and temperature showed that the greatest increase in the Degree of cellular inhibition (GX, %) was achieved in temperature of 34 °C and 2-PE concentration of 5 $g.L^{-1}$, resulting in a GX value of 98.18%. This suggests that decreasing temperature may be a positive factor in increasing yeast tolerance to this compound. Therefore, adjusting the cultivation temperature can be an effective strategy to optimize the large-scale production of compounds such as 2-Phenylethanol, considering its influence on the growth of the yeast *S. cerevisiae*.

5 ACKNOWLEDGMENTS

The authors are grateful for the support provided by Biotechnology Graduate Program – PPGBiotec, Biotechnological Processes Laboratory - LPBiotec, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq and Pró-Reitoria de Pesquisa e Inovação – PROPESQI.

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