INFLUENCE OF DIFFERENT 2-PHENYLETHANOL CONCENTRATIONS ON THE FERMENTATIVE CAPACITY OF *Saccharomyces cerevisiae*

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ABSTRACT

The cytotoxicity of 2-phenylethanol is a limiting factor in the large-scale production of this compound. This study investigated the effect of external 2-phenylethanol on the fermentative capacity of the yeast *S. cerevisiae*. Assays were carried out in shaken flasks (200 rpm and 28 ºC) with the exogenous addition of 2-PE (0, 1, 2, 3, 4 and 5 $g.L^{-1}$). The results indicated that higher concentrations reduced the consumption rate of the substrate, resulting in the presence of residual substrate at the end of 24 h of cultivation. Thus, the effect negative of 2-PE on the fermentative capacity of the yeast *S. cerevisiae* is evident.

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

1 INTRODUCTION

The growing interest in biotechnological production of flavors is largely driven by being classified as natural, which represents a strong marketing advantage. The 2-Phenylethanol (2-PE) is an aromatic alcohol with a delicate rose odor, widely used in the cosmetics, cleaning products and personal care industry [1]. Therefore, with the increasing population and growing consumer demand for anti-aging products, the 2-phenylethanol market is estimated in USD 370 million by 2028 [1].

Among the microorganisms that produce this alcohol, yeasts have great potential [2]. However, the production of 2-phenylethanol by the yeast *Saccharomyces cerevisiae* is limited by the strong inhibition effect of the product [4, 6, 7]. The 2-PE accumulated in the broth during the course of the production process is the main component toxic to microorganism, resulting in decreases in the growth, substrate consumption and consequently low concentration of the 2-PE product in the medium [4]. 2-PE concentrations greater than 4 $g.L^{-1}$ negatively influence cell physiology, affecting membrane fluidity and causing a reduction in cell viability [4, 6].

The final concentration of 2-PE is a critical factor in the production process. The study of the influence of 2-phenylethanol on cell growth and substrate consumption (fermentative capacity) is very important since this aromatic alcohol has great economic interest. Thus, the present work aimed to evaluate the influence of different concentrations of exogenous 2-phenylethanol in the fermentative capacity of *S. cerevisiae* during the fermentation process using sucrose whit substrate.

2 MATERIAL & METHODS

This work was carried out at the Biotechnological Processes Laboratory (LPBiotec) of the Department of Antibiotics at the Federal University of Pernambuco. All fermentative experiments were performed with commercial lyophilized *Saccharomyces cerevisiae* (Fleischmann, AB Brazil). The composition of the culture medium was ($g.L^{-1}$): sucrose (100.0), KH_2PO_4 (2.43), $MgSO_4.7H_2O$ (0.61), yeast extract (2.96), and urea (2.31). 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 temperature of 28 °C.

In order to evaluate the inhibitory effect of 2-PE on the yeast *S. cerevisiae*, erlenmeyer flask containing 160 mL of culture medium was inoculated with lyophilized yeast at a concentration of 5 $g{\cdot}L^{-1}$ (dry basis). Subsequently, the flasks were supplemented with different exogenous 2-Phenylethanol (Sigma) concentration, 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 the fermentative progress.

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.

The concentrations of sucrose, glucose, fructose, and ethanol in the supernatants were determined using an HPLC instrument (1100 series, Agilent Technologies, USA) equipped with a refractive index detector and an Aminex HPX-87H column (300x7.8 mm, Bio-Rad) operated at 35 °C using 5 mM H_2SO_4 as mobile phase, at a flow rate of 0.6 mL/min.

3 RESULTS & DISCUSSION

Saccharomyces cerevisiae cells are affected by the presence of the compound 2-phenylethanol in the fermentative medium [5]. This compound has a strong inhibitory effect on the growth of yeast cells and substrate consumption, as it has a negative impact on the oxidative capacity of yeast [3].

Figure 1 provide comparisons the experimental values obtained for substrate concentration (expressed in TRS), for the range of 2-PE concentrations evaluated.

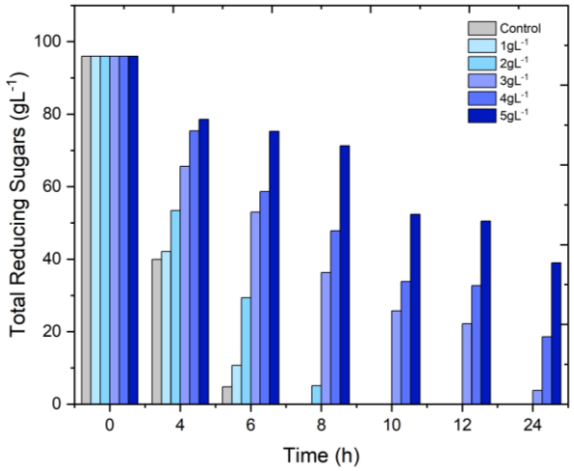

Figure 1. Total Reducing Sugar (TRS) concentrations over time for the inhibition experiments with different 2-FE concentrations

The sugar present in the control test was completely consumed in a period slightly longer than 6 hours of fermentation. This behavior was similar to that observed in the test that used a concentration of 1 $g.L^{-1}$ of 2-Phenylethanol due to the low concentration used in that test. However, for the highest concentrations of 2-PE (3, 4 and 5 $g.L^{-1}$) it was noted that after 24 hours of fermentation there was still the presence of residual substrate in the medium. This clearly indicates that the substrate uptake rate was reduced due to the presence of high 2-PE concentrations in the medium fermentative, indicating that they presented greater inhibitions [2, 3, 6].

When analyzing Table 1, it is clear that as 2-PE concentration values increase, there is a significant intensification in the degree of cellular inhibition [3, 6, 7]. This inhibitory effect of 2-PE may also be associated with the inhibitory effect of ethanol accumulated in the medium during the fermentation process [2,3,6]. The accumulation of ethanol in the fermentation broth significantly decreases cell growth rates [2, 3]. For the biotechnological production of 2-phenylethanol, these two inhibitory effects must be considered as they negatively influence cell physiology, affecting membrane fluidity, and consequently, cell viability and process efficiency [3, 6].

Table 1. Comparison of the performances of fermentation experiments with different exogenous 2-FE concentrations

2–PE ($g.L^{-1}$)	Degree of cellular inhibition (GX, %) (%)	$Y_{E/ART}$ ($g_E.g_{ART}^{-1}$)
0 (Control)	-	0.39
1	19.28	0.40
2	51.31	0.40
3	77.12	0.39
4	86.27	0.34
5	89.54	0.23

The value of the ethanol yield coefficients, $Y_{E/TRS}$ for the fermentation experiments carried out with the presence of up to 4 $g.L^{-1}$ of 2-PE did not vary compared to the control test ($C_{2\text{-}PE}$=0 $g.L^{-1}$). Except for the test conducted with 5 $g.L^{-1}$ of 2-phenylethanol

showed a reduction in the $Y_{E/TRS}$ value. Therefore, having detailed information about the inhibitory effect of 2-PE will allow us to adjust the biotechnological process of this aromatic alcohol, overcoming challenges and improving the viability and profitability of the production process.

4 CONCLUSION

The evaluation of the fermentative capacity of *Saccharomyces cerevisiae* under different 2-PE concentrations showed that the greatest effect of 2-PE was obtained at concentrations of 4 end 5 $g.L^{-1}$ (in the ranges evaluated). The results showed the presence of 18.57 and 38.98 g. L^{-1} of residual substrate for the fermentations carried out in the presence of 4 and 5 $g.L^{-1}$ of exogenous 2-PE. Another affected parameter was the volumetric ethanol productivity, which was 90,71% e 81,24% lower than the control fermentation for 4 and 5 g.L-1 of 2-PE respectively. These results are fundamental to understanding how the presence of 2-PE can affect yeast metabolism, potentially contributing to the development of strategies that increase the efficiency of biotechnological processes.

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