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Stress Factors and Fermentative Performance of Industrial Yeasts

Fatores de Estresse e Desempenho Fermentativo de Leveduras Industriais

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Abstract
Saccharomyces cerevisiae is essential in the production of ethanol, thanks to its fermentative efficiency. Meanwhile, in the fermentation process, stress conditions can alter the biochemical changes of these microorganisms, influencing the production of metabolites. In this sense, this study aims to investigate the stress factors present in the ethanolic fermentative process, as well as to analyze the influence of temperature on glycogen accumulation in industrial cells of *Saccharomyces cerevisiae*. It was a pre-inoculum with 0.10 g of the yeasts Catanduva-1 and Pedra-2 without the liquid YPSac 5%. The biomass obtained was inoculated in cane broth at 15 ºBrix and incubated in different cultivation conditions. The glycogen was extracted with sodium carbonate (Na_2CO_3 0.25 M) and determined by enzymatic method and readings at 525 nm. The results show that, during the ethanol fermentation process, the yeasts face several stress factors that can impair their fermentative performance. An analysis of glycogen accumulation revealed that there were differences in the profile between the cells, and Pedra-2 showed significant accumulation at 30 and 35 °C after 10 hours of fermentation.

Keywords: Fermentative Processes. Cellular Metabolism. Glycogenium.

Resumo
Saccharomyces cerevisiae são fundamentais na produção de etanol, graças à sua eficiência fermentativa. Entretanto, no processo fermentativo, as condições de estresse podem alterar as rotas bioquímicas destes microrganismos influenciando na produção de metabólitos. Neste sentido, este estudo visou investigar os fatores de estresse presentes no processo fermentativo etanólico, bem como analisar a influência da temperatura no acúmulo de glicogênio em linhagens industriais de *Saccharomyces cerevisiae*. Um pré-inóculo com 0,10 g das leveduras Catanduva-1 e Pedra-2 no meio líquido YPSac 5% foi preparado e a biomassa obtida foi inoculada em caldo de cana a 15 ºBrix e então, incubadas em diferentes condições de cultivo. O glicogênio foi extraído com Na_2CO_3 0,25 M e determinado por método enzimático e leituras em 525 nm. Os resultados

evidenciaram que, durante o processo de fermentação de etanol, as leveduras enfrentaram vários fatores de estresse que podem prejudicar sua performance fermentativa. A análise do acúmulo de glicogênio revelou que houve diferenças no perfil entre as linhagens, a Pedra-2 apresentou acúmulo significativo a 30 e 35 °C após 10 h de fermentação.

Palavras-chave: Processos Fermentativos. Metabolismo Celular. Glicogênio.

1 Introduction

The production of bioethanol from renewable sources has gained prominence as a more sustainable alternative to fossil fuels. In a scenario where the search for sustainable alternatives is imperative, various technologies have been employed to enable the energy transition. Among these approaches, the use of the yeast Saccharomyces cerevisiae has emerged as one of the most effective and well-established strategies for bioethanol production (Caballero-Sanchez *et al*., 2023). These yeasts are recognized for their versatility and have been widely explored in alcoholic fermentation due to their intrinsic ability to convert simple sugars into ethanol and carbon dioxide (Menezes; De Castro; Rocha, 2022).

The utilization of these microorganisms in bioethanol production presents several advantages. Beyond their inherent capacity for high sugar-to-ethanol conversion rates, *S. cerevisiae* is easily cultivable on a large scale, non-pathogenic, and genetically modifiable, allowing optimization through metabolic engineering. Additionally, it exhibits a high viability rate, making it feasible for industrial processes (De Moraes *et al*., 2022; Zheng *et al*., 2022). However, despite these advantages, some challenges persist, as fermentation efficiency and yeast performance are influenced by various factors that can induce cellular stress.

The fermentation process is affected by multiple interconnected factors that can directly impact yeast fermentative performance, such as temperature fluctuations, which influence *S. cerevisiae* metabolism during fermentation. Indeed, yeasts exhibit sensitivity to thermal changes, which results in distinct metabolic and physiological responses that can affect enzymatic activity, reaction kinetics, and, consequently, the yeast's metabolic profile. These responses can vary significantly depending on the stress factor and its interaction with other variables (Santos *et al*., 2022).

Nevertheless, the adaptability of this microorganism extends beyond ethanol production, encompassing a set of remarkable adaptive strategies that enable it to adjust to adverse environmental conditions. Under high-temperature conditions, *S. cerevisiae* enhances its production of self-protective compounds, such as trehalose and glycogen, which play fundamental roles in its survival and adaptation. According to Santos *et al*. (2018), elevated temperatures and prolonged fermentation times influence the production of these metabolites, consequently reducing yeast fermentative capacity and leading to decreased ethanol production.

Trehalose, a disaccharide, is typically synthesized in response to thermal stress and acts as an osmoprotective "shield," minimizing cell dehydration and preserving cellular structure and function against the detrimental effects of excessive heat (Yap *et al*., 2021). In parallel, glycogen accumulation represents another thermal stress response mechanism in yeast. As a polysaccharide energy reserve, glycogen provides yeast with a sustainable energy source during periods of low metabolic demand or stress conditions (Betlej *et al*., 2020).

The ability to accumulate trehalose and glycogen also enhances yeast adaptability to dynamic environmental conditions, contributing to its metabolic versatility and, ultimately, its evolutionary competitiveness. These adaptive strategies not only ensure yeast survival under thermal variations but also allow the resumption of vital metabolic activities once conditions stabilize (Elbakush; Güven, 2021). This demonstrates the efficiency of *S. cerevisiae* in adapting to diverse scenarios, ensuring its viability and evolutionary competence.

Understanding yeast physiology and analyzing its responses to different stress conditions is an approach that can provide highly relevant insights. These insights can be instrumental in the appropriate selection of strains for fermentative processes, particularly in bioethanol production. Furthermore, the knowledge generated is not limited to this application but also extends to other areas of biotechnology where yeasts play an essential role. Thus, this study aimed to investigate the stress factors present in the bioethanol fermentation process and analyze the influence of temperature on glycogen accumulation in industrial strains of *Saccharomyces cerevisiae*.

2 Material and Methods

2.1 Microorganism

The yeast strains Catanduva 1 and Pedra 2 were obtained from the company LNF Latino Americana Biotecnologia Aplicada, located in Bento Gonçalves, Rio Grande do Sul.

2.2 Pre-inoculum and fermentative conditions

The pre-inoculum was prepared using the classic YPSAC 5% culture medium, containing: 1.0% (w/v) yeast extract; 1.0% (w/v) peptone; 5.0% (w/v) sucrose; with pH adjusted to 5.0 using 1N HCl. All materials were autoclaved at 120 °C for 20 min. Subsequently, 0.10 g of lyophilized yeast was added and incubated for 24 h at 30 °C at 200 rpm. After this period, the cells were collected, centrifuged (800 g for 20 min), and washed three consecutive times in sterile saline solution (0.85%), resulting in a biomass concentration of 10 $mg.mL^{-1}$. This biomass was then inoculated into the fermentative medium, previously prepared with 50 mL of sterilized sugarcane juice at a concentration

of 15°Brix without pH adjustment, in 125 mL Erlenmeyer flasks, which were incubated at 30, 35, and 40 °C in an incubator without agitation. At predetermined fermentation times of 10, 20, 30, and 40 h, aliquots were taken for analysis.

2.3 Cell viability analysis

To evaluate cell viability, 10 µL of the samples were collected and added to methylene blue dye. Cell counting was performed using a Neubauer chamber with the aid of an optical microscope, following the method described by Lee *et al*. (1981).

2.4 Glycogen quantification

Glycogen was extracted using 0.25 M Na_2CO_3 and determined by the enzymatic method using glucose oxidase and peroxidase (Parrou; François, 1997). Readings were taken at 525 nm, and results were expressed as $nmoles.mg^{-1}.cells^{-1}$.

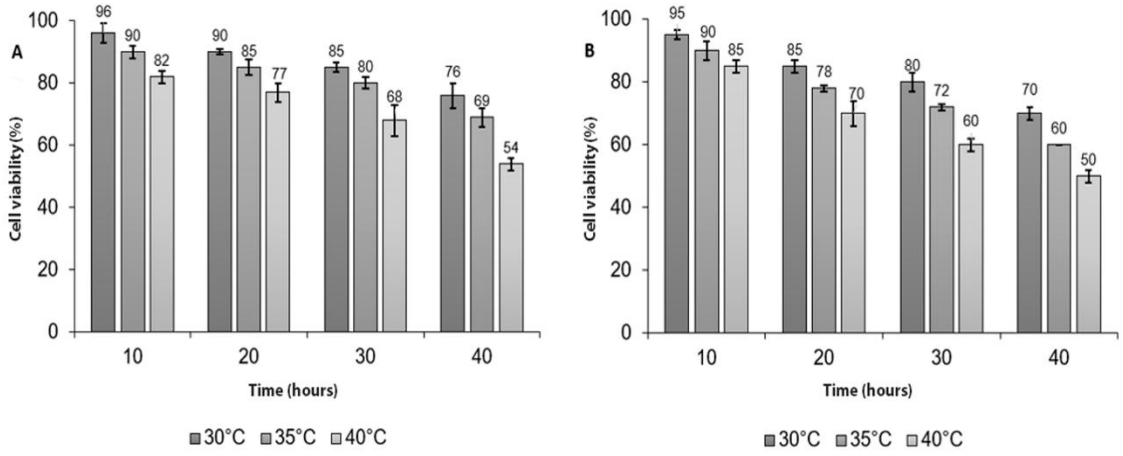
2.5 Statistical analysis

Data analysis, including mean and standard deviation calculations, as well as graphical representations, were performed using Excel Software (2019). The experiments were conducted independently in triplicate.

3 Results and Discussion

In the evaluation of cell viability of the industrial yeast strains Pedra-2 and Catanduva-1, both exhibited approximately 98% viability after 10 hours of fermentation under the assessed conditions. However, at longer fermentation times, a decline in cell viability was observed, with a more pronounced decrease at higher temperatures (Figure 1A and 1B). At 30 °C, both strains maintained viability above 60% after 40 hours of fermentation. However, at 40 °C, the Pedra-2 strain demonstrated greater stability, preserving approximately 60% viability after 40 hours, whereas Catanduva-1 exhibited viability below 40%. These results suggest that the Pedra-2 strain possesses greater resistance to thermal stress and prolonged fermentation periods.

Figure 1 - Evaluation of cell viability of the industrial yeast strains Pedra-2 (A) and Catanduva-1 (B) cultivated under different fermentation conditions.



Source: research data.

The results obtained in this study are consistent with the findings of Batistote and Santos (2020), who evaluated the cell viability of industrial yeast strains cultivated in sugarcane juice under temperatures of 30 and 40 °C. These authors observed that the Catanduva-1 strain exhibited an initial viability index of 90%, demonstrating its adaptation to the industrial fermentation process. However, at longer fermentation times, such as 20 and 40 hours, a significant reduction in cell viability was observed, particularly at higher temperatures, reinforcing the impact of thermal stress on yeast physiology.

Furthermore, the influence of temperature on cell viability was also reported in the studies by Santos *et al*. (2021), who investigated the response of the Pedra-2 and FT858 yeast strains in a sugarcane juice-based substrate with a concentration of 22°Brix. The authors found that yeasts exhibited better viability indices at 30 °C, reaching 79% for Pedra-2 and 76% for FT858. However, at 40 °C, there was a significant decline in cell viability, suggesting that thermal stress affected budding capacity and resulted in cell viability loss.

The literature highlights that *Saccharomyces cerevisiae*, the species to which the studied yeast strains belong, is widely used in biotechnological processes due to its metabolic capacity and resistance to different environmental conditions. However, factors such as high temperatures and prolonged fermentation periods can negatively impact cell viability. Studies indicate that temperatures above the optimal growth range can lead to protein denaturation and membrane dysfunction, ultimately reducing viability (Santos *et al*., 2021).

It is well established that the ability of certain yeast strains to tolerate environmental stresses is associated with the production of specific metabolites and the activation of secondary metabolic pathways. For instance, the production of trehalose and the expression of heat shock proteins are well-

known cellular protection mechanisms under stress conditions (Santos *et al*., 2021). The greater resistance observed in the Pe-2 yeast strain may be related to a more efficient expression of these defense mechanisms.

From a practical perspective, the selection of yeast strains for industrial fermentation processes should consider thermal stress resistance and the ability to maintain high viability during prolonged fermentations. The Pe-2 yeast strain, demonstrating greater stability under high-temperature conditions and extended fermentation periods, presents itself as a promising candidate for optimizing efficiency and productivity in industrial processes operating under such conditions.

Loss of cell viability is a direct consequence of these adverse effects. Yeasts exposed to high temperatures may experience irreparable damage that compromises their long-term survival. Cell death can result from a cascade of events triggered by protein denaturation and membrane damage. To mitigate these negative effects, cells often develop thermal stress response mechanisms, activating heat shock proteins and other defense systems (Silva *et al*., 2023). However, under extreme conditions, these mechanisms may be insufficient, leading to irreversible damage to the yeast cells.

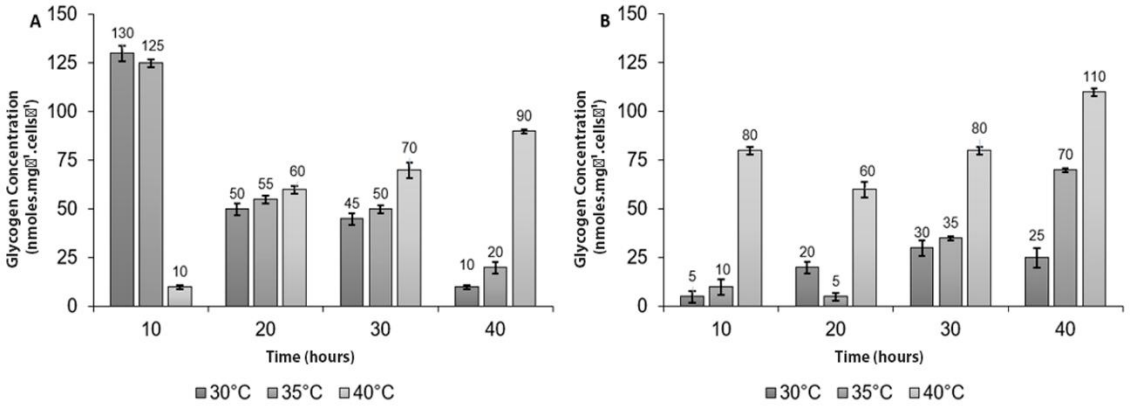
According to Mueller *et al*. (2020), elevated temperatures and high ethanol concentrations in the medium can cause more severe cellular damage, leading to alterations in deoxyribonucleic acid (DNA). In addition to ethanol, the fermentation medium may contain other toxic compounds, such as organic acids and aldehydes, which can impact the fermentative performance of yeasts. In this context, it is important to highlight that stress factors are crucial determinants of yeast metabolism and fermentative efficiency.

The effects of stress factors on ethanol fermentation vary depending on the intensity and duration of exposure. Over short periods, yeasts can adapt by activating their defense and cellular repair mechanisms. However, prolonged exposure may lead to irreversible damage and cell death (Yang; Tavazoie, 2020). According to Coertjens *et al*. (2023), the success of fermentation is linked to the yeast's ability to withstand and overcome the stress factors present in the fermentation environment. The ability to resist thermal stress stands out as one of the key qualities for preserving cellular integrity.

Saccharomyces cerevisiae produces metabolites that aid in the self-regulation of physiological functions and self-protection under extreme stress conditions. The analysis of glycogen concentration revealed differences in the metabolic profile between the studied yeast strains (Figure 2). The Catanduva-1 strain, when exposed to temperatures of 30 and 35 °C, exhibited a glycogen concentration lower than 50 nmoles.mg^{-1}.cells^{-1}. However, at 40 °C, a significant variation was observed, with values ranging between 60 and 75 nmoles.mg^{-1}.cells^{-1} (Figure 2A). In contrast, the Pedra-2 strain presented glycogen concentrations of 130 and 170 nmoles.mg^{-1}.cells^{-1} after 10 hours of fermentation at 30 and 35 °C, respectively, indicating a high concentration of this metabolite in the

cells. In subsequent fermentation times, glycogen concentrations in the Pedra-2 strain were more moderate (Figure 2B). This result may suggest that the lyophilization process induces cellular stress, reflected in elevated glycogen concentrations.

Figure 2 - Evaluation of Glycogen Concentration in Industrial Yeast Strains Pedra-2 (A) and Catanduva-1 (B) Cultivated Under Different Fermentation Conditions



Source: research data.

According to Berlowska, Kregiel, and Ambroziak (2013), immobilized yeast cells exhibit higher levels of glycogen and other structural compounds compared to free-state cells. This observation suggests that immobilization creates a more stable environment, promoting the accumulation of reserve carbohydrates that provide cells with protection against environmental fluctuations, leading to functional metabolic alterations. These changes can influence cell composition and result in significant implications for biotechnological processes, affecting the production of desired products such as ethanol.

The fermentation environment is characterized by a series of conditions that can cause stress in yeast cells. These stress factors have the potential to significantly interfere with the physiological metabolism of the cells, affecting their metabolic pathways and consequently influencing the production of desired metabolites. Yeast cells' response to these stresses may involve the activation of defense mechanisms. However, the activation of these defense mechanisms often competes with normal metabolic pathways, diverting cellular resources that could otherwise be directed toward the desired production. Therefore, understanding and optimizing the fermentation environment is crucial

to maximizing efficiency and the desired production while minimizing the negative impact of stress on yeast cells during the process.

4 Conclusion

The analysis of trehalose accumulation in the industrial strains Catanduva-1 and Pedra-2 indicates a similar pattern, with a gradual increase over time and at different temperatures. The yeast Pedra-2 shows a higher trehalose accumulation during prolonged fermentations at high temperatures.

The analysis of glycogen concentrations between the yeast strains Catanduva-1 and Pedra-2 reveals distinct response patterns to the experimental conditions. Pedra-2 exhibits high glycogen concentrations at 30 and 35°C after 10 hours of fermentation, suggesting a possible influence of the lyophilization process.

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