INFLUENCE OF FLOCCULENT YEAST ON THE PERFORMANCE OF INDUSTRIAL FERMENTATION OF MOLASSES.

João P. M. Souza[1,2*], Homero A. Neto[1], Suzana S. Francisco[3], Charles D. F. Jesus[1] Isadora A. C. Pupin[4], Teresa C. Zangirolami[1,4], Marcelo P. A. Ribeiro[1,4] Thais S. Milessi[1,4] & André Aguiar[2]

[1] *Graduate Program of Chemical Engineering (PPGEQ)/Federal University of São Carlos, São Carlos-SP, Brazil.*
[2] *Bioprocess Engineering/Institute of Natural Resources (IRN)/ Federal University of Itajubá, Itajubá-MG, Brazil.*
[3] *Chemistry/Department of Chemistry (DQ)/Federal University of São Carlos, São Carlos-SP, Brazil.*
[4] *Chemical Engineering/Department of Chemical Engineering (DEQ)/Federal University of São Carlos, São Carlos-SP, Brazil.*
* *jpzmarques@hotmail.com*

ABSTRACT

Brazil has a well-established bioethanol industry and is the second largest ethanol producer in the world. However, despite progress, challenges remain, such as the occurrence of flocculating yeasts in fermentation tanks during the harvest season, which can affect process efficiency. In this context, the present study aimed to analyze the impact of flocculating yeasts on fermentation performance under industrial conditions, using sugarcane molasses as substrate. The experiments were performed in a 5 L bench-scale bioreactor using molasses as substrate and mimicking industrial conditions (initial sugar ~120 g/L, 32°C, pH 5.0), using the yeast *Saccharomyces cerevisiae* Fermel® in its pure form (Experiment A) and also mixed with flocculating yeasts (FT2330L and FT2858L) at a 50% ratio (Experiment B). It was observed that Experiment B was significantly less efficient, with a 20% decrease in ethanol productivity (from 9.4 to 7.4 g/L/h), demonstrating the disadvantages of the presence of flocculating yeasts in the fermentation medium.

Keywords: Alcoholic fermentation 1. flocculating yeast 2. productivity 3. *S. cerevisae* 4. Industrial process 5.

1 INTRODUCTION

The global need for a sustainable energy matrix is becoming increasingly urgent, driven by growing concerns about climate change and the search for renewable energy sources. In this context, the role of ethanol as a viable alternative to fossil fuels stands out. In addition to reducing greenhouse gas emissions, ethanol diversifies the energy matrix, promotes energy security, and stimulates economic development in rural areas. Its production also drives research into sustainable technologies and fosters international cooperation, making it a critical component of a more sustainable and resilient energy future [1].

The ethanol industry in Brazil is widely recognized as one of the most developed and established in the world. Its main characteristics include a large availability of raw material, mainly sugar cane grown in favorable climatic conditions, and advanced technology that ensures efficiency and quality in production. This industry not only meets the domestic demand for vehicle fuel, but also plays an important role in international trade, strengthening the country's economy and its position in the global renewable energy market[2].

Most Brazilian sugarcane mills co-produce sugar, ethanol and electricity. In this context, molasses fermentation is essential for ethanol production from sugarcane due to its importance as a substrate rich in fermentable sugars. Molasses is a by-product of the sugar industry that contains a high concentration of sucrose, glucose and fructose, providing an efficient and economical source of sugars and nutrients for the yeasts used in fermentation [3]. This abundance of sugars allows yeasts to rapidly convert them into ethanol, resulting in high yields and efficiency in the biofuel production process. Therefore, molasses fermentation allows the commercial production of ethanol integrated with sugar production, contributing significantly to the country's energy and economic matrix. Despite the well-established ethanol industry in Brazil, there is always room for continuous improvement. Even small improvements in the process can result in significant gains due to the large volume of production.

Contamination is an unavoidable problem in Brazilian ethanol plants due to the large volume of the reactors (about 1000 m^3). It is usually caused by bacteria, mainly lactic acid bacteria, or native yeasts. The appearance of flocculating yeasts can hinder cell recovery for reuse in subsequent fermentation cycles, increasing operating costs and demand for inputs. When subjected to centrifugation, flocculent yeasts can form dense clusters that are more difficult to separate efficiently. In addition, centrifugal forces may not be sufficient to overcome the cohesion between yeast clusters, resulting in incomplete separation. Another challenge is that even after centrifugation, small amounts of flocculent yeast may remain suspended in the solution, especially if centrifugation is not performed for a sufficient time or under ideal conditions [4].

The excessive presence of flocculent yeasts can also lead to the formation of compact layers of their cells at the bottom of the tank, hindering heat and mass transfer and increasing the risk of microbiological contamination, which can directly affect the lower sugar-to-ethanol conversion efficiency, making the understanding of the impact caused by these yeasts on the process performance a key factor. Therefore, the aim of this study was to identify the effects of the presence flocculating yeasts in alcoholic fermentation carried out under industrial conditions using molasses as substrate.

2 MATERIAL & METHODS

The yeast *S. cerevisiae* Fermel®, an industrial yeast strain with high fermentative performance in molasses, and a mix of flocculating yeasts (FT2330L and FT2858L) were kindly provided in their lyophilized form by Fermentec (Piracicaba, Brazil) and used. Fermentation assays were conducted using only the Fermel yeast (Experiment A) or using a mixture of Fermel yeast with flocculating yeasts in a ratio of 1:1 (w/w) (Experiment B). The molasses was diluted to obtain a concentration close to 120 g/L of initial total reducing sugars (TRS), and yeast initial concentration was 20 g/L (in Experiment B, 10 g/L of each was used).

The lyophilized yeast was rehydrated and activated in a 5 L stirred-tank bench bioreactor following the procedure adapted from Mesquita [6]. First, 100 g of cells were suspended in 1,5 L of distilled water and kept under 0.5 L/min of aeration without agitation for 1 hour at 25°C. For the activation, 500 mL of medium composed by diluted molasses was added to the suspension to reach 60 g/L of TRS and kept at 250 rpm and 25°C for 4 hours. After activation, in Experiment B, the mix of flocculating yeasts was added to the tank.

The fermentation experiments were performed at 32ºC and 300 rpm in a 5 L stirred-tank bench bioreactor which was adapted to reproduce the industrial must recirculation system by installing a peristaltic pump that maintained the recirculation flow at 17 L/h[12]. The supervision system SUPERSYS_HCDC, developed in-house and programmed in Labview®, was used for real-time pH and temperature data acquisition[5]. The concentrations of sugars, ethanol and acetic acid were quantified by high-performance liquid chromatography (HPLC) using a refractive index and UV–VIS detectors (λ = 210 nm) with a RezexTM ROA-Organic acid H+ ion exclusion column at 65ºC with 5 mmol/L H_2SO_4 as eluent at a flow rate of 0.6 mL/min.

The overall substrate-to-ethanol yield (g/g, $Y_{P/S}$) and the volumetric ethanol productivity (g/L/h, Q_P) were calculated according to Shuler [7]. The substrate consumption rate ($-r_S$, g/L/h) was obtained by fitting a polynomial to the substrate concentration (C_S) over time curve and obtaining the tangent of the curve (dC_S/dt) through derivation of the polynomial, as described before. [8]

3 RESULTS & DISCUSSION

The monitoring of experiments A and B and the respective fermentation parameters are presented in Figure 1 and Table 1.

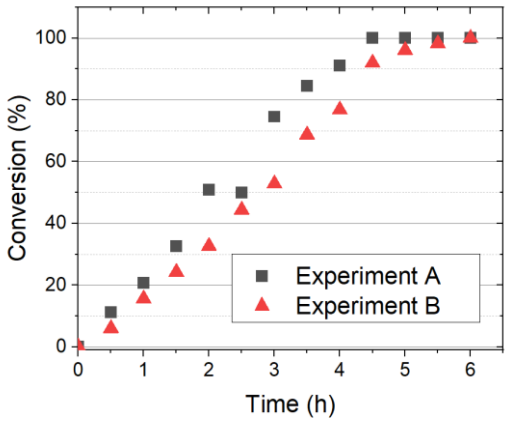

Figure 1 Total Reducing Sugars (TRS) (g/L) over time (h) for molasses fermentation in experiments A and B (32º, pH 5,5, 300 rpm, 120 g/L of initial ART, OD_0 = 40).

Table 1 Fermentation Parameters of Molasse fermentation in Experiments A and B (32º, pH 5,5, 300 rpm, 120 g/L of initial ART, OD_0=40).

Parameter	Experiment A	Experiment B
$Y_{P/S}$ [g/g]	0.46	0,35
Q_p [g/L/h]	9.43	7.40
Etanol [g/L]	53.0	53.0
$-r_S$ [g/L/h]	18.6	15.4
Lactic Acid (g/L)	0.1	4.1
Bacteria contamination (cells/mL)	7.6×10^9	8.9×10^9

It is evident that Experiment A, using pure Fermel yeast, showed superior performance compared to Experiment B, not only in terms of ethanol yield, but also in terms of productivity. Experiment A took 4.5 hours to consume all available TRS and produce 53 g/L ethanol, while Experiment B took 6 hours to achieve the same ethanol production. In addition, the substrate consumption rate was 17% lower when the flocculating yeasts were present (from 18.6 to 15.4 g/L/h). This is due to the fact that the flocculating yeast has difficulty diffusing the substrate and product through the cell pellet[9]. This phenomenon reduces fermentative activity and the contact area between the yeast and the cultivation medium, thereby affecting fermentation productivity and yield.

Both fermentations experienced bacterial contamination; however, Experiment A had lower production of lactic acid due to the more robust nature of the non-flocculating yeast strain. In contrast, the flocculating yeast strain suffered greater effects from the

acids, which can inhibit enzymatic activities, lower pH, and induce osmotic stress. Thus, although both experiments had the same bacterial contamination, the bacteria had a greater impact in Experiment B. This result is consistent with Guevara-Bravo et al.[10], who observed lower efficiency in a fermentation with flocculent yeast due to the presence of fermentation inhibitors such as organic acids and low fermentation pH, using 23.8 g/L yeast and 14.5 °Brix, attempting to produce ethanol from banana waste. Although its efficiency was 82%, giving a $Y_{P/S}$ of 0.41 g/g, which is greater than that of molasses with flocculant yeasts, the productivity was much lower, being only 1.74 g/L/h, confirming the low efficiency of fermentation parameters of flocculant yeast in relation to industrial ones.

Therefore, adequate control of fermentation conditions and selection of more suitable yeast strains are crucial to mitigate the problems associated with the presence of flocculent yeasts and optimize the performance of the ethanol production process from molasses. Considering the impact of the presence of flocculent yeasts in the process, one solution would be to use pre-selected yeasts in ethanol production, a technique that offers a number of critical advantages to the industry. These yeasts are carefully selected for their specific characteristics, such as high fermentation rate, stress tolerance, and efficiency in converting sugars to ethanol [11]. This results in a more consistent and efficient ethanol production, with shorter fermentation time and higher yield. In fact, Basso et al [11] compared the performance of baker's yeast and a selected yeast PE-2 and observed a superiority of the selected yeast in all evaluated parameters (ethanol yield, cell viability and biomass gain), in particular the trehalose content was more than 2 times higher for the selected yeast (9.5% dry basis). Trehalose plays a crucial role in ethanol production from molasses by protecting yeast cells from fermentation stress. Selecting yeast strains with higher trehalose levels increases cell resistance to adverse conditions such as temperature fluctuations and osmotic pressure, resulting in more efficient fermentation and increased ethanol production. Thus, increasing trehalose levels in selected yeast strains is a promising strategy to optimize ethanol production processes and mitigate problems associated with the presence of flocculating yeasts. In addition, pre-selected yeasts contribute to the reduction of microbiological contamination and the formation of flocculent yeasts, providing a possible solution to avoid this type of contamination.

4 CONCLUSION

It is evident that the presence of flocculating yeasts during the alcoholic fermentation of molasses affects the process in terms of both yield and ethanol productivity. The flocculating yeast used in Experiment B showed difficulties in substrate and product diffusion, resulting in a longer experimental time and lower overall performance. Therefore, the use of pre-selected yeasts represents an interesting solution to avoid the challenges associated with yeast flocculation and to improve the overall performance of the fermentation process in ethanol production.

5 REFERENCES

1 MOUCO, J. 2011. Tese de Doutorado. Universidade Tecnica de Lisboa. Lisboa, Portugal.
2 Banco Nacional de Desenvolvimento Econômico e Social. 2007. https://web.bndes.gov.br/bib/jspui/handle/1408/2527
3 PEREIRA, L. P. R. C. 2021. Dissertação (Mestrado em Engenharia Química) – Universidade Federal de São Carlos, São Carlos.
4 CAMARGOS, C. V. et al. 2019. Dissertação de mestrado. Universidade Federal de Uberlândia, Uberlândia,
5 HORTA, A. C. L. et al. Brazilian Journal of Chemical Engineering, v. 32, n. 4, p. 821–829, dez. 2015.
6 MESQUITA, T. J. B. Tese (Doutorado em Engenharia Química) – Universidade Federal de São Carlos, São Carlos, 2021.
7 SHULER, M. L., KARGI F., Bioprocess engineering: basic concepts. p. 276-277 2002.
8 Soares, R.C.; et al. T.S. *Polymers* 2022, *14*, 2400
9 S. Ranil et al. 2010. Advanced Powder Technology, Volume 21, Issue 4, Pages 374-379, ISSN 0921-8831,
10 GUEVARA-BRAVO, C; et al. 2014. Rev.Bio.Agro, Popayán , v. 12, n. 2, p. 151-159.
11 BASSO, L. C. et al. FEMS yeast research, v. 8, n. 7, p. 1155-1163, 2008.
12 SOUZA, J. P. M. et al. 2023. 23° Brazilian chemical engineering congress, Salvador.

ACKNOWLEDGEMENTS

The authors would like to thank Fermentec Tecnologias em Açúcar e Álcool LTDA, FINEP (Financiadora de Estudos e Projetos), and the Ministry of Science, Technology, Innovation, and Communications (MCTIC), with resources from FNDCT, for the financial support (UNIFEI PROEX No. 23088.017409/2022-61 and UFSCar 14.708 - ProEx No. 012827/2022-18). This study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - code 001; FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) Process 2016/10.636-8; and the Brazilian National Council for Scientific and Technological Development (CNPq) – process 130360/2024-4).