YARROWIA LIPOLYTICA: A VERSATILE YEAST WITH POTENTIAL FOR USE IN MULTIPRODUCT FERMENTATIONS IN THE CONTEXT OF BIOREFINERY

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ABSTRACT

Biorefineries aim to convert biomass into value-added products. These biofactories play a very important role in the environmental and economic aspects of a country, contributing to sustainability, helping to manage industrial by-products while also generating revenue. Multi-product fermentations can be interesting in this scenario, as they make it possible to obtain several products from a single fermentation. In this context, the behavior of the yeast *Yarrowia lipolytica* was evaluated in a fermentation process using a culture medium containing xylose and soybean oil as carbon sources. The results obtained were satisfactory, with the production of xylitol, a biosurfactant with emulsifying characteristics, and organic acids being observed.

Keywords: biorefinery, biosurfactant, yeast, xylose, *Yarrowia lipolytica*.

1 INTRODUCTION

In recent years, the biorefinery market has grown significantly, according to Research and Markets, this segment is expected to grow at an annual rate (CAGR) of 10.6% between 2022-2030[1]. With this advancement, investment opportunities and incentives for research in this area may arise, with the aim of improving the biotechnological processes involved. Biorefinery is an integration of processing all types of biomass – from agriculture, aquaculture, or industrial waste, for example – to obtain a variety of products[2]. Versatile yeasts, which can use different substrates to biosynthesize several molecules of industrial importance, such as the *Yarrowia lipolytica* species, can be a viable alternative for this segment, as they enable greater use of the by-products used.

Studies have reported that *Y. lipolytica*, an unconventional yeast, has become a valuable production platform due to some characteristics, including GRAS (Generally Considered Safe) status and versatility, due to the ability to use several hydrophobic and hydrophilic carbon sources and synthesize various value-added products, such as biosurfactants, organic acids and enzymes[3 4 5 6].

The aim of this work was to evaluate the behavior of the yeast *Y. lipolytica* using xylose and soybean oil as carbon sources to produce different bioproducts of industrial interest.

2 MATERIAL & METHODS

The inoculum was prepared in 50 mL Erlenmeyer flasks containing 10 mL of YMB medium composed of glucose (10 g/L), yeast extract (3 g/L), malt extract (3 g/L) and peptone (5 g/L), which were incubated in a rotary shaker at 150 rpm, 27 °C, for 24 hours. Then, the cultures were centrifuged at 3500 rpm for 10 minutes to separate the cell biomass. For inoculation into the fermentation medium, the cells were resuspended in sterile water. After appropriate dilutions, adjusting the inoculum to 1 unit of absorbance at 600 nm, 1.0 mL of the cell suspension was inoculated into Erlenmeyer flasks (approximately 0.7 g/L of total cells).

The fermentation trials were carried out in a modified culture medium from Sarubbo[7], evaluating the ability of the yeast *Y. lipolytica* to produce biosurfactant in a medium containing xylose and a hydrophobic carbon source (soybean oil). Thus, the medium was supplemented with 40.0 g/L of hydrophilic carbon source (xylose), 10% (v/v) of hydrophobic source (soybean oil), 1.0 g/L of ammonium nitrate, 0.2 g/L of monobasic potassium phosphate, 0.2 g/L of magnesium sulphate and 2.0 g/L of yeast extract. The pH of the culture media was adjusted to between 5.0 and 5.5.

The experiments were carried out in triplicate in 50 mL Erlenmeyer flasks containing 10 mL of medium. After inoculation, the flasks were incubated at 27 °C, 150 rpm for 168 hours in a rotary shaker. At the end of the fermentation process, to separate the cells, firstly the cultures were centrifuged at 4000 rpm for 20 minutes, then 2.0 mL of chloroform was added to the centrifuged culture media. Then the cells were separated using a Pasteur pipette (dropper type). The separated cells were resuspended in 10 mL of distilled water and dried in an oven, and the cell concentration was determined by gravimetry.

The cell-free supernatants were used to carry out analyses to determine xylose consumption, organic acid production and the emulsification index.

Xylose consumption was determined by HPLC on an Agilent Technology 1200 series chromatograph (USA). To determine the emulsification index, tests were carried out according to the methodology[8] in which 1.0 mL of the supernatant and 1.0 mL of kerosene were transferred to screw-top test tubes, which were shaken (9000 rpm) in a vortex for 2 minutes to obtain the emulsion. The production of organic acids was preliminarily verified based on the pH values obtained at the end of the cultivation.

3 RESULTS & DISCUSSION

The analyses carried out after the fermentation process showed that 31.72 % of xylose was consumed and 8.56 g/L of cell biomass was formed. A biosurfactant with emulsifying characteristics was obtained, since the emulsification index was 51.93 %. The emulsion obtained was stable for 7 days. During fermentation, the production of 6.65 g/L of xylitol was also observed. It should be noted that there are few studies in the literature showing the production of xylitol by this oleaginous yeast species. Furthermore, the yeast *Y. lipolytica*, under the conditions studied, may also have produced organic acids, with the reduction in pH to 1.89 being an indication. There are reports in the literature of this yeast species producing citric acid in considerable concentrations. Figure 1 shows the relation between xylose consumption, xylitol production and cell growth.

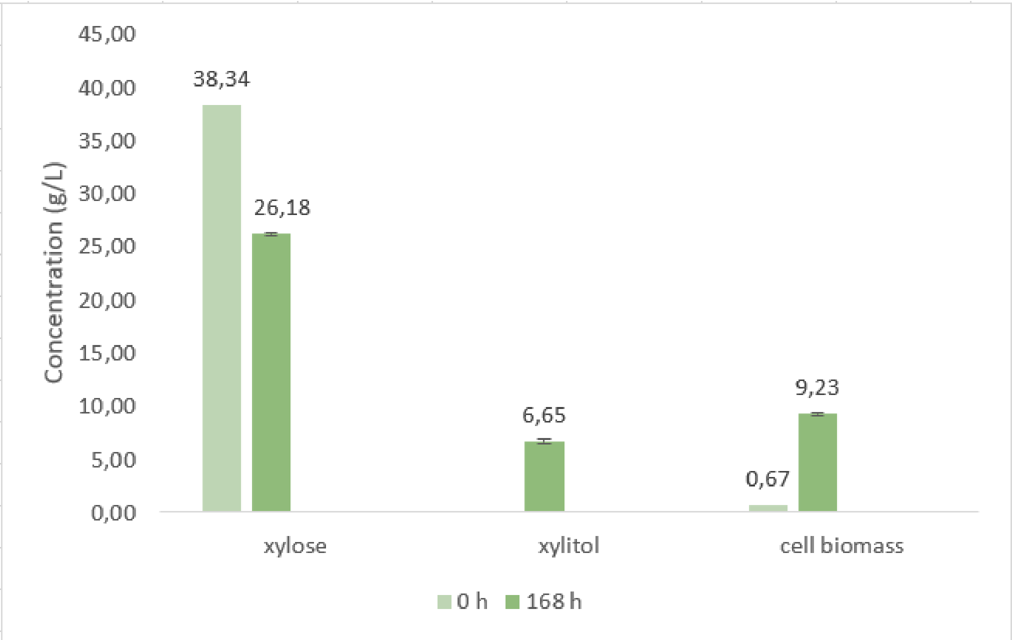

Figure 1: Sugar consumption, xylitol production and cell growth.

Figure 2 shows a picture of the test carried out to obtain the emulsification index.

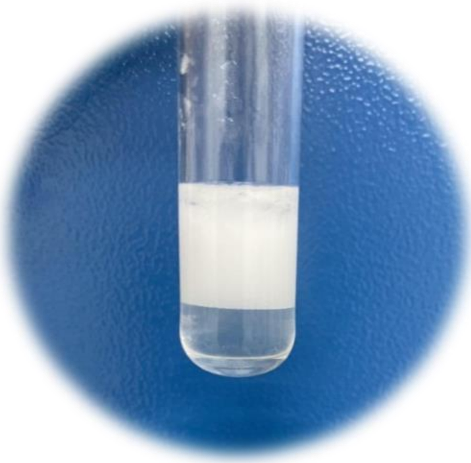

Figure 2: Emulsification test with the supernatant obtained from fermentation.

4 CONCLUSION

Given the growth of the biorefinery segment in the biotechnology area, the versatile yeast *Y. lipolytica* can be considered a promising alternative for use in these integration platforms, as there are indications that it can use hydrophilic agro-industrial by-products, such as sugarcane bagasse hydrolysate, which is rich in xylose, and hydrophobic ones, such as waste oils from biodiesel plants, to obtain various bioproducts, such as organic acids, biosurfactant and xylitol, in a single cultivation.

REFERENCES

1. MACHADO, N. Mercado global de biorrefinarias deve ultrapassar US$ 1,4 tri até 2030. **Newsletters epbr (2023)**. Disponível em: https://epbr.com.br/mercado-global-de-biorrefinarias-deve-ultrapassar-us-14-tri-ate-2030/
2. HINGSAMER, M.; JUNGMEIER, G. Biorefineries. In: **The role of bioenergy in the bioeconomy**. Academic Press, 2019. p. 179-222.
3. AMARAL, L.; JAIGOBIND, A. G. A.; JAISINGH, S. Detergente doméstico. **Instituto Tecnológico do Paraná**, 2007.
4. FONTES, G. C. et al. Renewable resources for biosurfactant production by *Yarrowia lipolytica*. **Brazilian Journal of Chemical Engineering**, v. 29, p. 483-494, 2012.
5. MADZAK, C.; GAILLARDIN, C.; BECKERICH, J. M. Heterologous protein expression and secretion in the non-conventional yeast *Yarrowia lipolytica*: a review. **Journal of biotechnology**, v. 109, n. 1-2, p. 63-81, 2004.
6. SUN, T. et al. Engineering *Yarrowia lipolytica* to produce fuels and chemicals from xylose: A review. **Bioresource Technology**, v. 337, p. 125484, 2021.
7. SARUBBO, L. A.; FARIAS, C. B. B.; CAMPOS-TAKAKI, G. M. Co-utilization of canola oil and glucose on the production of a surfactant by *Candida lipolytica*. **Current Microbiology**, v. 54, p. 68-73, 2007.
8. CHEN, S.Y. et al. Improved production of biosurfactant with newly isolated *Pseudomonas aeruginosa* S2. **Biotechnology Progress**, v.23, n.3, p.661–666, 2007.
9. PHILIPPINI, R. R. et al. Agroindustrial byproducts for the generation of biobased products: alternatives for sustainable biorefineries. **Frontiers in Energy Research**, v. 8, p. 152, 2020.

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