SCREENING OF YEASTS CULTIVATED ON BABASSU MESOCARP FLOUR HYDROLYSATE

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ABSTRACT

This study aimed to assess the viability of babassu mesocarp flour hydrolysate as a substrate for yeast biomass production. To this end, a screening of seven yeast strains was conducted to evaluate biomass production and substrate-to-biomass conversion efficiency. Cultivation experiments were conducted in 150 mL reagent bottles containing 50 mL of babassu mesocarp flour hydrolysate (80 %), with an initial pH of 5.0, an inoculum concentration of 4 g L^{-1}, at 32 °C, and 200 rpm for 48 h. Among the yeast strains evaluated, BFY-195 and BFY-207 exhibited promising results for biomass production, with 0.53 and 0.57 g of dry cell weight (DCW), respectively. These findings underscore the potential of babassu mesocarp flour hydrolysate as a viable substrate for use in bioprocesses.

Keywords: Sustainable. Substrate. Biomass. Conversion factor. Bioprocess.

1 INTRODUCTION

The babassu palm (*Orbignya phalerata*) is native to the Amazon region and northeastern Brazil, widely recognized for its economic and ecological significance.[1] In 2022, the annual production of babassu reached 30.5 tons, highlighting the economic significance of this fruit in the Amazon region and northeastern Brazil. The mesocarp flour of babassu is particularly notable for its nutritional composition, which includes proteins (1.5 %), lipids (0.18 %), ash (0.81 %), dietary fiber (14.3 %), and carbohydrates (83.21 %).[2]

Within this carbohydrate content, 72.9 % consists of starch, making the mesocarp flour a highly interesting matrix for use in bioprocesses.[2] However, the biotechnological potential of babassu mesocarp flour remains underexplored. Enzymatic hydrolysis presents an efficient alternative for converting starch into fermentable sugars, thereby tapping into the potential of this substrate in bioprocesses. The application of this technique can transform babassu mesocarp flour into a valuable source of fermentable sugars, providing a sustainable alternative to carbohydrates derived from other agricultural crops.[3]

In this context, it is known that the selection of yeast for the fermentation process is crucial, as different species and strains have varying capacities to utilize specific sugars and produce biomass. Yeasts are versatile microorganisms widely used in industrial bioprocesses due to their ability to grow under a wide range of conditions and convert substrates into high-value products.[4]

The use of babassu mesocarp flour hydrolysate as a fermentable substrate not only enhances the value of an underutilized regional resource but also contributes to the development of more sustainable industrial processes.[5] Yeast biomass production from renewable sources such as babassu can reduce dependence on conventional raw materials, promote a circular economy, and generate products with a lower environmental impact.[6] Therefore, the aim of this study was to evaluate the potential of babassu mesocarp flour hydrolysate as a substrate for yeast biomass production.

2 MATERIAL & METHODS

Babassu mesocarp flour was sourced from the Cantinas da Terra do Meio (Vem do Xingu), located in the city of Altamira, PA, Brazil. The enzymes α-amylase (LiqueStar) and glucoamylase (Fermentase HX) were provided by Suntaq International Limited, Brazil.

Babassu mesocarp flour hydrolysate was obtained through enzymatic hydrolysis using α-amylase and glucoamylase, simultaneously. The process conditions used were 20 % mass, 0.3 % of each enzyme, 200 rpm, temperature ramp of 65-85 ºC for 3 h. For the screening stage, 7 yeasts (*Saccharomyces cerevisiae* strains) from the BIOinFOOD culture collection were used (BFY-001, BFY-058, BFY-073, BFY-177, BFY-195, BFY-207 and BFY-218). Maintenance was conducted every 2 months, and the microorganisms were stored on YPD (Yeast-Peptone-Dextrose) agar and kept at 4 ºC (adapted from Machado et al. [7]).

For inoculum cultivation, a single loop of yeast colony from the previously prepared agar plates was transferred to a 150 mL reagent bottle using 50 mL YPS (Yeast-Peptone-Sucrose) medium (Adapted from Tahmasebi et al. [8]). Pre-culture was carried out at 32 ºC, 200 rpm for 24 h. Cultures were performed in 150 mL reagent bottle with 50 mL babassu mesocarp flour hydrolysate (80 %) at initial pH 5.0, 4 g L^{-1} inoculum, 32 °C, 200 rpm for 48 h (Adapted from Zhang et al. [9]).

The dry cell weight (DCW, g) of biomass was determined gravimetrically in accordance with the method of Zhang et al.[9]. In brief, after centrifugation was conducted at 4 ºC and 5000 rpm for 10 min, cell pellets were collected, washed with distilled water, and then dried at 65 ºC until constant weight. Total reducing sugars (TRS) was determined by a 3,5-dinitrosalicylic acid (DNS) colorimetric method in culture supernatants. A glucose standard curve was used, and absorbance was measured at 546 nm, as described by Miller[10]. The conversion factor of substrate to biomass ($Y_{X/S}$) was calculated as described by Bailey and Ollis[11].

3 RESULTS & DISCUSSION

The babassu mesocarp flour hydrolysate (80 %) contains approximately 110 g L^{-1} of total reducing sugars (TRS) in its composition. Thus, the high sugar content of the babassu mesocarp flour hydrolysate may be considered a stress factor for yeast growth due to its osmotolerance. In this context, a yeast screening was conducted to identify a strain with potential for growth on this substrate. The results obtained are presented in Table 1.

Table 1 Screening of different yeasts cultivated babassu mesocarp flour hydrolysate.

Yeasts	WCW* (g)	DCW** (g)	TRS consumed (g)	Yx/s (g g^{-1})
BFY-001	1.49	0.45	2.56	0.17
BFY-058	1.35	0.41	2.31	0.18
BFY-073	1.54	0.46	2.44	0.19
BFY-177	1.56	0.47	2.53	0.18
BFY-195	1.77	0.53	2.61	0.20
BFY-207	1.90	0.57	2.54	0.23
BFY-218	0.98	0.29	1.56	0.19

*WCW = Wet cell weight (g); **DCW = Dry cell weight (g).

Saccharomyces cerevisiae strains are widely used in industrial processes due to their adaptability to process conditions.[5] Among the yeasts evaluated, it was observed that strains BFY-195 and BFY-207 exhibited the highest biomass production, with 0.53 and 0.57 g of DCW, respectively. Additionally, both strains were the most efficient in substrate consumption, resulting in substrate-to-biomass conversion factors of 0.20 and 0.23 g g^{-1}, respectively.

It is known that babassu byproducts contain high levels of phenolic compounds (1975.14 mg GAE 100 g^{-1}), which are considered inhibitors in yeast biomass production.[12] Despite the presence of these inhibitors in the cultivation, the results were promising for the evaluation of these yeasts on a larger scale for biomass production. The results of this study demonstrate that under simple process conditions, the growth of these yeasts was observed, showcasing the potential of babassu mesocarp flour hydrolysate as a substrate for utilization in bioprocesses. Hence, further studies are necessary to optimize the process and achieve biomass production at larger scales and higher yields, including a fed-batch process approach.

4 CONCLUSION

The study demonstrated that babassu mesocarp flour hydrolysate is a promising substrate for yeast biomass production, achieving significant results. This finding highlights the importance of valorizing babassu byproducts, which are abundant and readily available, for industrial processes. By providing a sustainable alternative to conventional carbon sources derived from other agricultural crops, this study emphasizes the potential of utilizing babassu mesocarp flour hydrolysate to support environmentally friendly and cost-effective industrial biotechnology applications.

REFERENCES

[1] LIMA, R. C., CARVALHO A. P. A., SILVA, B. D., et al. 2023. Green ultrasound-assisted extraction of bioactive compounds of babassu (*Attalea speciosa*) mesocarp: effects of solid-liquid ratio extraction, antioxidant capacity, and antimicrobial activity. Appl Food Res. 3 (2). 100331. doi:10.1016/j.afres.2023.100331.

[2] NASCIMENTO, J. M., OLIVEIRA, J. D., LEITE, S. G. F. 2019. Chemical characterization of biomass flour of the babassu coconut mesocarp (*Orbignya speciosa*) during biosorption process of copper ions. Environ Technol Innov. 16 (1). 100440. doi:10.1016/j.eti.2019.100440.

[3] CINELLI, B. A., LÓPEZ, J. A., CASTILHO, L. R., FREIRE, D. M. G., CASTRO, A. M. 2014. Granular starch hydrolysis of babassu agro-industrial residue: a bioprocess within the context of biorefinery. Fuel. 124 (1). 41-48. doi:10.1016/j.fuel.2014.01.076.

[4] CHEN, A., QU, T., SMITH, J. R., LI, J., DU, G., CHEN, J. 2024. Osmotic tolerance in *Saccharomyces cerevisiae*: implications for food and bioethanol industries. Food Biosci. 60 (5). 104451. doi:10.1016/j.fbio.2024.104451.

[5] RODRIGUES, S. C. S., SILVA. A. S., CARVALHO, L. H., ALVES, T. S., BARBOSA, R. 2020. Morphological, structural, thermal properties of a native starch obtained from babassu mesocarp for food packaging application. J Mater Res Technol. 9 (6). 15670-15678. doi:10.1016/j.jmrt.2020.11.030.

[6] ONYEMA, V. O., AMADI, O. C., MONEKE, A. N., AGU, R. C. 2023. A Brief Review: *Saccharomyces cerevisiae* biodiversity potential and promising cell factories for exploitation in biotechnology and industry processes – West African Natural yeasts contribution. Food Chem Adv. 2 (7). 100162. doi:10.1016/j.focha.2022.100162.

[7] MACHADO, B.R., SILVA, P. G. P., GARDA-BUFFON J., SANTOS, L. O. 2022. Magnetic fields as inducer of glutathione and peroxidase production by *Saccharomyces cerevisiae*. Brazilian J Microbiol. 53. 1881–1891. doi:10.1007/s42770-022-00836-9.

[8] TAHMASEBI, Z., ZILOUEI, H., KOT, A. M. 2024. Investigating the concomitant production of carotenoids and lipids by the yeast *Rhodosporidium babjevae* using sugarcane bagasse hydrolysate or corn steep liquor. Renew Energy. 228 (11). 120618. doi:10.1016/j.renene.2024.120618.
[9] ZHANG, J., WANG, Y., GOU, Q., et al. 2022. Consolidated bioprocessing of cassava starch into microbial lipid for biodiesel production by the amylolytic yeast *Lipomyces starkeyi*. Ind Crops Prod. 177 (1). 114534. doi:10.1016/j.indcrop.2022.114534.
[10] MILLER, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 31 (3). 426-428. doi:10.1021/ac60147a030.
[11] BAILEY, J. E., OLLIS, D. F. 1986. Biochemical Engineering Fundamentals. Second. McGraw-Hill Education.
[12] FLETCHER, E., BAETZ, K. 2020. Multi-faceted systems biology approaches present a cellular landscape of phenolic compound inhibition in *Saccharomyces cerevisiae*. Front Bioeng Biotechnol. 8 (11). 1-13. doi:10.3389/fbioe.2020.53990.

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