IMPACT OF CELL ACCLIMATION ON LIGNOCELLULOSIC BIOTRANSFORMATION: AN INDUSTRIALLY RELEVANT IN SITU BIOPROCESS FOR PRODUCING ETHANOL FROM RESIDUAL SUGARCANE BIOMASS

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ABSTRACT

The production of second-generation ethanol (E2G) using lignocellulosic residues such as sugarcane bagasse is a sustainable alternative for renewable biofuels. This study highlights the importance of cell acclimation in cell propagation, aiming for efficient fermentation. Acclimation involves the gradual exposure of microorganisms to toxic components like hydroxymethylfurfural (HMF), furfural, and acetic acid, allowing for better adaptation to the fermentative medium. Using a genetically modified strain of *Saccharomyces cerevisiae* to ferment xylose, the study explored different propagation media, including lignocellulosic hydrolysate, mixtures with molasses, and commercial sugars. Results indicate that cells acclimated with hydrolysate showed significantly higher ethanol production rates in the first 16 hours (2.45 g/L.h) compared to other media. Cell acclimation proved crucial for the efficient utilization of xylose, an important monosaccharide in the biomass. Although the final yields after 36 hours were similar in the bioprocess, the impacts of acclimation were more pronounced in the first 20 hours of fermentation. Therefore, prior acclimation of the cells is essential to enhance the initial efficiency and robustness of the fermentation process, contributing to the sustainable production of second-generation ethanol.

Keywords: Bioprocess. Cell propagation. Lignocellulosic hydrolysate. Second-generation ethanol. E2G.

1 INTRODUCTION

The production of second-generation ethanol, using lignocellulosic residues such as sugarcane bagasse, has emerged as a sustainable alternative for obtaining biofuels. This process involves converting polysaccharides in the biomass into fermentable sugars, followed by fermenting these sugars into ethanol by specific microorganisms like *Saccharomyces cerevisiae*[1]. A crucial step in this process is the preparation of suitable propagation, ensuring efficient and productive fermentation.

Cell acclimation during pre-inoculum production plays a vital role in adapting microorganisms to the fermentation conditions. This process involves gradually exposing the microorganisms to toxic components, such as hydroxymethylfurfural (HMF) and furfural, derived from the acid hydrolysis of biomass, and high concentrations of inhibitory sugars. Acclimation allows cells to adjust their metabolism and defense mechanisms, resulting in greater resistance and efficiency in converting sugars into ethanol. Additionally, a critical aspect for the efficiency of second-generation fermentation is the microorganisms' ability to consume not only glucose but also xylose, one of the main monosaccharides derived from the hemicellulose in sugarcane bagasse. Efficient xylose fermentation is fundamental to maximizing ethanol production, as xylose represents a significant fraction of the available sugars after biomass hydrolysis. Microbial platforms capable of metabolizing both glucose and xylose can significantly increase ethanol yield, making the bioprocess more economical and sustainable[2].

Thus, understanding and optimizing cell acclimation in pre-inoculum, as well as ensuring good consumption rates of xylose and glucose, is essential for improving the productivity and robustness of ethanolic fermentations in second-generation ethanol production processes. Therefore, using a xylose-fermenting strain developed at the National Laboratory of Biorenewables of the National Center for Research in Energy and Materials (LNBR-CNPEM), this study aims to explore the conditions and strategies for cell acclimation that can maximize the performance of fermenting microorganisms, contributing to the development of more efficient and sustainable industrial processes.

2 MATERIAL & METHODS

The *S. cerevisiae* xylose-fermenting strain used in the present study was developed in previous works (unpublished data) and possesses the following genetic modifications: extra copies of four genes from the Pentose Phosphate Pathway, deletion of the *GRE3* gene, integration of one copy of the *XYL3* gene, and overexpression of the *xylA* gene encoding a xylose isomerase.

The strain was propagated in different culture media (commercial and industrial), always supplemented with YP (1% yeast extract, 2% peptone), with four different commercial or non-commercial carbon sources, namely: glucose and xylose (YPDX, where they represent 70% and 30%, respectively), molasses (YPM), lignocellulosic hydrolysate from sugarcane bagasse (YPH, where they

represent glucose 70% and xylose 30%), and a mixture of molasses with hydrolysate (YPMH, where they represent 25% and 75%, respectively); the carbon source was always provided in the culture medium at a concentration of 20 g/L.

The cell propagation process in the different culture media was carried out in bench-top bioreactors (stirred tank reactor – STR, Labfors5 - Infors HT), with a working volume of 2 L and batch mode operation, 30% dissolved oxygen (controlled by agitation cascade from 300-700 rpm and aeration from 0.7-1.0 L/min), pH of 5.5 and temperature of 30 °C (both with a dead band of 0.5). The initial optical density at 600_{nm} was set at 0.3, with a duration period of 16 hours. After propagation, the cells were centrifuged at 4000 *xg*, 4°C, for 15 minutes, and resuspended in sterile water. The cell suspension was used to inoculate fermentation batches where pure lignocellulosic hydrolysate from sugarcane bagasse was offered to the cells, and the process took place in bench-top bioreactors (stirred tank reactor – STR, Multifors - Infors HT), with a working volume of 0.25 L and batch mode operation, without air injection, agitation at 200 rpm, pH of 5.5 and temperature of 32 °C (both with a dead band of 0.5). The initial optical density at 600*nm* was set at 10, with a fermentation duration period of 36 hours. Aliquots were collected to monitor the bioprocess evolution, and ethanol production rates and respective yields were measured.

3 RESULTS & DISCUSSION

The results presented in Figure 1 suggest that the prior acclimation of the cultured cells plays a vital role in their response to the fermentation medium. The adaptation of microorganisms to the conditions of the fermentation medium is essential, especially due to the significant concentrations of acetic acid, hydroxymethylfurfural (HMF), furfural, and lignin residues present in hydrolysates derived from the acid hydrolysis of biomass[2].

The ethanol production rates from the main available sugars, glucose and xylose, were significantly better in cultures propagated solely with hydrolysate (YPH), reaching a production rate of 2.45 g/L.h in the first 16 hours of fermentation. In contrast, cultures propagated in a mixture of molasses with hydrolysate (YPMH) and molasses (YPM) showed production rates achieving 1.91 and 1.86 g/L.h, respectively. An ethanol production rate of 1.59 g/L.h was observed in fermentations conducted from control propagation with commercial sugars (YPDX).

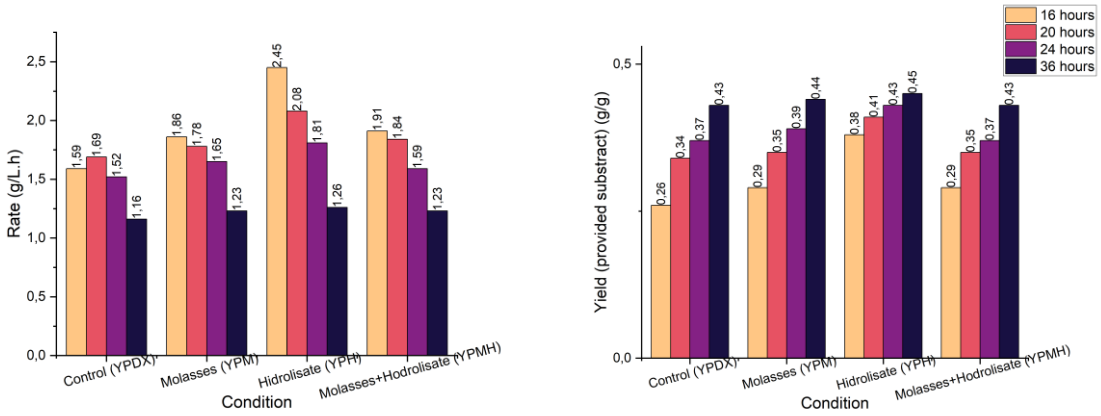

Figure 1 Rates and yields of ethanol production from lignocellulosic hydrolysate.

It is observed that the impact of the different propagation media is more pronounced in the first 20 hours of the process. In YPH, the cells had already been exposed to harmful agents, possibly preparing the cellular machinery to better handle the high concentrations of these compounds in the lignocellulosic hydrolysate. On the other, cells propagated in commercial medium, without the presence of these compounds, showed lower ethanol production rates in the initial hours of cultivation, indicating a period of cellular latency and adaptation.

Moreover, considering the ethanol yields from the sugars provided throughout the entire fermentation bioprocess (36 hours of fermentation), the data indicate similar yields among the different propagation media. Therefore, the impacts of acclimation are more pronounced at the beginning of the fermentation process.

4 CONCLUSION

The results of this study highlight the importance of investigating cell acclimation in the preparation of propagation for the fermentation of lignocellulosic hydrolysates, such as sugarcane bagasse, in the production of second-generation ethanol. Prior exposure of microorganisms to toxic components present in the hydrolysates significantly improves the cells' ability to withstand these inhibitors, resulting in higher ethanol production rates in the initial stages of fermentation.

REFERENCES

[1] ZHA, J., YUWEN, M., QIAN, W., WU, X. 2021. Yeast-Based Biosynthesis of Natural Products From Xylose. Front Bioeng Biotechnol.

[2] Zhang, K.; Wells P.; Liang Y.; Love J.; Parker, D. A.; Botella, C.; Effect of diluted hydrolysate as yeast propagation medium on ethanol production, Bioresource Technology, Volume 271, 2019, Pages 1-8, ISSN 0960-8524, https://doi.org/10.1016/j.biortech.2018.09.080.

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