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PRODUCTION OF 2,3-BUTANEDIOL FROM SUGARCANE MOLASSES USING A RECOMBINANT YEAST STRAIN

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ABSTRACT

A biorefinery aims to create productive pathways to convert by-products into high value-added products. Integrating the production of biocompounds, such as 2,3-butanediol (BDO), into a biorefinery portfolio is an interesting approach to achieve biorefineries feasibility. In this sense, the development of efficient microorganisms to produce BDO is a key factor for BDO production in biorefineries. In addition, the use of industrial byproducts without supplementation with expensive nitrogen sources is an interesting alternative to achieve BDO feasibility. Thus, in the present study the performance of the recombinant BDO-producing yeast *Saccharomyces cerevisiae* HGS37 was investigated in industrial culture media based on sugarcane molasses and the necessity of yeast extract and peptone (YP) supplementation as nitrogen source was accessed. The production of BDO in g/L in synthetic media and in molasse with and without YP was 75.7; 56.2 and 56.1, respectively. The BDO yield ($Y_{P/S}$) for the three studied media was the same (81% of theoretical). Additionally, the volumetric productivity of BDO (Qp) remained identical in molasses with and without YP, showing that the supplementation with these high-cost nitrogen source is not necessary for this yeast during molasse fermentation. Although lower concentrations of BDO were achieved compared to synthetic media, the *Saccharomyces cerevisiae* HGS37 strain produced 2,3-butanediol from molasses efficiently without the need of supplementation.

Keywords: 2,3-Butanediol. Biorefinery. Recombinant Yeast. Molasses.

1 INTRODUCTION

The production of renewable energy and products are the main guidelines for decarbonization and improvement of environmental quality.[1] In this context, the concept of biorefinery emerges as an important strategy for the global energy transition towards a renewable matrix, consisting of an industrial unit that contemplates a broad conversion of the entire plant biomass into biofuels and chemicals with high added-value with the aim of replacing fossil resources.[2]

In Brazil, most of the current biorefineries focus on the co-production of sugar, ethanol, and bioelectricity using sugarcane as raw material. Sugarcane processing in Brazilian biorefineries generates various by-products and residues, such as sugarcane straw and bagasse, molasses, vinasse, and CO_2, each of them suitable for different uses.[3] Sugarcane molasse is a byproduct of sugar production. It is a viscous liquid with a dark brown color and pronounced odor, composed of approximately 5% (w/w) of sugar content (mainly sucrose, glucose and fructose), along with other nutrients such as organic acids, vitamins, and minerals, as well as a nitrogen content ranging from 0.5% to 0.9% (w/v)[4,5], being an interesting by-product to be used as substrate in renewable bioprocess in integrated biorefineries.

An important compound that can be produced from sugarcane byproducts processing is 2,3-butanediol (BDO), a platform chemical with numerous industrial applications as plasticizer, polyester, drugs, cosmetics, and starting material for synthesis of several chemical products such as 1,3-butadiene and methyl ethyl ketone.[6] Currently, its production is related to chemical processes from petroleum through the hydrolysis of 2,3-butane oxide in a process that uses intense energy and is harmful to the environment.[7] Thanks to advances in genetic engineering, microbial production of BDO has become promising. The challenge faced in this type of production is the low yields and high production costs, which leads to the need to develop efficient strains, capable of using cheap and easily accessible substrates so that BDO becomes a feasible chemical platform.[8]

The *Saccharomyces cerevisiae* HGS37 strain, which was developed by Huo et al. 2022 stands out in literature due to its high yields and BDO concentrations using YPD medium (yeast extract, peptone and glucose) as substrate. Taking this into account, the use of molasses as a substrate for BDO production by this strain is an interesting low-cost alternative source of sugars. However, yeast extract and peptone reactants can add significant costs to the process once they are expensive nitrogen sources, with prices ranging from 5 to 6 times higher than other sources[9], which makes its removal from media composition or the use of alternative sources of nitrogen an important issue to BDO production feasibility.

Considering that Perez et al.[10] found that the supplementation of molasses with YP was not necessary for the performance of the xylose fermenting recombinant yeast *S. cerevisiae* MDS130 in ethanol production, operating the process without any supplementation, this study aimed to assess the production of BDO by the *Saccharomyces cerevisiae* HGS37 strain in a

molasses-based medium with and without YP supplementation, with the overall goal of replacing commercial components with industrial counterparts, intending to mitigate costs associated with BDO production through biotechnological routes.

2 MATERIAL & METHODS

Microorganism and inoculum: In all experiments, the yeast *Saccharomyces cerevisiae* HGS37 a BDO-producer strain developed by Huo et al.[7] was used. Initially, a yeast colony (taken from the stock culture) was transferred to 3 mL of YPD(E) 0.5% medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 5 g/L ethanol) and incubated for 12 hours at 200 rpm and 30°C in a rotary shaker. Subsequently, the 3 mL pre-inoculum was transferred to 50 mL of YPD(E) 0.5% medium in 300 mL Erlenmeyer flasks and maintained for 16-20 hours at 30°C and 200 rpm for cell propagation[7]. The cells were recovered by centrifugation (6000 rpm, 4ºC, for 10 minutes) and utilized in the BDO production assays.

BDO production in molasse based media: The BDO production by HGS37 yeast in molasse-based media was performed in Erlenmeyer flasks of 300 mL containing 50 mL of reaction volume at 30°C and 150 rpm.[7] The genetic modification performed on the HGS37 strain (deletion of PCD genes) resulted in auxotrophy for C_2 compounds due to the deficiency in cytosolic acetaldehyde production, which is circumvented by adding ethanol to the medium at the beginning of the process. In this sense ethanol was added in the beginning of all experiments (40 g/L ethanol). Experiments were carried out in sugarcane molasse-based media with 300 g/L of initial sugar and with or without YP supplementation (10 g/L yeast extract, 20 g/L peptone), M(E) and YPM(E) media, respectively. The experiments were conducted in duplicate, with the main response variable being the production of BDO. A control experiment was performed using synthetic media YPD(E) 2% (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 40 g/L ethanol).

Quantification of substrates and products: The quantification of sugars and products was carried out via High-Performance Liquid Chromatography (HPLC). Initially, the samples underwent treatment for removal of insoluble solids, being frozen for at least 24 hours and centrifuged twice according to Ramos et al.[2]. The analysis was performed using a Waters e2695 chromatograph equipped with refractive index and UV-VIS detectors (λ= 210 nm). For ethanol, glycerol and BDO quantification, the RezexTM ROA-Organic acid H+ ion exclusion column was used, with 5mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min and operating temperature of 65°C. Sugars (sucrose, glucose and fructose) were quantified using a Sugar Pak I 6.5×300 mm column (Waters, USA) with RID detection using ultrapure water with 50 mg/g Ca-EDTA as the mobile phase (0.5 mL/min) at 80°C[2].

Calculations: The fermentative parameters substrate conversion (X, %), BDO yield ($Y_{P/S}$, g/g), and the volumetric productivity (QP, g_{BDO}/L/h) were calculated according to SHULER and KARGI[11].

3 RESULTS & DISCUSSION

The *Saccharomyces cerevisiae* strain HGS37 was capable of efficiently produce BDO in both synthetic (YPD(E)) and industrial (YPM(E) and M(E)) culture media. The average production of BDO in g/L in the YPD(E), YPM(E), and M(E) media were 75.7; 56.2; and 56.1 respectively. The substitution of commercial glucose by sugarcane molasse led to lower productivities (Q_P) and sugar consumption (lower conversion X%) (Table 1). However, BDO yield ($Y_{P/S}$) in all three cultured media was the same, which suggests that the cell's metabolism was not altered by molasses use, as it produces the same amount of BDO for each gram of substrate consumed, regardless of the substrate. In this sense, the loss of productivity could be attributed to molasses being a more complex substrate than glucose, which may slightly delay the 2,3-butanediol production process. In addition, the available sugars were not completely consumed in all experiments which can be explained by the low initial cell load and by an inhibitory effect caused by the high initial ethanol concentration added to the medium.

Table 1 Fermentative parameters of BDO production by *Saccharomyces cerevisiae* HGS37 strain using molasse based media.

Medium	BDO (g/L)	$Y_{P/S}$ (g/g)	Q_P (g/L/h)	Y (%)	X (%)	$Y_{P/EtOH}$ (g/g)
YPD(E)	75.7	0.41	0.8	81%	65%	4.7
YPM(E)	56.2	0.41	0.6	81%	48%	2.7
M(E)	56.1	0.41	0.6	81%	49%	2.9

*$Y_{P/S}$: BDO yield; Q_P: BDO productivity; Y (%): percentage of the theoretical yield achieved (0.51 g/g)[6]; X: conversion of total reducing sugars; $Y_{P/EtOH}$: BDO yield per gram of ethanol consumption.

As can be seen in **Figure 1,** the removal of peptone (P) and yeast extract (Y) in experiments using molasses as a substrate did not affect the production of BDO, the volumetric productivity of BDO (Qp) remained identical in the YPM(E) and M(E) media (Table 1), indicating that molasses can meet the cells' requirements without YP supplementation. This is a very interesting finding once both yeast extract and peptone are expensive reactants to be used at large industrial scale.

Maina et al.[12] in their investigation on the production of BDO via microbial route using the native bacteria *Bacillus amiloliquefaciens* 18.025 and molasses as substrate, observed a productivity (g/L/h) of 0.83, a yield (g/g) of 0.4, and a titer (g/L) of 48.7. However, authors used yeast extract and peptone as a nitrogen source. In parallel with the results obtained in this study, it is noted that the present study achieved similar yields, although aforementioned study used media supplementation with YP.

Omeroglu et al.[13] achieved 31.6 g/L of BDO through non-sterile fermentation of sugarcane molasses supplemented with peptone using immobilized cells of *Bacillus licheniformis* during a 24-hour incubation period. The high productivity observed by the authors (1.3 g/L/h) can be a result of the use of immobilized cells, which is an interesting approach to achieve higher productivities due to

the use of high cell loads in the process[2], and could be further investigated for the HGS37 yeast aiming to increase process productivity.

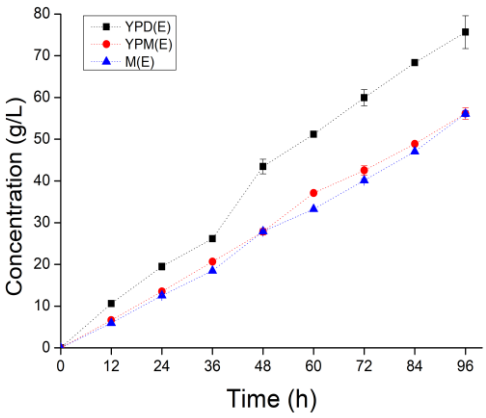

Figure 1 Production of BDO by the yeast S. cerevisiae HGS37 in different synthetic (YPD(E)) and industrial molasses based media with (YPM(E)) and without (M(E)) yeast extract and peptone supplementation.

4 CONCLUSION

Although in lower concentrations and productivities, the *Saccharomyces cerevisiae* HGS37 strain produces BDO from molasses efficiently without reduction on BDO yield due to the cell's metabolism deviation. Most importantly, the supplementation of molasses with yeast extract and peptone is not necessary for BDO production by this yeast. The results obtained in this study represent an advancement in the productive analysis of this yeast in particular, as previous tests had only been conducted in synthetic media.

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