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Xylitol production on sugarcane biomass hydrolysate by newly identified *Candida tropicalis* JA2 strain

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Abstract

Xylitol is a building block for a variety of chemical commodities, besides being widely used as a sugar substitute in the food and pharmaceutical industries. The aim of this work was to develop a microbial process for xylitol production using sugarcane bagasse hydrolysate as substrate. In this context, 218 non-*Saccharomyces* yeast strains were screened by growth on steam-exploded sugarcane bagasse hydrolysate containing a high concentration of acetic acid (8.0 g/L). Seven new *Candida tropicalis* strains were selected and identified, and their ability to produce xylitol on hydrolysate at low pH (4.6) under aerobic conditions was evaluated. The most efficient strain, designated *C. tropicalis* JA2, was capable of producing xylitol with a yield of 0.47 g/g of consumed xylose. To improve xylitol production by *C. tropicalis* JA2, a series of experimental procedures were employed to optimize pH and temperature conditions, as well as nutrient source, and initial xylose and inoculum concentrations. *C. tropicalis* JA2 was able to produce 109.5 g/L of xylitol with a yield of 0.86 g/g of consumed xylose, and with a productivity of 2.81 g·L·h, on sugarcane bagasse hydrolysate containing 8.0 g/L acetic acid and177 g/L xylose, supplemented with 2.0 g/L yeast nitrogen base and 4.0 g/L urea. Thus, it was possible to identify a new *C. tropicalis* strain and to optimize the xylitol production process using sugarcane bagasse hydrolysate as a substrate. The xylitol yield on biomass hydrolysate containing a high concentration of acetic acidobtained in here is among the best reported in the literature.

KEYWORDS

Candida tropicalis, fermentation, sugarcane biomass hydrolysate, xylitol

1 | INTRODUCTION

In recent years, there has been a considerable increase in studies seeking more efficient uses of agro-industrial residues for production of fuels and chemicals (Rocha, Rodrigues, Albuquerque, Gonçalves, & Macedo, 2014). Among several alternative uses of hexose and pentose sugars present in lignocellulosic hydrolysates, production of xylitol has been widely investigated (Albuquerque, Gomes, Marques, Silva, & Rocha, 2015). Xylitol is a pentose sugar alcohol with important applications because of its strong sweetening properties and capacity to inhibit microbial growth, as well as for its low calorie content, and lack of carcinogenic and cariostatic properties. It has been widely used as a replacement for sucrose in the food and pharmaceutical industries (Aguirre-Zero, Zero, & Proskin, 1993; Imark, Canisag, Vokoun, & Meryemoglu, 2017; Lynch & Milgrom, 2003; Ronda, Gómez, Blanco, & Caballero, 2005). In addition, xylitol is considered one of the 12

major high added-value intermediates that can be produced from biomass, due to it is being a building block for a variety of basic chemicals (Werpy & Petersen, 2004).

Xylitol is industrially produced by catalytic hydrogenation of pure xylose solution under high temperature and pressure (Dasgupta, Bandhu, Adhikari, & Ghosh, 2017). This production process is expensive and presents low yields due to the complexity of the product recovery stages (Bier, Maranho, Azevedo, & da Silva, 2007). Alternatively, biochemical production processes have been extensively evaluated using different microorganisms and substrates such as vegetable waste (Zhang, Geng, Yao, Lu, & Li, 2012), bamboo (Miura et al., 2013), corncob (Cheng et al., 2009; Hongzhi, Keke, Jingping, & Wenxiang, 2011; Ping, Ling, Song, & Ge, 2013; Rodrigues, Kenealy, & Jeffries, 2011), and sugar cane bagasse (Arruda, Rodrigues, Silva, & Felipe, 2011; Prakash, Varma, Prabhune, Shouche, & Rao, 2011). Fermentative processes have gained attention due to the possibility of using yeasts to produce xylitol with yields as high as 85% of theoretical maximum (Bier et al., 2007).

The major drawback of microbial production processes employing lignocellulosic-derived hydrolysates is the presence of inhibitory compounds in their composition, which may hinder yeast metabolism (Dasgupta et al., 2017). During pretreatment and hydrolysis of biomass, cellulose and hemicellulose are broken down, releasing monomeric sugars but also microbial-inhibitory compounds, such as furaldehydes, phenolic compounds, and acetic acid (Almeida et al., 2007; Kumar & Sharma, 2017). The composition and concentration of inhibitors vary depending on process conditions and biomass source. However, acetic acid is inherent to biomass hydrolysis conditions, as it is formed by deacetylation of hemicelluloses. This acid is a weak monocarboxylic acid (pKa 4.76) that shows antimicrobial effects mainly at low pH values (below pKa). Acetate penetrates the cell in its undissociated form, but once inside the cytoplasm, it dissociates, triggering high energy demand and an increase in cytoplasmic pH values (Hahn-Hägerdal, Karhumaa, Fonseca, Spencer-Martins, & Gorwa-Grauslund, 2007; Sousa, Ludovico, Rodrigues, Leão, & Côrtes-Real, 2012). In addition, it is capable of inhibiting yeast xylose metabolism (decreasing xylose reductase activity), and even at relatively low concentrations (5.4 g/L) it strongly inhibits xylitol production by *Candida tropicalis* (Rafiqul, Sakinah, & Zularisam, 2015). Thus, identification of microorganisms with higher tolerance to inhibitors, especially acetic acid, is essential for the development of an efficient xylitol production process (Bazoti et al., 2017; Xu & Hanna, 2010).

Yeast species from the *Candida*, *Pichia*, *Debaryomyces*, *Kluyveromyces*, and *Spathaspora* genera have been evaluated for xylitol production from biomass hydrolysates. Among these, *Candida* species have shown good performance with production yields varying from 19% to 72% of the theoretical maximum (Carvalheiro, Duarte, Medeiros, & Gírio, 2007; García-Diéguez, Salgado, Roca, & Domínguez, 2001; Miura et al., 2013; Rocha et al., 2014; Rodrigues et al., 2011; Zhang et al., 2012). *C. tropicalis* has shown good performance on lignocellulosic hydrolysates from corncob (Cheng et al., 2009; Guo et al., 2013; Ling, Cheng, Ge, & Ping, 2011; Misra, Raghuwanshi, & Saxena, 2013; Wang et al., 2011), rice straw (Liaw, Chen, Chang, & Chen, 2008), and sugarcane bagasse (Rao, Jyothi, Prakasham, Sarma, & Rao, 2006). The best reported value for xylitol production by *C. tropicalis* strains on sugarcane bagasse hydrolysate was 81.6 g/L of xylitol, with a yield of 0.57 g/g and productivity of 0.68 g/g h (Vallejos et al., 2016). However, under the conditions evaluated, the maximum acetic acid concentration was 1.39 g/L (Albuquerque et al., 2015). Although *C. tropicalis* is considered an opportunistic pathogen, the U.S. Food and Drug Administration has allowed its use for xylitol production (De Mello Lourenço, Dini-Andrade, Aguilar-Vildoso, & Basso, 2014).

In the current work, a collection of xylose-assimilating yeasts was screened for their ability to grow on sugarcane biomass hydrolysate. A strain with high tolerance towards hydrolysate was isolated and identified as *C. tropicalis* JA2. Subsequently, the xylitol production processed by this yeast was optimized through a series of experimental procedures in order to determine best pH and temperature conditions, as well as nutrient source, inoculum size, and substrate concentrations. Finally, a xylitol production process with high productivity and yield based on *C. tropicalis* JA2 and sugarcane biomass hydrolysate was obtained.

2 | MATERIAL AND METHODS

Yeast strains were kept in yeast peptone dextrose (YPD) composed of 10 g/L yeast extract, 10 g/L bacteriological peptone, and 20 g/L dextrose, or yeast peptone xylose (YPX) composed of 10 g/L yeast extract, 10 g/L bacteriological peptone, and 20 g/L xylose. In addition, minimal medium, yeast nitrogen base 6.7 g/L (YNB), was supplemented with 60 g/L xylose. When different concentrations of sugar were required, the value was specified appropriately.

The sugarcane bagasse hydrolysate, kindly provided by the Sugarcane Technology Center (CTC), São Paulo, Brazil, was obtained by steam explosion followed by acid hydrolysis of the hemicellulose-rich fraction. For each pretreatment batch, 8 kg of raw material (sugarcane bagasse) was processed in a steam explosion system for 8 min at 15 kgf/cm^2 (equivalent to 197.85°C), with 50% humidity. For the breakdown of the oligomers, the liquid fraction of the steam explosion was subjected to hydrolysis with 0.5% H_2SO_4 (w/w) at 130°C for 100 min. The final composition of the hydrolysate was (g/L):cellobiose, 2.21; glucose, 5.40; xylose, 90.32; acetic acid, 19.41; furfural, 2.93; and hydroxymethylfurfural (HMF), 0.54. This batch of hydrolysate was employed in all experiments described in this study, except in the experiment otherwise indicated. A second batch of hydrolysate was prepared following the same conditions employed before, and its composition was (g/L): cellobiose, 2.14; glucose, 4.96; xylose, 80.6; acetic acid, 19.62; furfural, 2.2; and hydroxymethylfurfural (HMF) 0.36.

2.1 | Screening of yeast strains

The 218 non-*Saccharomyces*yeast strains used in this work belong to the "Collection of Microorganisms and Microalgae Applied to

Agroenergy and Biorefining" from Embrapa Agroenergia. The yeast strains were previously isolated from soil and decaying wood samples collected in Brazilian Cerrado, and from fermentation broth in sugarcane mills. Their ability to grow on minimal medium supplemented with xylose as the sole carbon source was previously determined (unpublished data). In addition, the diversity among the 218 species was analyzed by sequencing of the D1/D2 region in some strains, and the following species were identified: *Cyberlindnerafabianii*, *Wickerhamomycesanomalus*, *Kluyveromyces marxianus*, *Spathaspora brasiliensis*, *Meyerozyma guilliermondii*, and *Hanseniasporauvarum*.

In order to identify strains with a high xylose consumption rate and tolerance to sugarcane biomass hydrolysate, a serial screening in minimal medium and hydrolysate was performed. Initially, each purified strain was grown in 200 μL of 10% YPX medium (10 g/L yeast extract, 20 g/L bacteriological peptone and 100 g/L xylose) at 30°C for 24 hr in 96-well microtiter plates on a rotating incubator at 200 rpm. After this, all 218 yeast strain suspensions were transferred to Erlenmeyer flasks (1 L) containing 400 ml of defined medium (6.7 g/L of yeast nitrogen base (YNB) and 100 g/L of xylose), which was incubated under the same conditions as the preculture for 48 hr. A second and third cycles of cultivation were carried out by inoculating the same fresh minimal medium with 10% of the previous culture. Cycles 4, 5, and 6 were performed under the same conditions, except that the minimal medium was supplemented with 15%, 30%, and 45% (v/v) sugarcane bagasse hydrolysate, respectively. Samples were taken at the end of each cycle for metabolite quantification.

After Cycle 6, the cultures were diluted and plated on YPD agar medium for isolation of single colonies. The plates were incubated at 30°C for 48 hr and morphologically different colonies were isolated.

The selected yeasts were deposited in the Collection of Microorganisms of Agricultural and Environmental Importance (CMAA) of the Brazilian Agriculture Research Corporation—EMBRAPA, located in Jaguariúna, Brazil. This collection is registered in the Culture Collection Information Worldwide of the World Data Centre for Microorganisms. The accession number is CMAA1716.

2.2 | Yeast taxonomy

Selected yeast strains were grown in 5 ml of YPD, at 28°C for 20 hr, and afterwards genomic DNA (gDNA) was extracted using the PureLink Genomic DNA Kit (Invitrogen), following the manufacturer's instructions. The D1/D2 region from the large-subunit of the rRNA gene was amplified by polymerase chain reaction using primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCG TGTTTCAAGACGG-3′; Kurtzman & Robnett, 1998) and gDNA as template.

Amplified DNA was purified and submitted to DNA sequencing at Eurofins Genomics (Louisville, KY, United States). Sequences were aligned, and the consensus sequences were compared with other yeast sequences deposited in GenBank with the following accession numbers: MH230894 (6.2), MH230895 (6.3), MH230896 (6.4), MH230897 (6.5), MH230898 (6.6), MH230899 (6.7) e MH229983 (6.8).

2.3 | Optimization of fermentation

2.3.1 | Preculture procedure

Single yeast colonies were propagated in 400 ml (1-L flask) of YPX (100 g/L) and incubated at 30°C for 72 hr in a rotating incubator at 200 rpm. Afterwards, the cells were separated by centrifugation at 4,000 g for 5 min.

The inoculum concentration was quantified by dry weight analysis. Samples were added to predried (105°C in oven, 30 min) and preweighed glass pads and then dried at 80°C overnight in a convection oven. Before determining the final weight after drying using an analytical balance, the glass pads and samples were cooled in a desiccator. The loss of weight was calculated as grams of dry weight per liter.

2.3.2 | Fermentation of biomass hydrolysate

After the screening and taxonomy steps, seven strains were tested for tolerance on sugarcane biomass hydrolysate, xylose consumption, and xylitol production. For this, 8.0 g/L of each strain was inoculated separately (with viability greater than 92%) into 125-ml Erlenmeyer flasks containing 40 ml of medium composed of sugarcane bagasse hydrolysate (described in Section 2), diluted to 40% (v/v). The final hydrolysate-fermentation medium contained YNB (6.7 g/L), xylose (75 g/L), glucose (12 g/L), and acetic acid (7.8 g/L), at pH 4.6. The pH value of biomass hydrolysate of sugarcane was adjusted with calcium hydroxide. The experiments were carried out at 30°C under stirring in a rotating incubator at 180 rpm. Samples were withdrawn periodically for biomass and metabolite quantification.

2.3.3 | Plackett–Burman experimental design for salts and nitrogen sources

To determine which salts and nitrogen sources are required to improve the xylose consumption rate and xylitol production, a Plackett–Burmam (PB) design with 16 trials was used to screen the medium components. The design included 11 input variables and three replicates at the central point (Plackett & Burmam, 1946; Rodrigues & Iemma, 2012). The medium salts components and their respective ranges were KH_2PO_4 from 0.0 to 4.0 g/L, $MgSO_4$ from 0.0 to 2.0 g/L, $(NH_4)_2SO_4$ from 0.0 to 4.0 g/L, $ZnSO_4$ from 0.0 to 1.0 g/L, $MnSO_4$ from 0.0 to 1.0 g/L, Fe_2SO_4 from 0.0 to 0.8 g/L, NaCl from 0.0 to 1.0 g/L, $CuSO_4$ from 0.0 to 0.1 g/L. For the nitrogen sources, the ranges were yeast extract from 0.0 to 5.0 g/L, YNB from 2.0 to 4.0 g/L, and urea from 0.0 to 2.0 g/L.

All fermentations were carried out using 8.0 g/L (dry weight) of *C. tropicalis* JA2 cells with viability greater than 94%, inoculated into 40 ml (125-ml flasks) of medium containing 40% sugarcane biomass hydrolysate, with final concentration of 40 g/L xylose and

12 g/L glucose. The medium was supplemented with nutrients according to the PB design with 16 trials. The experiments were carried out at 30°C under stirring in a rotating incubator at 180 rpm. Samples were withdrawn periodically for biomass and metabolite quantification.

2.3.4 | Effect of pH on xylitol production

In order to evaluate the effect of pH on xylitol production, the pH of the fermentation medium was adjusted to 4.6, 5.5, 6.0, 6.4, and 7.0 with calcium hydroxide. Based on the PB results, the medium composition was adjusted to 40% sugarcane biomass hydrolysate supplemented with 1.0 g/L of $(NH_4)_2SO_4$, 5.0 g/L of yeast extract, 4.0 g/L of YNB, and 0.8 g/L of urea, with xylose and glucose concentration of 50.0 and 8.0 g/L, respectively. All other fermentation parameters were the same as those described in Section 2.3.3. Each experiment was carried out in triplicate. Samples were withdrawn periodically for biomass and metabolite quantitation.

2.3.5 | Optimization using central composite design

Central composite design (CCD) was used to optimize xylitol production by *C. tropicalis* JA2 by evaluating different concentrations of supplementary nutrients that significantly affected xylitol production according to PB (Section 2.2.3). Thus, the first CCD (central composite design 1) with four variables and three replicates at central point was applied to optimize the xylitol production after 39 hr. Twenty-seven experiments were conducted. The alpha value used for orthogonality was 2.0.

The independent variables considered were $(NH_4)_2SO_4$ from 0 to 6.0 g/L (X_1), yeast extract from 0 to 10.0 g/L (X_2), YNB from 2.0 to 7.0 g/L (X_3), and urea from 0 to 4.0 g/L (X_4).

Fermentations were carried out with 8.0 g/L (dry weight) of *C. tropicalis* cells (with viability greater than 94%), inoculated into 40 ml (in 125-ml flasks) of medium containing 40% sugarcane biomass hydrolysate, supplemented with 40 g/L xylose and 8.0 g/L of glucose, at pH 6.4. The experiments were carried out at 30°C and 180 rpm in a rotating incubator.

After optimization of composition and nutrient concentration in the medium, two new CCDs were performed to evaluate the effects of initial xylose and yeast concentration on the fermentation medium and operating temperature. The responses analyzed were xylose consumption and xylitol production after 39 hr. A sequential strategy was used for the second and third CCDs, with 17 experiments conducted in each CCD, and for both, the alpha value used for orthogonality was 1.68.

For CCD2, we considered the independent variables of initial xylose from 50 to 150 g/L (X_1), inoculum concentration from 5 to 25 g/L (X_2), and operating temperature from 25 to 40°C (X_3).

CCD3 was constructed having as a central point the conditions that maximize xylitol production as determined in the second CCD (CCD2). For CCD3, we considered the independent variables of initial xylose from 80 to 220 g/L (X_1), inoculum concentration from 10 to 40 g/L (X_2), and operating temperature from 25 to 55°C (X_3).

The fermentations were carried out in 125-ml flasks with 40 ml of medium containing 40% sugarcane biomass hydrolysate with glucose concentration at 8 g/L, cellobiose (0.88 g/L), acetic acid (7.76 g/L), furfural (1.17 g/L), and HMF (0.22 g/L). The medium was supplemented with YNB (2 g/L) and urea (4 g/L), at pH 6.4. The experiments were carried out by incubating the flasks under stirring in a rotating incubator at 180 rpm.

Finally, the yeast performance was evaluated under the optimized fermentation conditions in nondiluted hydrolysate. A fermentation experiment employing the same conditions described above was carried out with 100% sugarcane biomass hydrolysate (second batch with composition described in Section 2). To minimize any dilution of hydrolysate, it was only supplemented with 0.32 g of YNB (to reach 2 g/L) and 0.64 g urea (to reach 4 g/L) and yeast biomass. The experiment was carried out in triplicate.

2.3.6 | Statistical analysis

The statistical analysis of experimental data obtained in the PB and CCD were performed using the software STATISTICA 12.0. Due to the variability of the bioprocesses, a significance level of 90% ($p < .1$) was considered (Haaland, 1989). To validate the model predictions, fermentations were carried out under conditions predicted by the models.

2.4 | Metabolite and biomass quantification

Samples from fermentation experiments were centrifuged at 16,873 g for 10 min, and the supernatant was used in quantification. Acetic acid, glucose, xylose, xylitol, and ethanol were quantified by HPLC (Agilent-1260 Infinity) coupled with an index detector RID 10-A using a reverse-phase column (Aminex® HPX87H, BioRad). Products were eluted using a solution of 5 mM H_2SO_4 as mobile phase at 0.6 ml/min flow rate at 45°C. The concentrations of compounds were determined using calibration curves, and values were calculated from peak areas.

Yeast concentration was monitored by optical density at 605 nm and correlated with dry weight. The dry cell weight was calculated according to optical density using the following linear correlation: Concentration (g/L) = dilution × 0.518 × OD_{605}. The correlation coefficient R^2 was 0.998.Yeast viability was determined by counting cells stained with erythrosine B in a Neubauer chamber using optical microscopy.

3 | RESULTS AND DISCUSSION

3.1 | Yeast selection and identification

To isolate a yeast strain able to efficiently metabolize xylose in the presence of biomass hydrolysate with a high concentration of

acetic acid (8.0 g/L), 218 yeast strains were screened for growth on defined medium (DM) and on medium supplemented with sugarcane bagasse hydrolysate. Initially, all strains were inoculated in 10% YPX (xylose, 100 g/L) medium, and after 48 hr of growth, they were transferred to fresh medium. After six cycles of cultivation (three in DM and three in increasing concentrations of hydrolysate), the xylose-consuming yeasts were isolated on agar plates containing 2% YPX (xylose, 20 g/L). Thus, seven different colonies, named as 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, and 6.8, were picked for taxonomic identification and physiological characterization.

For molecular identification, the D1/D2 domain of the large-subunit rRNA gene was amplified by polymerase chain reaction and submitted for sequencing. Analysis of these sequences showed that all strains belong to the *C. tropicalis* species. These results indicate the efficiency of *C. tropicalis* strains in growth on xylose, either in defined or hydrolysate-containing medium. However, some differences could be noted among the seven strains. Strain 6.2 showed an identical sequence to *C. tropicalis* isolate GX06 (accession number MF148900.1), whereas one indel was observed in the 6.6 and 6.7 strain sequences. Strain 6.8 differs in the D1/D2 sequence by a two-nucleotide deletion compared with the same *C. tropicalis* strain. The D1/D2 sequences from the 6.3 and 6.5 strainswere, respectively, 100% and 99% identical to *C. tropicalis* isolate NBT8 (accession number MF461173.1), whereas the 6.4 sequencewas identical to *C. tropicalis* strain SA-1 (accession number JN185908.1).

The analyses of xylose reductase activity with NADH and NADPH in the selected *C. tropicalis*strain JA2 was carried out by Trichez, Steindorff, Soares, Formighieri, and Almeida (2018), and the results showed that the xylose reductase (XR) activity was strictly NADPH-dependent. High XR activities improve the rate of xylose consumption and when associated with dependence on the NADPH cofactor, they favor the production of xylitol (Cadete et al., 2016; Karhumaa, Garcia-Sanchez, Hahn-Hägerdal, & Gorwa-Grauslund, 2007).

3.2 | Fermentation of sugarcane biomass hydrolysate by the isolated yeast strains

The fermentation capacities of the seven new *C. tropicalis* strains were evaluated in medium containing sugarcane biomass hydrolysate. Fermentation assays were carried out under aerobic conditions, in medium containing 8.0 g/L of acetic acid with an initial pH of 4.6. All yeasts were able to grow on the hydrolysate. After 65 hr of cultivation, growth was similar for the seven strains ($p < .05$; Figure 1). The 6.8 strain was the most efficient xylose-consuming strain (49.1 ± 0.89 g/L) and the most efficient producer of xylitol (22.16 ± 1.47 g/L) and ethanol (6.9 ± 0.73 g/L). On the other hand, the yeast 6.7 consumed only 22.9 ± 0.66 g/L of xylose the lowest in comparison with the other strains, producing 12.5 ± 0.51 g/L xylitol and 5.3 ± 0.09 g/L ethanol. The other five strains showed similar performances, consuming approximately 45 g/L of xylose (Figure 1). These results indicate that although the strains belong to the same species, they showed significantly different hydrolysate fermentation capabilities. These results are in good agreement with previous observations that different yeast strains respond distinctly in presence of hydrolysate (Almeida, Karhumaa, Bengtsson, & Gorwa-Grauslund, 2009).

The xylose consumption rate, and xylitol production and yield, varied significantly among the strains ($p < .05$). Strain 6.8 showed the highest xylose consumption rate (0.77 ± 0.02 g·L·h) and the highest xylitol yield 0.45 ± 0.01 g/g consumed xylose, whereas the strain 6.7 showed the lowest values for xylose consumption

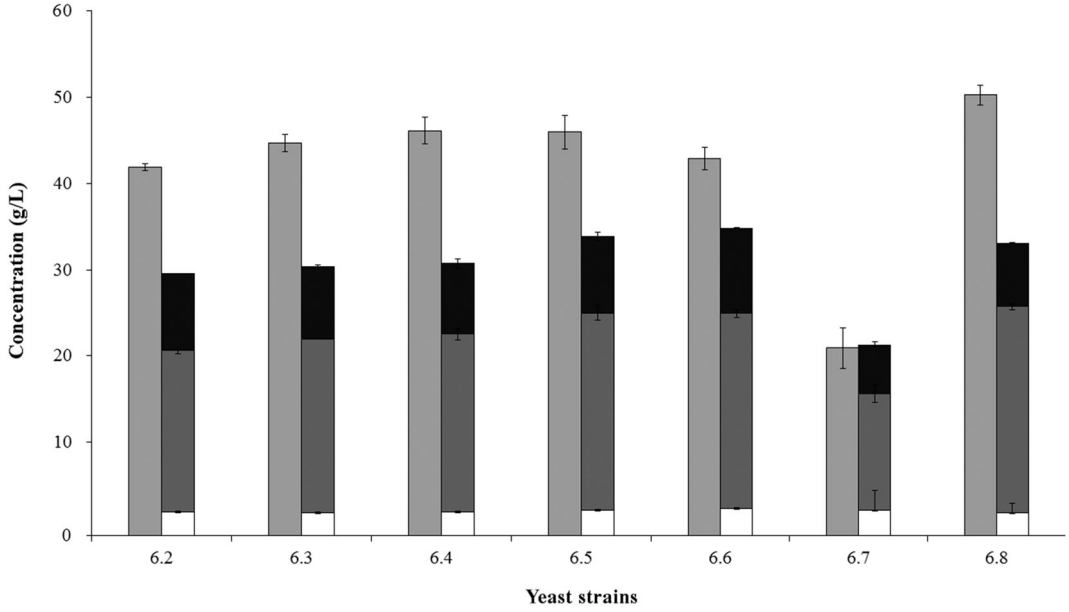

FIGURE 1 Cell growth (OD), xylose consumption and product formation after 65 hr of fermentation in medium containing sugarcane biomass hydrolysate. (□) Biomass, (▒) xylose consumed, (▒) xylitol, and (■) ethanol. Experiments were performed in triplicate and standard errors are less than 5%

rate and xylitol yield. Despite the low cell density and relatively high concentration of acetic acid in these experiments, the xylitol yields described here are superior to values reported in other studies with *C. tropicalis*. Mateo, Puentes, and Moya (2015) used *C. tropicalis* NBRC 0618, achievinga xylitol yield of 0.23 g/g of consumed xylose in the fermentation of acid hydrolysate from olive pruning. Swain and Krishnan (2015) reached a xylitol production yield of 0.19 g/g of consumed xylose using *C. tropicalis* in the fermentation of rice straw. However, higher xylitol concentration (16.2 g/L) and yield (0.67 g/g) values were obtained by fermentation of *C. guilliermondii* in medium supplemented with sugarcane straw (Hernández-Perez, Arruda, & Felipe, 2016).

Based on the obtained results of higher xylose consumption rate and xylitol yield, strain 6.8 was named *C. tropicalis* JA2 and was chosen for optimization of xylitol production.

3.3 | Plackett–Burman design for xylitol production

As *C. tropicalis* JA2 was able to produce xylitol in the presence of sugarcane hydrolysate containing a considerable amount of acetic acid, we aimed to improve the yeast performance by optimizing the fermentation medium. Thus, a Plackett and Burman (PB) design was performed to analyze the influence of the salts KH_2PO_4, $MgSO_4$, $(NH_4)_2SO_4$, $ZnSO_4$, $MnSO_4$, Fe_2SO_4, NaCl, and $CuSO_4$, and the nitrogen sources urea, yeast extract, and YNB in medium at an initial pH of 4.6.

The effect of each variable on the dependent variable xylitol production was evaluated after 39 hr of fermentation (Table 1). Considering statistical significance of $p < .1$, in the concentration range evaluated, $(NH_4)_2SO_4$, yeast extract, YNB, and urea had a significant positive effect, indicating that it is possible to increase xylitol production by *C. tropicalis* JA2. On other hand, Fe_2SO_4, $MnSO_4$, $CuSO_4$, and KH_2PO had a significant negative effect, indicating that they do not contribute to xylitol production (Table 1). It was also observed that the salts $MgSO_4$ and NaCl did not show statistically significant effects on xylitol production.

TABLE 1 Estimated effect, regression coefficient, and corresponding *t* and *p* values for xylitol concentration in 39 hr of fermentation in Plackett–Burman (PB) design experiment

Factor	Effect	Coefficient	Std. Error	*t* value	*p* value
Mean/Interc.	59.481	59.481	0.0789	753.514	0.00000
Urea	19.108	0.9554	0.0860	111.064	0.00001
Yeast Ext.	13.150	0.6575	0.0860	76.433	0.00012
Fe_2SO_4	−12.950	−0.6575	0.0860	−75.271	0.00013
$(NH_4)_2SO_4$	0.7613	0.3807	0.0860	44.252	0.00306
YNB	0.5819	0.2910	0.0860	33.824	0.01172
$MnSO_4.1H_2O$	−0.3647	−0.1824	0.0860	−21.199	0.07173
$CuSO_4$	−0.3039	−0.1520	0.0860	−17.666	0.12064
KH_2PO_4	−0.1044	−0.0522	0.0860	−0.6070	0.56303
$ZnSO_4$	−0.0577	−0.0288	0.0860	−0.3352	0.74727
NaCl	−0.0562	−0.0281	0.0860	−0.3265	0.75358
$MgSO_4.7H_2O$	0.0518	0.0259	0.0860	0.3012	0.77199

The PB design evaluates only which independent variables influence xylitol production. In this case, it can be suggested that $(NH_4)_2SO_4$, yeast extract, YNB, and urea promote improved xylitol production in sugarcane biomass hydrolysate by *C. tropicalis*. Subsequently, a CCD was performed to evaluate the effects of different concentrations of these nitrogen sources on xylitol production. Before this, the effect of variation in medium pH on yeast performance was evaluated.

3.4 | Effects of medium pH on xylitol production

The pH of fermentation media has been reported as one of the most important factors that affect xylitol production by yeasts (Sampaio et al., 2006). Yeasts usually grow well in acidic medium with pH between 4.0 and 6.0, though the tolerance range for pH variation is wide, ranging from pH 2.5 to 8.0 (Cao, Tang, Gong, & Chen, 1994; Sampaio et al., 2006; Walker, 1998). However, acetic acid present in sugarcane bagasse hydrolysate is a potent inhibitor of yeast metabolism, especially at pH values below its pKa of 4.75(Almeida et al., 2007; Lawford & Rousseau, 1993). The optimum pH for xylitol production is species-specific and medium composition-dependent (Hahn-Hägerdal et al., 2007). Thus, in order to evaluate the influence of initial pH on xylitol production by *C. tropicalis* JA2, the yeast was inoculated in sugarcane bagasse medium with a final concentration of: 1.0 g/L $(NH_4)_2SO_4$, 5.0 g/L yeast extract, 4.0 g/L YNB, and 0.8 g/L urea, favorable conditions indicated in the PB design, and pH adjusted to 4.6, 5.5, 6.0, 6.4, or 7.0. Xylose consumption and xylitol production were measured after 42 hr of fermentation (Figure 2).

C. tropicalis JA2 consumed all xylose present in the medium after 42 hr of cultivation at pH 6.4, whereas at pH 5.5, 6.0, and 7.0, it consumed approximately 87.5% of the provided sugar. At pH 4.6, the xylose consumption decreased significantly to 22.0%. The higher xylose consumption correlated with xylitol concentration (Figure 2), and yields, which varied from 0.47 ± 0.08 g/g total xylose at pH 6.4, to 0.19 ± 0.03 g/g total xylose at pH 4.6. These observations corroborate previous reports that acetic acid toxicity increases at pH values below its pKa. Under this condition, acetic acid in its undissociated form penetrates the cell membrane, and once in the cytoplasm, which as a neutral pH, it dissociates, causing toxicity to the cell (Hahn-Hägerdal et al., 2007; Sousa et al., 2012).

Therefore, the results show that *C. tropicalis* JA2 is more efficient for xylitol production in medium containing sugarcane bagasse hydrolysate with an initial pH of 6.4.

3.5 | Optimization of xylitol production

3.5.1 | Central composite design 1

To maximize xylitol production, a CCD was carried out with the nitrogen sources that showed significant positive effects on the PB

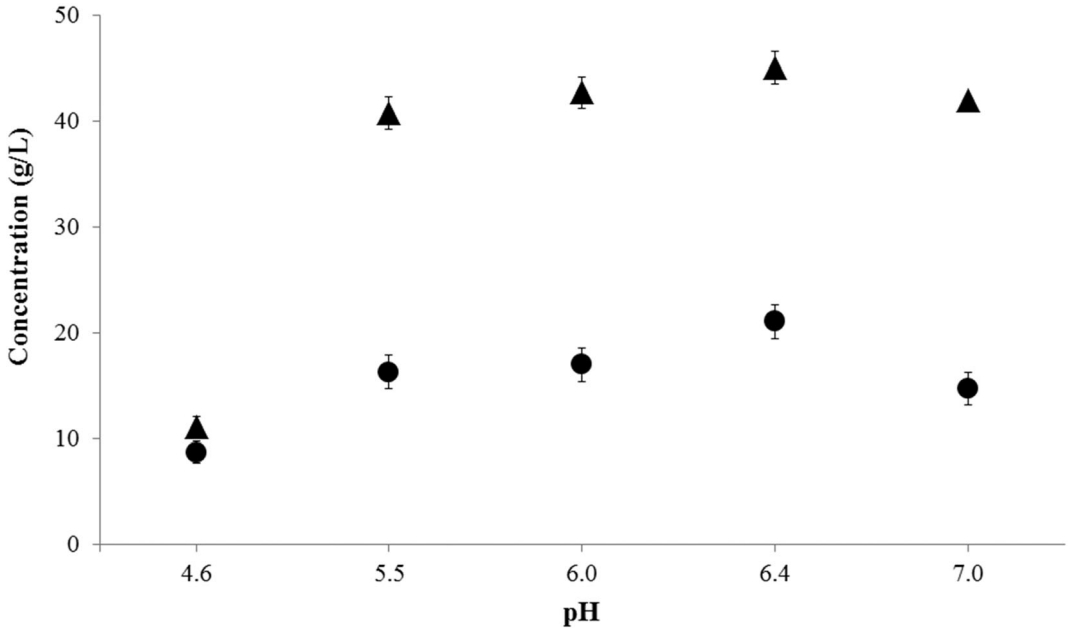

FIGURE 2 Fermentation profile of *Candida tropicalis* JA2 in hydrolysate medium with different initial pH values, after 42 hr of fermentation. (▲) Xylose consumed and (●) xylitol produced. Initial xylose concentration was 50 g/L. Experiments were performed in triplicate and standard errors are less than 5%

design ($(NH_4)_2SO_4$, yeast extract, YNB, and urea). Table 2 presents the 27 trials carried out in the design and the experimental values obtained for the responses xylose consumption and xylitol production after 39 hr of fermentation.

The regression equations for xylose consumption and xylitol production with coded variables considering statistically significant parameters ($p < .1$) are given in Equations 1 and 2, respectively. The analysis of variance indicated that the calculated F value for regressions was highly significant. The F ratio (Fcalc/Ftab) was 5.88 for xylose consumption and 9.42 for xylitol production. The coefficients of determination (R^2) by the models were 75% and 86%, respectively. Despite the low observed variation for the xylose consumption model, the results predicted by the model are in agreement with experimental values. This happens because the variables and interactions effects are much smaller than the mean in this model. The highest residual value, when the predicted value is subtracted from the observed value for this model, was 1.85.

Thus, we can conclude that the models fit the experimental data well. The lack of fit was not significant for both models. The value of pure error was low, which indicates good reproducibility of the obtained data. Therefore, it is possible to use techniques for optimization of the responses in the evaluated ranges.

$$\textit{Xylose consumption}\ (g/L) = 5.52 + 0.78\,X_1 + 0.29\,X_2 + 0.74\,X_3 - 0.48\,X_4 + 0.29\,X_4^2, \quad (1)$$

$$\textit{Xylitol}\ (g/L) = 18.38 - 0.99\,X_1 - 0.53\,X_2 - 1.31\,X_3 + 0.58\,X_4 - 0.39\,X_1X_4 + 0.63\,X_2X_3, \quad (2)$$

where, in coded values, X_1 corresponds to $(NH_4)_2SO_4$, X_2 to yeast extract, X_3 to YNB, and X_4 to urea.

Using the complete models, the eigenvalues and adjustments of the model to the canonical form were determined in order to verify the nature of the stationary point. For both responses, the given values positive and negative values simultaneously, indicating that the inflection point is a saddle. Then, using the models as objective functions and the experimental region as constraints, the maximum in each regression equation was found by a nonlinear iterative numerical solution optimization algorithm, which returns the highest value that the function assumes in the evaluated interval.

For the iterative numerical solution, xylitol production is maximized in medium containing 2.0 g/L YNB, 4.0 g/L urea, and without $(NH_4)_2SO_4$ and yeast extract (in coded values $X_1 = -2.0$, $X_2 = -2.0$, $X_3 = -2.0$, and $X_4 = 2.0$). The same maximum condition was found for the xylose consumption model, indicating that the substrate consumption rate and xylitol production are favored by the same nitrogen sources. Replacing the coded values of these parameters in Equation 1, predicted xylitol production is 29.28 g/L over 39 hr of fermentation. Equation (2) predicts total xylose consumption. To validate the maximum xylitol production predicted by the model, fermentation was carried out, in triplicate, in medium containing sugarcane bagasse hydrolysate with 7.8 g/L acetic acid, xylose concentration of 40.0 g/L, and 12.0 g/L of glucose, supplemented with 2.0 g/L YNB, and 4.0 g/L urea. After 39 hr of fermentation, *C. tropicalis* JA2 produced 28.79 ± 0.64 g/L xylitol (corresponding to a yield of 0.72 ± 0.05 g/g consumed xylose). The xylose was completely consumed. The results obtained experimentally and the small deviations (1.70%) from the predicted values confirm the quality of the model to fit the data.

It is known that the type and concentration of nitrogen source in the medium play a key role in xylitol production by

TABLE 2 Central composite design 1 to optimize nitrogen sources concentrations for xylitol production with *Candida tropicalis* JA2

Tests	Real variable (coded variable)				Xylose consumption (g/L)	Xylitol (g/L)	Yield (g/g)	Productivity (g·L·h)
	$(NH_4)_2SO_4$ (g/L)	Yeast Ext. (g/L)	YNB (g/L)	Urea (g/L)				
1	1.5 (−1)	2.5 (−1)	3.25 (−1)	1.0 (−1)	36.0	20.5	0.45	0.53
2	1.5 (−1)	2.5 (−1)	3.25 (−1)	3.0 (+1)	37.3	23.1	0.49	0.59
3	1.5 (−1)	2.5 (−1)	5.75 (+1)	1.0 (−1)	33.7	17.5	0.40	0.45
4	1.5 (−1)	2.5 (−1)	5.75 (+1)	3.0 (+1)	34.3	18.8	0.42	0.48
5	1.5 (−1)	7.5 (+1)	3.25 (−1)	1.0 (−1)	34.5	20.1	0.45	0.52
6	1.5 (−1)	7.5 (+1)	3.25 (−1)	3.0 (+1)	36.3	21.2	0.46	0.54
7	1.5 (−1)	7.5 (+1)	5.75 (+1)	1.0 (−1)	33.1	16.9	0.39	0.43
8	4.5 (+1)	7.5 (+1)	5.75 (+1)	3.0 (+1)	35.4	19.3	0.42	0.49
9	4.5 (+1)	2.5 (−1)	3.25 (−1)	1.0 (−1)	32.9	21.0	0.49	0.54
10	4.5 (+1)	2.5 (−1)	3.25 (−1)	3.0 (+1)	35.0	20.1	0.45	0.51
11	4.5 (+1)	2.5 (−1)	5.75 (+1)	1.0 (−1)	32.0	15.4	0.37	0.39
12	4.5 (+1)	2.5 (−1)	5.75 (+1)	3.0 (+1)	33.2	16.2	0.37	0.41
13	4.5 (+1)	7.5 (+1)	3.25 (−1)	1.0 (−1)	33.5	16.7	0.38	0.43
14	4.5 (+1)	7.5 (+1)	3.25 (−1)	3.0 (+1)	34.1	18.6	0.42	0.48
15	4.5 (+1)	7.5 (+1)	5.75 (+1)	1.0 (−1)	33.8	17.2	0.39	0.44
16	4.5 (+1)	7.5 (+1)	5.75 (+1)	3.0 (+1)	32.1	16.5	0.39	0.42
17	0.0 (−α)	5.0 (0)	4.5 (0)	2.0 (0)	36.1	20.3	0.44	0.52
18	6.0 (+α)	5.0 (0)	4.5 (0)	2.0 (0)	33.7	16.4	0.37	0.42
19	3.0 (0)	0.0 (−α)	4.5 (0)	2.0 (0)	35.8	20.0	0.44	0.51
20	3.0 (0)	10.0 (+α)	4.5 (0)	2.0 (0)	33.0	16.6	0.39	0.42
21	3.0 (0)	5.0 (0)	2.0 (−α)	2.0 (0)	36.4	20.2	0.43	0.52
22	3.0 (0)	5.0 (0)	7.0 (+α)	2.0 (0)	33.6	16.3	0.37	0.42
23	3.0 (0)	5.0 (0)	4.5 (0)	0.0 (−α)	32.6	16.5	0.39	0.42
24	3.0 (0)	5.0 (0)	4.5 (0)	4.0 (+α)	34.1	19.2	0.44	0.49
25 (C)	3.0 (0)	5.0 (0)	4.5 (0)	2.0 (0)	34.2	18.1	0.41	0.46
26 (C)	3.0 (0)	5.0 (0)	4.5 (0)	2.0 (0)	33.8	16.9	0.38	0.43
27 (C)	3.0 (0)	5.0 (0)	4.5 (0)	2.0 (0)	33.7	17.1	0.39	0.44

Note. Real and coded values for the variables studied (ammonium sulfate, yeast extract, YNB and urea) xylose consumption and xylitol production and experimental yield and productivity (three replicates at the central point) after 39 hr of fermentation.

microorganisms (Barathikannan, Khusro, & Paul, 2016). Similar to the results reported in this research, previous studies reported an increase in xylose consumption and xylitol production by recombinant *Saccharomyces cerevisiae* when fermentation medium was supplemented with urea (Tesfaw & Assefa, 2014). When the fermentation medium was supplemented with urea, the xylitol production by mutant *Candida* sp. increased up to twofold (Lu, Tsai, Gong, & Tsao, 1995). Because YNB and urea are expensive compounds when compared with yeast extract (for example), further studies should be carried out to analyze the components of YNB individually, and to evaluate which have the greatest influence on improvement of xylitol production by the yeast *C. tropicalis* JA2.

The model obtained through statistical analysis for cell growth (data not shown) was not significant in the description of the experimental data. The coefficient of determination was low and residues very high. The Pearson correlation coefficient obtained between xylitol production and cell concentration, considering the dry weight, was rho = 0.267, indicating that there is no correlation between these variables. This behavior showed that yeast growth was not associated with xylitol production and nitrogen source concentration within the range studied in this work.

3.5.2 | Central composite design 2

A new CCD2 was carried out with initial xylose and inoculum concentration, and incubation temperature, as independent variables. The responses xylose consumption and xylitol production were measured over 39 hr, and results are shown in Table 3.

The models obtained for xylose consumption and xylitol production were statistically significant in the description of the experimental

TABLE 3 Central composite design 2 to optimize nitrogen sources concentrations for xylitol production with *Candida tropicalis* JA2

Tests	Real variable (coded variable)			Xylose consumption (g/L)	Xylitol (g/L)	Yield (g/g)	Productivity (g·L·h)
	Xylose (g/L)	Inoculum (g/L)	Temperature (°C)				
1	73.6 (−1)	9.7 (−1)	28.5 (−1)	65.1	35.9	0.65	0.92
2	73.6 (−1)	9.7 (−1)	36.5 (+1)	73.0	46.9	0.64	1.20
3	73.6 (−1)	20.3 (+1)	28.5 (−1)	66.3	34.6	0.52	0.89
4	73.6 (−1)	20.3 (+1)	36.5 (+1)	74.1	55.5	0.75	1.42
5	126.4 (+1)	9.7 (−1)	28.5 (−1)	79.6	31.9	0.64	0.82
6	126.4 (+1)	9.7 (−1)	36.5 (+1)	70.3	51.5	0.73	1.32
7	126.4 (+1)	20.3 (+1)	28.5 (−1)	63.5	40.0	0.63	1.03
8	126.4 (+1)	20.3 (+1)	36.5 (+1)	105.1	74.0	0.70	1.90
9	50.0 (−α)	15.0 (0)	32.5 (0)	46.0	26.4	0.57	0.68
10	150.0 (+α)	15.0 (0)	32.5 (0)	70.5	47.2	0.67	1.21
11	100.0 (0)	5.0 (−α)	32.5 (0)	52.9	34.2	0.65	0.88
12	100.0 (0)	25.0 (+α)	32.5 (0)	87.4	55.8	0.64	1.43
13	100.0 (0)	15.0 (0)	25.0 (−α)	57.7	30.1	0.52	0.77
14	100.0 (0)	15.0 (0)	40.0 (+α)	88.2	62.7	0.71	1.61
15 (C)	100.0 (0)	15.0 (0)	32.5 (0)	66.0	42.6	0.64	1.09
16 (C)	100.0 (0)	15.0 (0)	32.5 (0)	71.1	43.3	0.61	1.11
17 (C)	100.0 (0)	15.0 (0)	32.5 (0)	69.9	43.9	0.63	1.13

Note. Real and coded values for the variables studied (xylose, inoculum and temperature) xylose consumption and xylitol production and experimental yield and productivity (three replicates at the central point) after 39 hr of fermentation.

data, and the calculated F value was highly significant for both. The F ratio was 2.96 for xylose consumption and 5.58 for xylitol production, and the regression could be used for predictive purposes (Box, Hunter, & Hunter, 1978). The lack of fit was not significant, and the value of pure error was low, which indicates good reproducibility of the obtained data, allowing the use of techniques for optimization of the responses in the evaluated ranges.

In the evaluated range for both models, the stationary point is a saddle. Subsequently, we used the nonlinear iterative numerical solution optimization algorithm to find the maximum value for the functions over the studied range. Equations (3) and (4) (empirical equation of second order calculated in the software STATISTICA 12.0, based on the results of CCD2) show the fitted model for xylose consumption and xylitol production after 39 hr of fermentation.

$$Xylose\ (g/L) = 69.23 + 8.24\,X_2 + 9.96\,X_3 + 5.79\,X_1X_2 + 5.81\,X_1X_3, \quad (3)$$

$$Xylitol\ (g/L) = 42.29 + 10.82\,X_3 + 5.65\,X_2 + 4.45\,X_1 + 3.03\,X_2X_3 + 2.93\,X_1X_2 + 2.71\,X_1X_3 + 2.66\,X_3^2, \quad (4)$$

where, in coded values, X_1 corresponds to xylose concentration, X_2 to inoculum concentration, and X_3 to temperature. The correlation coefficients for the xylitol production model (R^2 = 0.93) suggest that there is close agreement between the experimental results and the theoretical values predicted by polynomial model. However, the correlation coefficient for the xylose consumption model (R^2 = 0.76) was below the desired value. There were relative errors of approximately 1% to 16%, with one case of almost −36% for the lowest xylose concentration. This often occurs, but in the region of interest, the relative errors are very low considering processes involving microorganisms (Rodrigues & Iemma, 2012).

Based on Equation (4), xylitol production is maximized in medium with X_1 = +1.68; X_2 = 0.94; and X_3 = +1.68, corresponding to 150 g/L xylose, inoculating 20 g/L of cells, and fermentation carried out at 40°C. Under these conditions, the model predicts a maximum xylitol production of 97.9 g/L (0.81 g/g) with productivity of 2.51 g·L·h. In the xylose consumption model, the maximum value occurs with initial xylose concentration of 150 g/L, yeast concentration of 25 g/L, and temperature at 40°C (X_1 = 1.68, X_2 = 1.68, and X_3 = 1.68). Analyzing models and optimum conditions indicated that higher values for independent variables could promote higher response values. After this, a sequential strategy was used to obtain the condition that leads to maximum production and the most appropriate operating conditions. A new CCD (CCD3) was performed with the maximum conditions for xylitol production as a central point. A new range for the variables was proposed and three repetitions at the central point in CCD3 were used in order to validate the previous experiment.

3.5.3 | Central composite design 3

The CCD3 experiments were conducted in medium containing sugarcane bagasse hydrolysate diluted to 40% (v/v) with glucose

concentration of 8 g/L, supplemented with 2.0 g/L YNB and 4.0 g/L urea, as in previous experiments. In this case, initial xylose concentration (X_1) was evaluated from 80 to 220 g/L, yeast concentration (X_2) from 10 to 40 g/L, and temperature (X_3) from 25°C to 55°C. The real and coded values for the variables and the responses xylose consumption and xylitol production are presented in Table 4.

Analyzing the results obtained in the central point experiments (trials 15–17), it was observed that under conditions determined by CCD2, *C. tropicalis* JA2 yeast would produce 100.3 ± 1.44 g/L xylitol (representing a yield of 0.83 g/g of consumed xylose) with a productivity of 2.57 g·L·h. The predicted xylose consumption in this condition was 119.25 g/L, and the experimental value was 119.03 ± 0.47 g/L. The results obtained experimentally and the less than 1% deviation from the predicted values confirm that the models obtained in CCD2 fit the data well.

The regression equations in CCD3 for xylose consumption and xylitol production considering the significant parameters ($p < .1$) are given in Equations (5) and (6), respectively.

$$\text{Xylose}\ (g/L) = 113.35 - 33.80\,X_3^2 + 18.13\,X_1 + 15.68\,X_2 - 13.48\,X_3 - 10.83\,X_1^2 - 10.52\,X_2^2, \quad (5)$$

$$\text{Xylitol}\ (g/L) = 101.46 - 29.14\,X_3^2 + 13.22\,X_1 + 10.39\,X_2 - 10.14\,X_1^2 - 9.26\,X_2^2 - 5.77\,X_3, \quad (6)$$

where, in coded values, X_1 corresponds to xylose concentration, X_2 to inoculum concentration, and X_3 to temperature.

The analysis of variance corresponding to xylose consumption and xylitol production after 39 hr fermentation indicates that the explained variances (R^2 = 0.94 and 0.93, respectively) and F calc (30.61 and 20.69, respectively) are suitable to evaluate the tendency of this response. The calculated F value was six times greater than the value tabulated for F for both models.

The models are statistically significant and show good agreement between experimental and predicted results. The sum of squares for pure error was low, which indicates good reproducibility of the obtained data.

Using the complete model for xylitol production response, the eigenvalues and adjustments of the model for the canonical form were determined to verify the nature of the stationary point, which presented only negative values, indicating a maximum point that corresponds to 177 g/L xylose, 30 g/L of inoculum, and 39.3°C (X_1 = 0.66, X_2 = 0.58, and X_3 = −0.09). Substituting the coded values of these parameters into Equation 5 results in a predicted xylitol production of 108.96 g/L (0.86 g/g) with productivity of 2.79 g·L·h after 39 hr of fermentation. The maximum stationary point was also found in the xylose consumption model when X_1 = 0.94, X_2 = 0.88, and X_3 = −0.17, corresponding to 189.1 g/L of xylose, 32.83 g/L of inoculum, and 38.5°C. In this condition, predicted xylose consumption is 130.00 g/L. We can observe that the maximum conditions for both models are very similar.

The results obtained from CCD3 were experimentally verified at optimal xylitol production conditions. The xylose concentration was 177 g/L, inoculum with 30.0 g/L cells, and fermentation at

TABLE 4 Central composite design 3 to optimize nitrogen sources concentrations for xylitol production with *Candida tropicalis* JA2

Tests	Real variable (coded variable)			Xylose consumption (g/L)	Xylitol (g/L)	Yield (g/g)	Productivity (g·L·h)
	Xylose (g/L)	Inoculum (g/L)	Temperature (°C)				
1	108.4 (−1)	16.1 (−1)	31.1 (−1)	35.1	30.5	0.56	0.78
2	108.4 (−1)	16.1 (−1)	48.9 (+1)	9.6	21.1	0.53	0.54
3	108.4 (−1)	33.9 (+1)	31.1 (−1)	65.7	52.5	0.72	1.35
4	108.4 (−1)	33.9 (+1)	48.9 (+1)	20.0	34.4	0.64	0.88
5	191.6 (+1)	16.1 (−1)	31.1 (−1)	71.3	53.5	0.84	1.37
6	191.6 (+1)	16.1 (−1)	48.9 (+1)	35.3	41.0	0.82	1.05
7	191.6 (+1)	33.9 (+1)	31.1 (−1)	95.0	70.9	0.97	1.82
8	191.6 (+1)	33.9 (+1)	48.9 (+1)	77.7	63.4	0.97	1.63
9	80.0 (−α)	25.0 (0)	40.0 (0)	63.3	55.9	0.75	1.43
10	220.0 (+α)	25.0 (0)	40.0 (0)	121.9	109.5	0.86	2.81
11	150.0 (0)	10.0 (−α)	40.0 (0)	61.7	65.4	0.72	1.68
12	150.0 (0)	40.0 (+α)	40.0 (0)	125.3	105.1	0.86	2.69
13	150.0 (0)	25.0 (0)	25.0 (−α)	45.3	38.3	0.66	0.98
14	150.0 (0)	25.0 (0)	55.0 (+α)	9.9	19.6	0.33	0.50
15 (C)	150.0 (0)	25.0 (0)	40.0 (0)	116.2	101.1	0.82	2.53
16 (C)	150.0 (0)	25.0 (0)	40.0 (0)	112.9	101.3	0.81	2.51
17 (C)	150.0 (0)	25.0 (0)	40.0 (0)	107.5	98.7	0.80	2.50

Note. Real and coded values for the variables studied (xylose, inoculum and temperature) xylose consumption and xylitol production and experimental yield and productivity (three replicates at the central point) after 39 hr of fermentation.

TABLE 5 Xylitol production by different yeasts on different substrates

Feedstocks	Microorganisms	Acetic acid (g/L)	Xylitol (g/L)	Xylitol yield (g/g xylose)	Productivity (g·L·h)	Reference
Corncob hydrolysate	*Candida tropicalis* W103	1.00	68.4	0.70	0.95	Cheng et al. (2009)
Corncob hydrolysate	*C. tropicalis*	0.35	16.8	0.50	0.28	Misra et al. (2013)
Acid hydrolysate from olive pruning	*C. tropicalis* NBRC 0618	-	9.0	0.23	0.06	Mateo et al. (2015)
Rice straw	*C. tropicalis*	-	1.8	0.19	0.06	Swain and Krishnan (2015)
Corn straw biomass hydrolysate	*C. tropicalis*	3.93	56.4	0.72	0.94	Wang et al. (2015)
Sugarcane straw	*Candida guilliermondii* FTI 20037	2.87	17.0	0.41	0.34	Hernández-Perez et al. (2016)
Corncob hemicellulosic hydrolysate	*C. tropicalis*	-	58.8	0.77	2.45	Jia et al. (2016)
Hemicellulosic liquor from sugarcane bagasse	*C. tropicalis*	1.39	81.6	0.57	0.68	Vallejos et al. (2016)
Cashew apple bagasse hydrolysate	*Kluyveromyces marxianus*	4.50	13.2	0.36	0.11	Albuquerque et al. (2015)
Sugarcane biomass hydrolysate	*C. tropicalis* JA2	7.76	109.5	0.86	2.81	This study
Sugarcane biomass hydrolysate	*C. tropicalis* JA2	17.9	41.5	0.95	1.04	This study

40°C. After 39 hr of fermentation, *C. tropicalis* JA2 produced 109.5 ± 0.63 g/L xylitol, with a yield of 0.86 ± 0.04 g/g of consumed xylose and productivity of 2.81 ± 0.01 g·L·h. For this condition, a xylose consumption of 128.01 g/L was predicted, and up to 127.32 ± 4.1 g/L xylose was experimentally consumed. During the fermentations, the concentrations of acetic acid and glycerol were also monitored, and it was observed that the values did not change considerably. The small deviations from the predicted values confirmed that the models developed could reliably predict xylose and xylitol concentration.

Finally, *C. tropicalis* JA2 performance was evaluated in nondiluted hydrolysate. For this, a fermentation experiment was carried out with 100% sugarcane biomass hydrolysate, containing approximately 80 g/L xylose and 18 g/L acetic acid. After 40 hr, the yeast *C. tropicalis* JA2 was able to produce 41.5 ± 1.3 g/L xylitol, with a yield of 0.95 ± 0.04 g/g of consumed xylose, and productivity of 1.04 ± 0.03 g·L·h (Table 5). For this condition, up to 43.6 ± 1.5 g/L xylose was consumed. The hydrolysate toxicity, which has a very high concentration of acetic acid, reduced the amount of xylose consumed (from 71% to 53%) and productivity (from 2.81 to 1.04 g/L.h) when compared with the previous experiments. However, the xylitol yield increased from 0.86 to 0.95 g/g of consumed xylose (Table 5). The inhibitory effects of the hydrolysate completely hampered yeast growth (data not shown), and the carbon was directed almost exclusively to xylitol production. These results confirm the potential of *C. tropicalis* JA2 to produce xylitol even in hydrolysates with very high concentration of acetic acid (Table 5).

Optimization of the medium composition and the fermentation parameters by three sequential CCDs allowed a significant improvement in xylitol production by the new isolated *C. tropicalis* JA2 strain. Indeed, it was possible to increase xylitol production from 11.2 ± 0.02 g/L xylitol (0.47 ± 0.03 g/g consumed xylose) to 109.5 ± 0.63 g/L (0.86 ± 0.04 g/g consumed xylose). These results show the promising performance of *C. tropicalis* JA2 for xylitol production in media containing sugarcane bagasse hydrolysate. Indeed, the high xylitol yield of0.86 ± 0.04 g/g of consumed xylose and productivity of2.81 ± 0.01 g·L·h obtained in this study are among the best results reported for microbial production of xylitol, especially when using biomass hydrolysate with high content of acetic acid (Table 5).

C. tropicalis CCTCC M2012462 was able to produce xylitol with yields of 0.72 g/g of consumed xylose; however, this was only after 60 hr of fermentation and in a medium containing corn straw biomass hydrolysate with only 3.93 g/L acetic acid (Wang, Ling, & Zhao, 2015). The xylitol concentration values obtained are higher than those found by Jia et al. (2016), who obtained 0.77 g/g of consumed xylose and productivity of 2.45 g·L·h after 40 hr of fermentation, using corncob hydrolysate as substrate. They are also higher than the value found by Vallejos et al. (2016), who achieved a maximum xylitol production of 0.57 g/g, after 120 hr, produced by *C. tropicalis* in liquor obtained from sugarcane bagasse with 1.39 g/L acetic acid. Finally, Albuquerque et al. (2015) reported a maximum xylitol production of 0.36 g/gand productivity of 0.11 g·L·h by *Kluyveromyces marxianus* in cashew apple bagasse hydrolysate with 4.5 g/L acetic acid.

4 | CONCLUSIONS

The strategy used to select a non-*Saccharomyces* yeast strain tolerant to a hydrolysate with high acetic acid concentration was successful, because the newly identified *C. tropicalis* strains showed promising results for xylitol production on sugarcane biomass hydrolysate. The optimization of the fermentation parameters (pH, salt, and nitrogen sources, inoculum and xylose concentration, and temperature of fermentation) allowed a considerable increase in xylitol production from 11.20 ± 1.47 to 109.5 ± 0.63 g/L and yield from 0.47 ± 0.03 to 0.86 ± 0.04 g/g. Higher yield and productivity

values were observed when the hydrolysate was supplemented with 2.0 g/L YNB and 4.0 g/L urea, and fermentation was carried out with inoculum of 30 g/L, 177 g/L xylose, at pH 6.4, and at 40°C.

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