Short-Term Adaptation Strategy Improved Xylitol Production by *Candida Guilliermondii* on Sugarcane Bagasse Hemicellulosic Hydrolysate

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Research Article

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Abstract

One of the major bottlenecks of the biotechnological production of xylitol by pentose-fermenting yeasts is the presence of toxic compounds in the hemicellulosic hydrolysates, which inhibit the bioconversion of xylose into xylitol. In this work, short-term adaptation was evaluated as a strategy to minimize the toxicity of the sugarcane bagasse hemicellulosic hydrolysate to *Candida guilliermondii* FTI 20037. Yeast adaptation improved xylose assimilation as well as xylitol production. The beneficial effects of adaptation were more pronounced in the hydrolysate with higher concentration of toxic compounds, leading to an increase of 62.5% in the xylitol volumetric productivity in comparison to the use of non-adapted cells. In this condition, it was also verified the reduction of glycerol production (about 102%), a by-product formed as consequence of cellular stress, indicating a greater tolerance of adapted cells to the toxicity of hydrolysates. Short-term adaptation proved to be a promising strategy to improve considerably the microbial tolerance and overcome the toxicity of hydrolysates.

1. Introduction

In the last decades, the bioeconomy emerged and has gained space as an alternative economic system to the use of non-renewable and non-sustainable resources [1]. Sugarcane is considered as one of the most competitive carbon sources for application as raw material in this context due to its high efficiency in the low-cost carbon generation and its contribution to mitigate the effects caused by the use of fossil fuels [2]. The use of sugarcane in a biorefinery context also allows the expansion of the portfolio of products of the sugarcane agro-industry [1]

Xylitol is a polyol classified as one of the top 12 high value-added chemicals capable of supporting technical and economic viability of biorefineries [3]. Due to its interesting properties, this polyol has consolidated applications in food, dental, pharmaceutical, and cosmetics industries. In addition, it can also be used in the medicine and as a chemical platform for the production of new molecules and materials [4]. Xylitol commercial production is carried out from rich xylan materials through a chemical route [5]. Due to the disadvantages associated to this route, the biotechnological production proves to be an attractive alternative of better cost-benefit, less energy demand, and more environmentally friendly [6]. The high content of xylose in the hemicellulose of vegetal biomasses associated with microorganisms that assimilate pentoses, as well as the establishment of operational fermentation conditions, are essential for the bioconversion of xylose into xylitol.

The deconstruction of the plant cell wall and the solubilization of hemicellulosic sugars is one of the key stages of xylitol production by the biotechnological route [7]. Diluted acid hydrolysis is commonly used for this purpose due its low cost, easy operation, short reaction times, and effectiveness as it allows the recovery of 70 to 95% of the monomeric sugars [4]. However, in addition to sugars, this process results in the release/formation of toxic compounds to microorganisms, which reduce the yield and productivity of fermentation process [8–11].

Furfural, 5-hydroxymethylfurfural (5-HMF), acetic acid, phenolics compounds, and inorganic ions are often found in hemicellulosic hydrolysates obtained through diluted acid hydrolysis [8, 11, 12]. Furfural and 5-HMF are generated from the thermochemical degradation of pentoses and hexoses, respectively. Their toxicity is associated with inhibition of metabolism enzymes, disturbance of the integrity of the plasma membrane, DNA damage, and inhibition of RNA and protein synthesis [13, 14]. The toxicity of acetic acid, which is generated from the deacetylation of hemicellulose [15], is related to the penetration of its non-dissociated form in the cell and acidification of intracellular pH, leading to protein inactivation, inhibition of metabolic processes, and induction of oxidative stress [16]. However, low concentrations of this acid can favor xylose metabolism by *Candida guilliermondii* [17, 18]. Phenolic compounds, considered as the main inhibitors found in hydrolysates [14], are generated from the partial degradation of lignin and extractives [15]. Their toxicity are mainly associated with damage to the plasma membrane, resulting in loss of its integrity and function as a selective barrier [19]. Inorganic ions generated from biomass, chemical reagents or corrosion of the hydrolysis reactor [15, 20], can inhibit enzymes and metabolic pathways and form cytotoxic non-specific compounds [21].

Detoxification of hemicellulosic hydrolysates is often performed to reduce their toxicity to microorganisms, being the adsorption with activated carbon commonly used for this purpose [9, 10, 22]. However, this method has some disadvantages, such as the loss of sugars and waste generation, which are difficult to recycle and regenerate [10, 23]. Alternative methodologies to detoxification methods, such as cell adaptation techniques, have been suggested to develop robust and tolerant strains and overcome the toxicity of hydrolysates [19, 24]. Short-term adaptation strategies have been described as efficient adaptative techniques to increase microbial tolerance to inhibitors found in hydrolysates, being carried out through the pre-exposure of the microorganism to non-lethal concentrations of the inhibitors [19, 25]. Unlike evolutionary adaptation that leads to genetic modifications, short-term adaptation mainly affects metabolism [25], resulting in the induction of a cell phenotype more resistant to the inhibitors and the consequent preparation of the microorganism to grow in the presence of a harmful environment [26]. Short-term adaptation strategies are described as a capable of increasing microbial tolerance to lignocellulosic inhibitors, leading to an increase in fermentative performance [19, 27]. However, few studies report their use for xylitol production. Thus, the objective of this study was to evaluate the effect of short-term adaptation of *C. guilliermondii* FTI 20037, grown in increasing concentrations of toxic compounds present in the sugarcane bagasse hemicellulosic hydrolysate (SBHH), on the bioconversion of xylose to xylitol.

2. Materials And Methods

2.1 Preparation of SBHH

The sugarcane bagasse, gently donated by Usina Guarani, Olímpia, SP, Brazil, was submitted to diluted acid hydrolysis in a 250-L stainless steel reactor under the following conditions: 1.0% (w/v) H₂SO₄, 1:10 solid/liquid ratio (dry biomass/acid solution) at 121°C for 10 min. The hydrolysate was filtered and

concentrated two, three, four, and five-fold under vacuum at 70°C [28]. Next, the hydrolysates were treated by pH adjustment to 7.0 and 2.5 with CaO and H₃PO₄, respectively, followed by new filtration and an adsorption treatment with 1.0% (w/v) activated charcoal at 60°C, 100 rpm for 30 min [29]. In the sequence, the pH was adjusted to 5.5 using 6 molL⁻¹ NaOH and the treated (detoxified) hydrolysates were autoclaved at 115°C for 15 min (Table 1). A portion of twice concentrated and untreated hydrolysate (pH 5.5) was used as a control to evaluate the effect of fermentation on the non-detoxified hydrolysate.

Table 1

Composition of sugarcane bagasse hemicellulosic hydrolysates used in cell adaptation and fermentation stages. Non-concentrated (NC), two-fold concentrated and non-treated (H2N) and concentrated two (H2) three (H3), four (H4) and five-fold (H5) and treated hydrolysates.

Compounds (gL ⁻¹)	NC	H2N	H2	H3	H4	H5
Xylose	14.72 ± 0.41	30.23 ± 0.84	29.50 ± 0.76	39.15 ± 1.23	56.38 ± 1.42	68.54 ± 0.91
Arabinose	0.86 ± 0.06	2.60 ± 0.24	2.14 ± 0.17	3.78 ± 0.07	5.45 ± 0.28	7.01 ± 0.35
Glucose	0.08 ± 0.01	0.70 ± 0.00	0.60 ± 0.01	0.72 ± 0.03	1.18 ± 0.13	1.37 ± 0.09
Acetic acid	1.65 ± 0.18	2.45 ± 0.22	2.33 ± 0.25	2.74 ± 0.20	2.92 ± 0.31	3.21 ± 0.17
Phenolic compounds	0.70 ± 0.07	4.84 ± 0.35	1.65 ± 0.24	2.45 ± 0.18	3.55 ± 0.42	4.07 ± 0.11
5-HMF ^a	-	-	-	-	-	-
Furfural ^a	-	-	-	-	-	-
^a Not detected.						

2.2 Inoculum preparation and short-term adaptation

Candida guilliermondii FTI 20037 was preserved at 4°C on malt extract agar (Difco, BD, France) to be used for inoculum preparation in a medium containing (gL⁻¹): xylose (30.0), (NH₄)₂SO₄ (2.0), CaCl₂·H₂O (0.1) and rice bran extract (20.0). All hydrolysates used were also supplemented with ammonium sulfate, calcium chloride, and rice bran extract. A loop of cells grown on malt extract agar was aseptically transferred to the medium (50 mL) in Erlenmeyer flasks (125 mL) and incubated in an orbital shaking incubator (New Brunswick Scientific Co. Inc.) at 30°C, 200 rpm for 24 h. Cells were recovered by centrifugation (800×g for 15 min), washed, and resuspended in sterile distilled water to be used as inoculum in the non-concentrated hydrolysate at initial concentration of 0.4 g_{DCW}.L⁻¹. After 24 h of growth (30°C, 200 rpm) the cells were recovered from the non-concentrated hydrolysate by centrifugation as described above, and transferred to the two-fold concentrated hydrolysate at initial concentration of

0.4 g_{DCW}·L⁻¹. This process was carried out consecutively until five-fold concentrated hydrolysate in order to adapt the cells to the same concentration factor of the hydrolysate used in the fermentation, thus obtaining different degrees of cell adaptation.

2.3 Fermentation conditions

Batch fermentations were carried out on untreated and two-fold concentrated hydrolysate (H2N) and two (H2), three (H3), four (H4), and five-fold (H5) concentrated and treated hydrolysates using adapted and non-adapted cells to the same hydrolysate concentration factor. Fermentations were performed in a 2.4 L bench scale KLF 2000 bioreactor (BioEngineering Co., Switzerland), filled with 1.6 L of hydrolysate, at initial pH 5.5, $k_L a$ 20 h⁻¹ and 30°C during incubation time corresponding to the consumption of at least 80% of the xylose present in the hydrolysates. Samples were periodically collected to monitor cell growth, substrate consumption, and product and byproducts formation.

2.4 Analytical methods

The concentrations of xylose, glucose, arabinose, ethanol, glycerol, and acetic acid were determined by High Performance Liquid Chromatography (HPLC) (Waters 786), using a refractive index detector and an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 45°C with 0.01 molL⁻¹ H₂SO₄ as eluent at a flow rate of 0.6 mL min⁻¹. Xylitol yield ($Y_{P/S}$) was determined as the ratio between xylitol produced and xylose consumed (gg⁻¹). Xylitol volumetric productivity (Q_P) was defined as the ratio between xylitol produced and fermentation time (gL⁻¹h⁻¹), while xylose/arabinose consumption rates (Q_{XS} and Q_{AS} , respectively) were calculated as the ratio between xylose/arabinose consumed and fermentation time (gL⁻¹h⁻¹).

Furfural and 5-hydroxymethylfurfural were also quantified by HPLC (Shimadzu-LC1110AD) equipped with an RP-18 column (Hewlett-Packard, Palo Alto, CA, USA) and UV detector (SPD-10A UV-Vis, Waters Corp., Milford, MA, USA) operating at 25°C and using acetonitrile/water (1:8) with 1% acetic acid (w/v) as eluent at a flow rate of 0.8 mL min⁻¹. Total phenolics were determined spectrophotometrically at 280 nm after adjusting the pH of the samples to 12.0 [30]. Cell concentration was monitored by measuring the absorbance at 600 nm which was correlated to the dry weight using a calibration curve previously established.

3. Results And Discussion

3.1 Effect of short-term adaptation on bioconversion of xylose to xylitol

Adapted and non-adapted *Candida guilliermondii* FTI 20037 cells were grown in the hydrolysates to evaluate the effect of short-term adaptation on the bioconversion of xylose to xylitol. Fermentations carried out with non-adapted cells had the purpose to evaluate the behavior of the yeast against different

hydrolysate concentration factors. According to the results in Fig. 1, xylose assimilation occurred from the first hours and a similar consumption profile was observed in all adaptation conditions evaluated. However, it is interesting to observe the favoring of xylose consumption in H2N (two-fold concentrated and non-treated) and H5 (five-fold concentrated and treated) hydrolysates (Figs. 1a and 1e, respectively) which had the highest content of total phenolic compounds, that are known inhibitors of the fermentative activity of the studied yeast [11]. The favoring of xylose consumption after cell adaptation is supported by an 22.5% increase in Q_{XS} that varied from 0.40 (non-adapted cells) to 0.49 $\text{g L}^{-1} \text{h}^{-1}$ (adapted cells) in the cultivations with H2N hydrolysate (medium with higher inhibitors concentrations, Table 1). Similarly, a 10.4% increase in this parameter also was found (Fig. 1e) in the five-fold concentrated hydrolysate and treated (H5). In the fermentations with more concentrated hydrolysate (H5) a complete consumption of xylose of the medium was observed after 96 h of cultivation. In contrast, when non-adapted cells were used as inoculum there was a negligent consumption of xylose (70.1%) after 96 h (Fig. 1e). In regard to the H2N hydrolysate, the adapted cells promoted a 96.9% xylose consumption in 60 h of fermentation, corresponding to an increase of 15.6% in relation to the consumption by the non-adapted cells (Fig. 1a). These results indicate that short-term adaptation may be a technique capable of helping microorganisms to overcome toxicity caused by high concentrations of toxic compounds found in hemicellulosic hydrolysates (Table 1).

Corroborating with the results found in the present study, Tomás-Pejó and Olsson (2015) [31] described an increase in xylose consumption by a recombinant strain of *Saccharomyces cerevisiae* when short-term adaptation in wheat straw hydrolysate was performed. The authors observed after 120h fermentation of wheat straw hydrolysate containing 4.25 g L^{-1} acetic acid, 0.65 g L^{-1} 5-HMF, 3.85 g L^{-1} furfural and 0.025 g L^{-1} vanillin, a xylose consumption between 40 and 98%, respectively, while the consumption of this pentose by the non-adapted yeast did not occur. van Dijk et al. (2019) [25] also reported the increase of xylose consumption by a recombinant *Saccharomyces cerevisiae* strain in fermentations using wheat straw hydrolysate containing about 3.76 g L^{-1} acetic acid, 0.48 g L^{-1} 5-HMF, and 2.4 g L^{-1} furfural. After 48 h fermentation, the authors observed a xylose consumption of 46% by the adapted cells, while the consumption by non-adapted cells was only 22%. Zhang et al. (2019) [32] observed the highest consumption of xylose by cells adapted to sweet sorghum hydrolysate. After 24 h cultivation more than 80% of the xylose had been consumed by adapted recombinant *S. cerevisiae*, while non-adapted cells consumed only 45%. In another study, an increase in the total xylose consumption (from 26.3 to 62.7 %), was also observed for *Escherichia coli* adapted in increasing concentrations of kenaf hydrolysate [33].

Positive effect of short-term adaptation at the beginning of fermentation was also found in this work. In the first 12 h fermentation, an increase in the assimilation of xylose was observed, especially in the hydrolysates with higher levels of toxic compounds (H2N and H5). The greater tolerance to inhibitors promoted by the cell adaptation to the hydrolysate results in a faster and more complete xylose consumption [26]. In response to previous exposure to inhibitors, microorganisms reprogram their metabolic pathways and regulatory machineries, developing tolerant phenotypes to harmful

environments [34]. In this sense, adapted strains might induce stress response faster than non-adapted, allowing a faster development [19].

The importance of the detoxification stage was also evidenced in this study, since the consumption of xylose in H2N hydrolysate (two-fold concentrated and not treated) by non-adapted cells was 16.1% lower than the consumption in the H2 hydrolysate (two-fold concentrated and treated) at 60 h fermentation. For adapted cells, the decrease in xylose assimilation was only 2.17%, showing the benefit of adaptation strategy against the toxic effect of hydrolysates.

The profile of xylitol production followed a similar behavior to xylose consumption for all adaptation conditions evaluated. In addition, the production of this polyol by cells adapted to hydrolysates with higher toxic levels (H2N and H5) was clearly increased (Fig. 1a and 1e). The results showed an increase in xylitol production of about 58.7% (from 23.5 to 37.3 gL⁻¹) by cells adapted to H5 hydrolysate in 96 h fermentation, corresponding to a yield ($Y_{P/S}$) increase of 15.7% (0.51 to 0.59 gg⁻¹) (Fig. 2e) and a xylitol volumetric productivity (Q_P) increase of 62.5% (0.24 to 0.39 gL⁻¹h⁻¹) (Fig. 3e). With regard to H2N hydrolysate, the increase in xylitol production by the cell adaptation was of approximately 60.9% (from 6.4 to 10.3 gL⁻¹) in 60 h of fermentation, which led to an increase in both yield ($Y_{P/S}$: 29.6%) and productivity (Q_P : 54.5%) (Fig. 3a). No relevant difference was observed in these fermentation parameters in the cultivations with the hydrolysates H2, H3, and H4, making it clear that cell adaptation is not necessary when these hydrolysates are used, since the increase in $Y_{P/S}$ and Q_P was much lower (up to 13.5 %). This is probably due to the lower levels of toxic compounds in these hydrolysates in comparison to H2N and H5 hydrolysates (Table 1). According to Sene et al. (1998) [35], the greater the degree of adaptation of cells, the greater their capacity to metabolize toxic compounds. The production of this polyol by non-adapted cells in H2N hydrolysate fermentation in 60 h was 123.4% lower than that observed in H2 hydrolysate (Fig. 1a and 1b). For adapted cells, the reduction in xylitol production was only 17.5%. There was also a delay in the start of xylitol production when increasing the concentration factor of the hydrolysate (Fig. 1), possibly due to the increased toxic content (Table 1). However, the performance of adapted *C. guilliermondii* improved in H5 hydrolysate fermentation (Fig. 1e), coinciding with the highest final xylitol concentration, highest volumetric productivity, and maximum xylose consumption in 96 h of fermentation. Regarding the cell growth, there were no differences among the adaptation conditions evaluated (data not shown). Similar behavior was reported by Sene et al. (2001) [36] when adapting *C. guilliermondii* to increasing concentration factors of sugarcane bagasse hemicellulosic hydrolysate.

An improvement in xylitol production by cell adaptation was also observed by Shah et al. (2020) [33]. These authors carried out the adaptation of a recombinant strain of *E. coli* in increasing concentrations of kenaf hemicellulosic hydrolysate, and consequently of toxic compounds, observing at the end of the cultivation a two-fold increase in xylitol production and an improvement of 10.7 and 100% in the xylitol yield and volumetric productivity, respectively. Sene et al. (2001) [36] observed a 34% increase in xylitol production by *C. guilliermondii* adapted to sugarcane bagasse hemicellulosic hydrolysate four-fold

concentrated in comparison to non-concentrated hydrolysate. Wang et al. (2011) [37] observed that the number of propagation steps during cell adaptation in increasing concentrations of corn cob hemicellulosic hydrolysate is important to gradually improve the tolerance of *Candida tropicalis*, increase xylitol yield and decrease residual xylose concentration. Similar behavior was reported by Kim (2019) [34] when adapting *C. tropicalis* to empty palm fruit bunch fiber hydrolysate. Tomás-Pejó and Olsson (2015) [31] reported a 33.3% increase in ethanol yield when a recombinant strain of *S. cerevisiae* was adapted in wheat straw hydrolysate (23% vv⁻¹) in comparison to the adaptation in less concentrated hydrolysate (12% vv⁻¹). Silva et al. (2014) [38] observed an increase of 22 and 49% in ethanol yield and volumetric productivity, respectively, by *Scheffersomyces stipitis* adapted to sugarcane bagasse hemicellulosic hydrolysate. An 50% increase in lactic acid volumetric productivity was also observed when *Bacillus coagulans* was adapted to wheat straw hemicellulosic hydrolysate [39].

Based on the results of xylose consumption and xylitol production, it is possible to observe the existing correlation between the levels of toxic compounds in the hydrolysates and the need for cell adaptation. When comparing to non-adapted cells, adapted *C. guilliermondii* had a better performance in H2N and H5 hydrolysates, which contained higher inhibitors levels than in H2, H3, and H4 (Table 1). So, it is reasonable to assume that there is a need to adapt *C. guilliermondii* cells to increase xylitol production from hydrolysates, mainly in that ones with higher inhibitors levels. According to Sene et al. (1998) [35], the higher the concentration factor of the hydrolysate, and consequently of inhibitors levels, the greater the need for adaptation of the cells. Nouri et al. (2018) [40] observed in their study with concentrated and non-concentrated sugarcane bagasse hydrolysate, the importance of cell adaptation in face of the concentration factor of the hydrolysate. According to the authors, *Barnettozyma californica* adapted to the concentrated hydrolysate exhibited a highest increase in ethanol yield and productivity than in the non-concentrated hydrolysate, as well as in growth rate. The need for adaptation in face of increased toxicity was also observed for *S. stipitis* grown in semi-defined media containing glucose and xylose and increasing concentrations of acetic acid [41].

Regarding the consumption of glucose and arabinose, it was found that short-term adaptation favored both of them (data not shown). However, while glucose was completely consumed in the first 12 h cultivation, regardless of hydrolysate concentration factor, arabinose was slowly and partially consumed. Tomás-Pejó and Olsson (2015) [31] also observed the favoring of glucose consumption by an adapted *S. cerevisiae* recombinant strain. The yeast consumed all the glucose present in the wheat straw hydrolysate in less than 24 h, while the consumption of this hexose by non-adapted cells was partial. Sene et al. (2001, 1998) [35, 36] also observed a slow assimilation of arabinose by *C. guilliermondii* adapted in different concentration factors of sugarcane bagasse hemicellulosic hydrolysate. This behavior may be justified by the catabolite repression of arabinose assimilation caused by xylose. *Pichia guilliermondii*, the teleomorph form of *Candida guilliermondii*, and *Candida arabinofermentans* have two arabinose transportation systems: proton symport and facilitated diffusion. The first one has a high arabinose affinity, however it is strongly inhibited by xylose in the case of *C. arabinofermentans*, while for *P. guilliermondii* the presence of xylose leads to a reduction in arabinose transportation once the proton

symport system has similar affinities for both pentoses. In facilitated diffusion transport, although xylose does not cause inhibition, it has a low affinity by arabinose [42]. In another work, arabinose assimilation by *Kluyveromyces marxianus* and *P. guilliermondii* induced by arabinose transporters expressed in *S. cerevisiae* was strongly inhibited by xylose (75 and 100 %, respectively), indicating that this pentose is preferentially transported than arabinose [43]. The consumption of arabinose by some yeasts seems to be attached to the reduction of xylose levels in the medium, as this pentose can inhibit arabinose assimilation. In this sense, xylose consumption over the fermentation could relieve the repression in arabinose assimilation, favoring its use as observed in other studies [44, 45]. In this work, Q_{AS} was 250 % and 117 % increased by short-term adaptation in H2N and H5 hydrolysates, respectively. According to the results, the improvement in xylose assimilation may be the reason of the favoring of arabinose assimilation.

3.2 Effect of short-term adaptation on acetic acid consumption

In addition to sugars present in the hydrolysates, *C. guilliermondii* was able to completely consume acetic acid with a consequent increase in the medium pH (Supplementary Fig. S1). Similar behavior was reported for this yeast in semi-defined media [17] and sugarcane bagasse [18], barley straw [12], and rapeseed straw [9] hemicellulosic hydrolysates. The complete consumption of acetic acid occurred regardless the concentration factor of hydrolysates and acid concentration in the medium, as well as the cell adaptation. There were no changes in the assimilation profile of acetic acid due to the adaptation and the depletion of this acid occurred together with that of xylose (Fig. 1) for the all hydrolysates used in this study.

3.3 Effect of short-term adaptation on ethanol and glycerol production

The formation of by-products such as ethanol and glycerol were observed during fermentations, regardless the concentration factor of the hydrolysate and yeast adaptation (Fig. 4). Ethanol formation occurred from the first fermentation hours, with its maximum production between 12 and 24 h fermentation. The highest ethanol concentration (2.14 g L^{-1}) was verified in 12 h in the hydrolysate with the highest toxic level (H5). This higher ethanol production is related to the increase of glucose concentration in the hydrolysates, as demonstrated by *C. tropicalis* in synthetic medium containing xylose and increasing concentrations of glucose [46, 47]. Sene et al. (2001) [36] also reported an increase in ethanol formation by *C. guilliermondii* adapted to increasing concentrations of sugarcane bagasse hydrolysate. An increase of 40% in ethanol production was found with cells adapted to the four-fold concentrated hydrolysates in comparison to the not concentrated one. In addition, the production of ethanol coincides with the period of glucose consumption (first 12 h of fermentation - data not shown). Consumption of ethanol over fermentation time was also observed. Likewise, there was production of glycerol in the first hours of fermentation in all cultivation conditions, especially in hydrolysates with higher concentrations of toxics. Maximum glycerol production (2.52 g L^{-1}) was verified for non-adapted

cells in 60 h fermentation in the media based on H2N hydrolysate (Fig. 4a). There is a tendency of increasing glycerol generation by non-adapted cells due to the increase of the concentration factor of the hydrolysates and consequently of inhibitors. The increase in glycerol production due to the increase in the concentration of toxic compounds in hydrolysates has been reported by Zhang et al. (2019) [32]. It is worth to highlight the lower production of this by-product by adapted cells, when compared to non-adapted, notably in the fermentations of hydrolysates with higher concentrations of toxic substances. A decrease of approximately 300% in glycerol production was observed by cells adapted to the hydrolysate H2N after 60 h fermentation (Fig. 4a), while a decrease of 102% was observed for the hydrolysate H5 after 96 h fermentation (Fig. 4e). Even with the increased toxicity of the hydrolysates, the production of glycerol by adapted cells was still lower. Unlike ethanol, glycerol was not consumed by the cells, thus accumulating in the medium throughout the entire fermentation.

Glycerol is a compatible solute typically produced by yeasts under stress conditions. The lower glycerol formation by adapted cells indicates that short-term adaptation made *C. guilliermondii* more tolerant to the toxic compounds present in the hydrolysates, promoting a decrease in its toxic effects on cell metabolism and favoring fermentative performance. According to Nevoigt and Stahl (1997) [48], in addition to cell osmoregulation, glycerol generation plays an important role in the regeneration of NAD⁺ and maintenance of the cell redox balance. Zhang et al. (2019) [32] reported a higher glycerol production by the recombinant strain of *S. cerevisiae* non-adapted to sweet sorghum hydrolysate than by the adapted yeast. This byproduct was produced in highest amounts by the yeast in fermentations of hydrolysates of greater toxicity, corroborating to the relationship between increased cellular stress and glycerol production. Narayanan et al. (2016) [49] and Sánchez I Nogué et al. (2013) [50] also observed a decrease in glycerol production by adapted cells of *S. cerevisiae* in a semi-defined medium containing inhibitors usually found in hydrolysates.

4. Conclusions

This work evidenced the short-term adaptation as a technique capable to improve *C. guilliermondii* FTI 20037 tolerance to the inhibitors present in sugarcane bagasse hemicellulosic hydrolysate. Results showed the enhancement of xylose assimilation and xylitol production as well as the reduction of glycerol formation, a cellular stress response provoked mainly by the presence of phenolic compounds. This is a simple, promising, and inexpensive technique that could be applied to other microorganisms to overcome the hydrolysates toxicity, enabling the commercial use of non-recombinant strains for the industrial scale production of high value-added products from hydrolysates.

5. Declarations

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Conflict of interest

The authors declare no competing interests.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available in the Engineering School of Lorena – University of São Paulo (EEL-USP) repository, [<http://sistemas.eel.usp.br/bibliotecas/antigas/2004/BIT04003OCR.pdf>].

Code availability

Not applicable.

Author's contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Maria das Graças de Almeida Felipe. The first draft of the manuscript was written by Italo de Andrade Bianchini and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent

Informed consent was obtained from all individual participants included in the study

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Figures

Figure 1

Xylose assimilation (circles) and xylitol production (triangles) by non-adapted (open color) and adapted (closed color) in fermentations of sugarcane bagasse hemicellulosic hydrolysates. (a) H2N [two-fold concentrated and non-treated], (b) H2, (c) H3, (d) H4 e (e) H5 [concentrated and treated hydrolysates]

Figure 2

Xylitol yield over fermentation time of sugarcane bagasse hemicellulosic hydrolysates. (a) H2N [two-fold concentrated and non-treated], (b) H2, (c) H3, (d) H4 and (e) H5 [concentrated and treated hydrolysates] by non-adapted (light bars) and adapted (dark bars) *C. guilliermondii*

Figure 3

Xylitol volumetric productivity in fermentations of sugarcane bagasse hemicellulosic hydrolysates. (a) H2N [two-fold concentrated and non-treated], (b) H2, (c) H3, (d) H4 and (e) H5 [concentrated and treated hydrolysates] by non-adapted (light bars) and adapted (dark bars) *C. guilliermondii*

Figure 4

Ethanol (triangles) and glycerol (circles) production by non-adapted (open color) and adapted (closed color) in fermentations of sugarcane bagasse hemicellulosic hydrolysates. (a) H2N [two-fold concentrated and non-treated], (b) H2, (c) H3, (d) H4 and (e) H5 [concentrated and treated hydrolysates]

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