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Short Communication

Biochemical conversion of sugarcane straw hemicellulosic hydrolyzate supplemented with co-substrates for xylitol production

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HIGHLIGHTS

- Biotechnological production of xylitol is a valuable route for using sugarcane straw.
- Co-substrate utilization is a strategy to improve xylitol production.
- Sucrose supplementation improved xylose consumption and xylitol production.
- Use of sugarcane molasses could favor bioprocess feasibility.

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ABSTRACT

Biotechnological production of xylitol is an attractive route to add value to a sugarcane biorefinery, through utilization of the hemicellulosic fraction of sugarcane straw, whose availability is increasing in Brazil. Herein, supplementation of the sugarcane straw hemicellulosic hydrolyzate (xylose 57 g L^{-1}) with maltose, sucrose, cellobiose or glycerol was proposed, and their effect as co-substrates on xylitol production by *Candida guilliermondii* FTI 20037 was studied. Sucrose (10 g L^{-1}) and glycerol (0.7 g L^{-1}) supplementation led to significant increase of 8.88% and 6.86% on xylose uptake rate (1.11 g L^{-1} h^{-1} and 1.09 g L^{-1}), respectively, but only with sucrose, significant increments of 12.88% and 8.69% on final xylitol concentration (36.11 g L^{-1}) and volumetric productivity (0.75 g L^{-1} h^{-1}), respectively, were achieved. Based on these results, utilization of complex sources of sucrose, derived from agro-industries, as nutritional supplementation for xylitol production can be proposed as a strategy for improving the yeast performance and reducing the cost of this bioprocess by replacing more expensive nutrients.

1. Introduction

Development of sustainable technologies for integral utilization of lignocellulosic biomass in thermochemical and biochemical processes to produce bioenergy and high-value chemicals in a biorefinery, under economic, social and environmental sustainability, is a major challenge for a transition to a bio-based economy (Ghatak, 2011; Lago et al., 2012). A sugarcane-based biorefinery is a suitable option through integral utilization of the lignocellulosic byproducts, bagasse and straw (tops, dry and green leaves) (Lago et al., 2012). Differently from sugarcane bagasse, which is widely used in energy production, sugarcane straw is becoming an available lignocellulosic biomass due to the progressive introduction of the non-burning harvest in Brazil, which aims to improve the crop sustainability (Leal et al., 2013). Besides the agronomic benefits of keeping this biomass in the field, sugarcane straw can be used as feedstock in a future sugarcane biorefinery (Lago et al., 2012; Leal et al., 2013).

Although bioenergy production has been suggested as the main use for sugarcane straw (Leal et al., 2013), alternative bioprocesses can be proposed mainly for conversion of the pentoses into high-value products and valorization of its hemicellulosic fraction, as xylitol production, which can replace the chemical process of commercial production (Silva and Chandel, 2012). Xylitol is a sugar-alcohol employed in food and pharmaceutical industries, with a growing market (Silva and Chandel, 2012) and one of the top valuable chemicals that can support economically and technically the production of low-value biofuels in an integrated biorefinery (Werpy and Peterson, 2004).

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Biotechnological production of xylitol is based on xylose metabolism in pentose-assimilating yeast, in which NAD(P)H-depending xylose reductase (XR) reduces xylose to xylitol, followed by oxidation of xylitol to xylulose catalyzed by NAD^+-depending xylitol dehydrogenase (XDH) (Granström et al., 2007). Xylitol accumulation is promoted by a restriction on oxygen availability, condition in which XDH activity is limited due to a NADH/NAD^+ redox imbalance, resulting in the reduction of xylulose formation (Granström et al., 2007). As a result, carbon flux through the central metabolic pathways is reduced (Kim et al., 1999; Granström et al., 2007), particularly through PPP, which is the main NADPH-producing pathway (Bruinenberg et al., 1983). Consequently, NADPH regeneration becomes an important challenge in xylitol production in yeasts whose XR is exclusively dependent on NADPH, such as *Candida guilliermondii* (Granström et al., 2007).

A strategy to overcome this issue is the utilization of co-substrates, since simultaneous metabolism of xylose and a co-substrate can maintain continuous regeneration of NADPH and meet the demand for energy and carbon intermediates for cell growth and maintenance that is insufficiently fulfilled by xylose uptake under oxygen-limited conditions, improving xylitol production (Kim et al., 1999; Tamburini et al., 2010). Other carbon sources in hemicellulosic hydrolyzates besides xylose, mainly glucose and arabinose, are not suitable co-substrates because of the catabolic repression of xylose metabolism in the case of the hexose (Kim et al., 1999; Tamburini et al., 2010), and the poor assimilation of arabinose (Silva and Felipe, 2006). Therefore, the supplementation of the hemicellulosic hydrolyzate with complementary carbon sources is a promissory strategy to improve xylitol production, which also can benefit the bioprocess sustainability since co-substrates can be part of complex materials, such as byproducts from agro-industries, which can replace more expensive nutrients (Tamburini et al., 2010).

Nonetheless, to the best of our knowledge, the effect of co-substrates utilization on xylitol production has not been evaluated using hemicellulosic hydrolyzates as fermentation medium, but defined media with a native strain of *Candida tropicalis* (Tamburini et al., 2010) and mainly recombinant yeasts, such as *C. tropicalis* BSXDH-3 (Ko et al., 2006), *Debaryomyces hansenii* DBX 11 (Pal et al., 2013) and *Kluyveromyces marxianus* YZJ015 (Zhang et al., 2014).

In the present work, a biochemical route for xylitol production by *C. guilliermondii* FTI 20037 from sugarcane straw hemicellulosic hydrolyzate supplemented with co-substrates was proposed. Compounds studied as co-substrates were maltose, sucrose, cellobiose or glycerol, which were selected based on the possibility of being obtained from agro-industrial byproducts, which could be integrated to biotechnological production of xylitol from sugarcane straw hemicellulosic hydrolyzate and replace more expensive nutrients.

2. Methods

2.1. Materials

Sugarcane straw was kindly provided by Usina Pederneiras, Tietê, São Paulo, Brazil. The structural composition was (% w/v): cellulose (31.70), hemicellulose (27.00), lignin (31.10) and ash (1.50), determined according to Gouveia et al. (2009). All chemicals were obtained from Vetec (Sigma–Aldrich, Brazil), unless otherwise stated.

2.2. Preparation of the hemicellulosic hydrolyzate

Dilute-acid hydrolysis of the sugarcane straw was performed with 1.0% (w/v) H_2SO_4 at 1:10 solid/liquid ratio in a 40-L steel reactor at 121 °C for 20 min. The sugarcane straw hemicellulosic hydrolyzate (SSHH) was filtered and concentrated under vacuum at 70 °C. Next, SSHH was detoxified by pH adjustment (initial pH 0.97) to 7.0 and 2.5 with CaO (commercial grade) and H_3PO_4, respectively, followed by treatment with 1.0% (w/v) activated charcoal (refined powder, Synth, Brazil) at 60 °C, 100 rpm for 30 min (Marton et al., 2006). After treatment, SSHH was autoclaved at 111 °C for 15 min to be used as fermentation medium.

2.3. Microorganism and inoculum preparation

C. guilliermondii FTI 20037 was preserved at 4 °C on malt extract agar (Difco, BD, France) slants. The cultivation medium used for inoculum preparation contained (g L^{-1}): xylose (30.0), rice bran extract (20.0), $(NH_4)_2SO_4$ (2.0) and $CaCl_2$ $2H_2O$ (0.1). A loopful of cells grown on malt extract agar was transferred to the cultivation medium (50 mL) in Erlenmeyer flasks (125 mL) and incubated in a rotary shaker (New Brunswick Scientific Inc., Edison, NJ, USA) at 30 °C, 200 rpm for 24 h. Later, the cells were recovered by centrifugation (2000*g*, for 20 min), rinsed twice with sterile distilled water and the cell pellet was re-suspended in an adequate volume of sterile distilled water to be used as an inoculum.

2.4. Xylitol production from sugarcane straw hemicellulosic hydrolyzate supplemented with co-substrates

Co-substrates (maltose, sucrose, cellobiose and glycerol) were individually supplemented to the fermentation medium (xylose 57.50 g L^{-1}, glucose 7.05 g L^{-1}, arabinose 9.54 g L^{-1}, acetic acid 2.38 g L^{-1}, 5-HMF 0.45 g L^{-1}, furfural 0.01 g L^{-1} and total phenolic compounds 2.19 g L^{-1}) to reach an initial concentration (g L^{-1}) of 5.0, 10.0 or 15.0 in the case of the disaccharides, and 0.7, 1.0 or 1.5 for glycerol. Medium without co-substrates was used as control. The same nutritional supplementation employed in inoculum preparation was used, except xylose. The initial pH was adjusted at 5.5 using 6 M NaOH solution and it was not controlled during experiments. Batch fermentations were carried out in 125-mL Erlenmeyer flasks containing 50 mL of fermentation medium. The initial cell biomass concentration in each flask was 1 g L^{-1}. The flasks were incubated in a rotary shaker at 30 °C, 200 rpm for 48 h.

2.5. Analytical methods

Xylose, glucose, arabinose, xylitol, ethanol, glycerol and acetic acid were determined by high-performance liquid chromatography (HPLC) (Shimadzu LC-10AD, Kyoto, Japan), using a refractive index detector and a Bio-Rad (Hercules, CA, USA) Aminex HPX-87H column, with 0.01 N H_2SO_4 as an eluent, at 45 °C and a flow rate of 0.6 mL min^{-1}. Furfural and 5-HMF were also determined by HPLC, employing an ultraviolet light detector (SPD-10A UV–VIS, Waters Corp., Milford, MA, USA), a RP-18 column (Hewlett-Packard, Palo Alto, CA, USA) with acetonitrile:water (1:8) and 10% acetic acid as an eluent, at 25 °C and a flow rate of 0.8 mL min^{-1}. Cell concentration was monitored by measuring absorbance at 600 nm (DU 640B spectrophotometer, Beckman Coulter, Brea, CA, USA) and calculated using a calibration curve previously established between absorbance and cell dry weight.

3. Results and discussion

Fermentation results regarding the effect of the supplementation of maltose, sucrose, cellobiose and glycerol to the SSHH on xylose consumption and xylitol production by *C. guilliermondii* FTI 20037 are summarized in Table 1. Regarding xylose consumption, significant increments on xylose uptake rate were obtained

when SSHH was supplemented with sucrose or glycerol ($p < 0.05$). As shown in Table 1, the maximum value in this parameter (1.11 ± 0.02 g L^{-1} h^{-1}) was achieved when sucrose (10.0 g L^{-1}) was used, which represented an increment of 8.88% compared with the control (1.02 ± 0.02 g L^{-1} h^{-1}) ($p < 0.05$). Based on these results, the duration of fermentation can be reduced by 5 h if the xylose uptake rate kept constant after 48 h with the supplementation of sucrose (10.0 g L^{-1}).

Significant increases on final xylitol concentration and volumetric productivity were achieved only when SSHH was supplemented with sucrose (10 g L^{-1}). Table 1 shows that the maximum values of these parameters (36.11 ± 0.83 g L^{-1} and 0.75 ± 0.02 g L^{-1} h^{-1}, respectively) represented increments of 12.88% and 8.69%, respectively, relative to the control (31.99 ± 0.21 g L^{-1} and 0.69 ± 0.01 g L^{-1} h^{-1}, respectively). The beneficial effect of supplementation of SSHH with sucrose was not evidenced on fermentation efficiency (Table 1), since significant differences with the control or the other experiments were not observed ($p > 0.05$). Based on these results, it is possible to suggest that the xylitol production was enhanced due to a higher production rate, coherent with higher xylose consumption rate when SSHH was supplemented with sucrose (10 g L^{-1}).

Regarding net cell biomass production (data not shown), only in three conditions the value of this parameter was significantly higher than the one obtained in the control (5.70 ± 0.12 g L^{-1}): with 15 g L^{-1} of maltose (7.22 ± 0.63 g L^{-1}), 10 g L^{-1} of sucrose (6.94 ± 0.07 g L^{-1}) and 15 g L^{-1} of cellobiose (6.81 ± 0.74 g L^{-1}), which are 26.67%, 21.75% and 19.47% higher than the control ($p < 0.05$), respectively.

To the best of our knowledge, this is the first study addressing the biotechnological production of xylitol from sugarcane straw hemicellulosic hydrolyzate supplemented with co-substrates; therefore, it is not possible to compare the results achieved with other obtained in similar fermentation medium and conditions. Furthermore, studies focused on the influence of co-substrates on this bioprocess have been performed mainly with recombinant yeasts in defined media, but not in hemicellulosic hydrolyzates as in this work (Ko et al., 2006; Pal et al., 2013; Zhang et al., 2014).

Results on consumption of the compounds studied as co-substrates are summarized in Table 2. Most of the supplemented sucrose was consumed between 8 h and 24 h of fermentation, after glucose exhaustion and simultaneously with xylose, regardless the concentration supplemented to the fermentation medium. On the other hand, maltose was mostly consumed during the first 8 h of fermentation, simultaneously with glucose and xylose uptake (Table 2). These results indicate that profiles of consumption of sucrose and maltose seem to be opposite, a fact which is similar to that already reported by Tamburini et al. (2010) in batch fermentation with *C. tropicalis* in a defined medium. Cellobiose consumption was observed from 8 h, after glucose exhaustion, and the time for its exhaustion depended on the concentration added, indicating similar uptake rates independent of the concentration.

The differences evidenced on consumption of the disaccharides are possibly related to the uptake mechanisms of each. According to Flores et al. (2000), in both *Saccharomyces* and non-*Saccharomyces* species, firstly sucrose is hydrolyzed by an extracellular enzyme and then the products, glucose and fructose, are transported to the interior of the cell. Antuña and Martínez-Anaya (1993) suggested that maltose is transported by a symport system and hydrolyzed intracellularly in *C. guilliermondii*. Same mechanism was suggested for cellobiose uptake in *Candida queiroziae* (Santos et al., 2011).

Considering both the results on consumption of the disaccharides and those regarding the effect of each one on yeast performance, it can be stated that the improvements in xylose consumption and xylitol production achieved with sucrose supplementation (10 g L^{-1}) are related to the simultaneous metabolism of xylose and also sucrose, which led to increase in cell growth. However, considering the fact that with maltose (15 g L^{-1}) and cellobiose (15 g L^{-1}) increments in cell growth were also obtained but not in xylose consumption and xylitol production, it is possible to attribute the improvement in these parameters when sucrose was supplemented to SSHH also to an increase on NADPH availability due to an increment of carbon flux through PPP. Furthermore, it can be supposed that the rapid sucrose consumption after glucose exhaustion had a more favorable impact on NADPH regeneration than the slow uptake of maltose or cellobiose after glucose depletion.

Non-consumption of glycerol when supplemented to SSHH was observed (Table 2), which is similar to that observed by Tamburini et al. (2010), with *C. tropicalis* in a defined medium. In this sense, Flores et al. (2000) indicated that in a condition of limited oxygen availability glycerol production is favored over its consumption as an alternative pathway to NAD^+ regeneration.

Results obtained in the present study indicate that sucrose was the most suitable co-substrate among the compounds evaluated

Table 1
Effect of co-substrate supplementation to sugarcane straw hemicellulosic hydrolyzate on xylose consumption and xylitol production by *C. guilliermondii* FTI 20037.

Co-substrate (g L^{-1})		Xylose uptake rate (g L^{-1} h^{-1})[a]	Final xylitol concentration (g L^{-1})	Xylitol volumetric productivity (g L^{-1} h^{-1})[b]	Fermentation efficiency (%)[c]
Control	–	1.02 ± 0.02	31.99 ± 0.21	0.67 ± 0.00	70.03 ± 1.03
Maltose	**5.0**	0.96 ± 0.03	29.35 ± 0.71	0.61 ± 0.01	68.66 ± 1.19
	10.0	0.91 ± 0.02	28.26 ± 1.20	0.59 ± 0.03	70.03 ± 4.49
	15.0	0.93 ± 0.01	26.06 ± 0.64	0.54 ± 0.01	63.51 ± 1.57
Sucrose	**5.0**	1.07 ± 0.02	32.73 ± 1.89	0.68 ± 0.04	63.17 ± 3.15
	10.0	1.11 ± 0.02	36.11 ± 0.83	0.75 ± 0.02	70.37 ± 2.59
	15.0	1.05 ± 0.02	32.64 ± 0.48	0.68 ± 0.01	66.94 ± 1.46
Cellobiose	**5.0**	0.95 ± 0.03	29.35 ± 0.62	0.61 ± 0.01	68.66 ± 3.15
	10.0	0.96 ± 0.01	30.16 ± 0.78	0.63 ± 0.02	67.28 ± 3.31
	15.0	0.95 ± 0.03	31.79 ± 1.32	0.66 ± 0.03	75.52 ± 2.14
Glycerol	**0.7**	1.09 ± 0.02	31.82 ± 0.82	0.66 ± 0.02	66.25 ± 2.97
	1.0	0.98 ± 0.03	31.76 ± 0.82	0.66 ± 0.02	72.09 ± 3.09
	1.5	1.08 ± 0.02	33.25 ± 2.05	0.69 ± 0.04	70.72 ± 6.21

Bold values are correspond to the initial concentration of the co-substrates in the fermentation medium.
[a] Xylose uptake rate was calculated as the slope of the plot of residual concentration of xylose against time.
[b] Xylitol volumetric productivity was calculated as the ratio between final xylitol concentration and time.
[c] Fermentation efficiency was calculated as the ratio between experimental and theoretical xylitol yield, which corresponds to 0.917 gg^{-1} according to Barbosa et al. (1988).

Table 2
Consumption of the co-substrates supplemented to the sugarcane straw hemicellulosic hydrolysate by *C. guilliermondii* FTI 20037.

Co-substrate	Concentration ($g\ L^{-1}$)	Co-substrate consumption (%)		
		8 h	24 h	48 h
Maltose	**5.0**	65.17 ± 2.79	100.0 ± 0.0	100.00 ± 0.0
	10.0	63.70 ± 0.56	100.00 ± 0.0	100.00 ± 0.0
	15.0	60.94 ± 1.24	83.90 ± 1.36	100.00 ± 0.0
Sucrose	**5.0**	33.21 ± 1.36	100.00 ± 0.0	100.00 ± 0.0
	10.0	22.96 ± 3.75	100.00 ± 0.0	100.00 ± 0.0
	15.0	8.11 ± 0.79	100.00 ± 0.0	100.00 ± 0.0
Cellobiose	**5.0**	1.38 ± 0.13	60.93 ± 1.61	100.00 ± 0.0
	10.0	7.99 ± 0.12	55.83 ± 1.13	69.79 ± 2.19
	15.0	5.39 ± 1.10	35.49 ± 4.37	52.91 ± 5.86
Glycerol	**0.7**	0	0	0
	1.0	0	0	0
	1.5	0	0	0

Bold values are correspond to the initial concentration of the co-substrates in the fermentation medium.

and its supplementation to SSHH rises as a promising strategy to improve xylitol production by *C. guilliermondii* FTI 20037. Furthermore, these results lead to the possibility of studying the utilization of complex sources of sucrose as supplementation of the hemicellulosic hydrolyzate, such as sugarcane molasses. This byproduct of the sugarcane agroindustry is rich in sucrose (25–40% w/v), nitrogenous compounds, inorganic salts and vitamins (Dai et al., 2015). Thus, xylitol bioproduction from SSHH can be coupled to sugarcane agroindustry processes in a biorefinery context, through utilization of the molasses as source of sucrose and nutrients for supplementation of the hemicellulosic hydrolysate.

4. Conclusion

Biochemical conversion of the hemicellulosic fraction of the sugarcane straw for xylitol production by *C. guilliermondii* FTI20037 was favored by the supplementation of the hemicellulosic hydrolyzate with sucrose. Simultaneous consumption of this co-substrate with xylose promoted significant improvements in xylose consumption and xylitol production. These results lead to the possibility of using sugarcane molasses as nutritional supplementation of the medium to supply sucrose as co-substrate and replace nutrients already used. This strategy can contribute with the feasibility of this bioprocess as an alternative to the commercial xylitol production by chemical process and as a valuable route within a biorefinery.

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