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Research Article

Biotechnological Production of Xylitol from Oil Palm Empty Fruit Bunches Hydrolysate

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Abstract: This study evaluated the biomass and xylitol production using Oil Palm Empty Fruit Bunches (OPEFB) hydrolysates. *Candida tropicalis* ATCC 13803, which produces xylitol, was cultivated in the synthetic media Yeast extract Peptone Xylose (YPX), Medium Minimum Xylose (MMX) and hemicellulosic Hydrolysates medium (HR). Biomass concentration was evaluated in the synthetic media to identify the best medium for the biomass production. Hemicellulosic hydrolysates were obtained by dilute acid hydrolysis of OPEFB. All fermentations were performed in 100 mL Erlenmeyer flasks containing 40 mL of medium with 20 g/L xylose, initial pH 5, 6, 119 rpm and 30°C for 72 h. During the fermentations the cellular concentration was determined spectrophotometrically by Optical Density (OD) at 620 nm and the kinetics parameters and xylitol production were evaluated. The best synthetic medium for biomass production was YPX with 2.52 g/L at 30 h. The xylitol yield and yield values for HR media were 0.41 g/g and 0.10 g/L.h, respectively.

Keywords: Acid hydrolysis, *Candida tropicalis*, fermentation, hydrolysate, oil palm empty fruit bunches, xylitol

INTRODUCTION

In the last few decades, there has been a growing interest for the use of lignocellulosic residues as a consequence of its low costs and high potential as raw materials for the production of biofuels and high value-added products (Escalante *et al*., 2010).

Colombia is the main producer of palm oil in Latin America and significant amounts of biomass wastes are also produced from this industry (Shafawati and Siddiquee, 2013). It has an annual production of 1'143,500 tons of oil palm, which produces a total of 1'660.074 tons of lignocellulosic residues. The main residue is known as Oil Palm Empty Fruit Bunches (OPEFB), is the fibrous mass left behind after separating the fruits (Shinoj *et al*., 2011). This residue has been an environmental problem because it must be burned or transformed into compost for its final disposition (Dishington, 2016).

Lignocellulosic residues consist mainly of a complex matrix of hemicellulose, cellulose and lignin. Thus, an acid or enzymatic hydrolysis allows to obtain low molecular weight compounds, including fermentable sugars such as xylose (Piñeros, 2014).

Xylose is the precursor of Xylitol, a naturally occurring five-carbon sugar alcohol, has applications in the pharmaceutical, food and odontological industries owing to its similar high sweetening power, but fewer calories, relative to sucrose (Mardawati *et al*., 2015).

Currently, xylitol is synthetized by a chemical process by the catalytic hydrogenation of xylose at high pressure and temperature. This process generates many by-products that hinder the separation and purification processes, whereby it is considered as an expensive, inefficient production process and environmentally unfriendly (Dasgupta *et al*., 2017). In the last few years, an effort has been made to produce xylitol biotechnologically using different yeast, bacteria and fungi (Mohamad *et al*., 2015).

Different studies have demonstrated the capability of *Candida guilliermondii*, *Candida tropicalis* and *Pichia guilliermondii* to produce xylitol from lignocellulosic hydrolysates by batch fermentations (Manjarres-Pinzón *et al*., 2016). Furthermore, there is widely evidence of the effect of different factors that could influence the yield, including: pH, residence time and xylose initial concentration (Mohamad *et al*., 2015).

The objective of this study was to evaluate the biotechnology xylitol production with *Candida tropicalis* ATCC 13803 using OPEFB hydrolysates.

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Additionally, different pre-culture medium were evaluated for the biomass production.

MATERIALS AND METHODS

Raw material: The OPEFB were collected from a local oil palm mill (Palmar del Oriente, Colombia). The OPEFB characterization was carried out in a previous study (Manjarres-Pinzón *et al*., 2017).

Acid hydrolysis: The dilute-acid hydrolysis of the OPEFB biomass was carried out in 500 mL Erlenmeyer flasks at 121°C, 20 psi for 30 min, with a solid/liquid ratio of 1:8 and aqueous H_2SO_4 solution of 2% (w/v). Solids were separated from the aqueous solution through filtration. Filtrate was stored at 4°C for further xylose quantification.

Yeast strain: *Candida tropicalis* ATCC 13803 was kept at 4°C on Sabouraud Dextrose Agar plates and sub-cultured at 30°C before each experiment. Two strains were evaluated, adapted and non-adapted. Non-adapted strain was obtain by transfera colony to a 100 mL Erlenmeyer flask containing 40 mL of YPG (yeast, peptone and glucose) medium. At the same time adapted strain was obtain by gradual substitution of glucose by xylose until cell growth was sufficiently obtained with 100% substitution of glucose by xylose (YPX medium-yeast, peptone, xylose).

Media and fermentation conditions: When the xylose-adapted strains were obtained, the flask-scale fermentations were performed in YPX medium, minimum xylose medium (MXM-xylose 1.5%, $(NH_4)_2SO_4$ 0.5%, KH_2PO_4 0.5% and sterile water) and OPEFB hydrolysates. Each one supplemented with an initial xylose concentration of 20 g/L. Fermentations were performed in 100 mL Erlenmeyer flasks containing 40 mL of medium with initial pH of 5.6. Erlenmeyer flasks were agitated at 119 rpm and incubated at 30°C for 72 h. Samples were taken periodically until the stationary phase was reached.

Analytical methods:

Cell growth: Cellular concentration was determined spectrophotometrically by Optical Density (OD) at 620 nm (Genesys 20, Thermo Scientific) and it was correlated with dry weight method.

Xylose and xylitol quantification: Xylose and xylitol were analyzed using a HPLC system (Shimadzu Prominence), with a RI detector; equipped with an Aminex HPX-87H (Biorad) column. Elution was carried out with Aqueous H_2SO_4 (0.005M) at a flow rate of 0.6 mL/min. Oven temperature was maintained at 65°C. Injection volume was 20 µL (Piñeros, 2014). Samples were prepared in duplicates and filtered through a 25 mm nylon membrane syringe filter (pore size 0.45 µm) before analysis.

All experiments were performed in triplicate and the data were analyzed with Statgraphics Plus 5.1.

RESULTS AND DISCUSSION

Effect of medium composition on xylitol and biomass production: No significant differences were observed in xylitol production for adapted and non-adapted strain in synthetic YPX and MMX medium (data not shown). These results are consistent with those reported by Manjarres-Pinzón *et al*. (2017), who concluded that the adaptation of the strain is not necessary to increase the production of xylitol. Furthermore, the production of xylitol by strains depends on other factors associated with fermentation conditions such as nitrogen source, temperature, pH and aeration (Dasgupta *et al*., 2017).

According to the previous results, non-adapted *Candida tropicalis* ATCC 13803 was chosen to study the effect of medium composition (YPX and MMX) on biomass production. Biomass and growing phases during fermentation can be observed in Fig. 1. YPX medium favored the kinetic behavior with the highest biomass concentration of 2.52 g/L at 30 h. Exponential growth phase in YPX media was17 h, while in MMX media was 48 h. Therefore, the YPX became an attractive medium for suitable biomass production. These results showed the importance of the yeast extract in the culture media and its effect on biomass production (Dasgupta *et al*., 2017). Some studies had shown that nitrogen is required not only for biomass production but also xylitol production; specifically the yeast extract has a significant effect on the production of this metabolite (Ling *et al*., 2011).

Xylose fermentation for xylitol production using non-adapted *Candida tropicalis* ATCC 13803innon-detoxified HR media is illustrated in Fig. 2. During the first 34 h xylose uptake was mainly for biomass production, after this time a significant increase in the production of xylitol was observed from 1.67 g/L to 5.5 g/L in 14 h and reaching a maximum concentration of

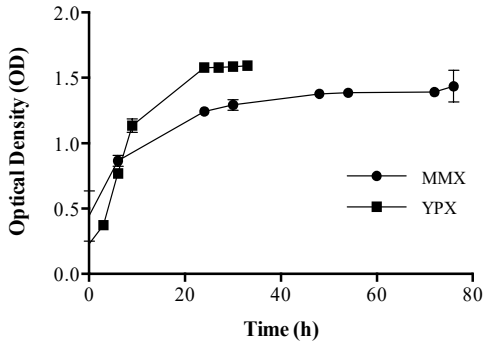

Fig. 1: Kinetic behaviour of *Candida tropicalis* in synthetic YPX and MMX medium

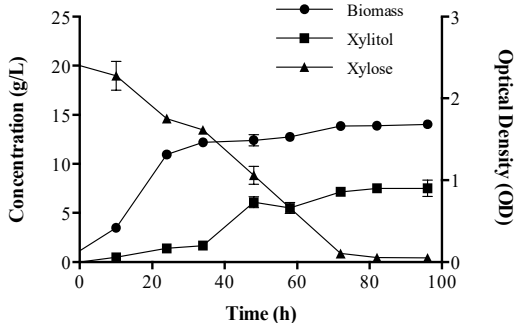

Fig. 2: Kinetic behavior of *Candida tropicalis* during xylitol production from non-detoxified Hydrolysate medium (HR) in Erlenmeyer flask for 96 h

Table 1: Fermentative parameters using HR medium

Parameters	HR
S_o (g/L)	20.00
S_f (g/L)	2.68
Fermentation time (h)	72.00
Xylose consumed (%)	86.60
P_f (g/L)	7.15
X_f (g/L)	3.40
Q_P (g/L-h)	0.10
$Y_{p/s}$ (g/g)	0.41
μ_{max} (h^{-1})	0.45

S_o: Initial xylose concentration; P_f: Maximum xylitol concentration; X_f: Maximum cell concentration; Q_P: Xylitol volumetric productivity; $Y_{P/S}$: Xylitol yield coefficient with respect to xylose

7.15 g/L at 72 h of fermentation. During the acid hydrolysis of OPEFB toxic compounds such as hydroxymethylfurfural, acetic acid and glucose are released (Manjarres-Pinzón *et al*., 2017). Inhibitors may affect mildly the yeast metabolic activity during the hydrolysate fermentation (Lenihan *et al*., 2010). Biomass and xylitol production could be higher with a detoxification process, such as overleaming, activated charcoal, pH adjustment or enzyme treatment, removing between a 4-95% of some of these inhibitory compounds (Mussatto and Roberto, 2004).

Xylitol production using *Candida tropicalis* ATCC 13803 in HR medium was obtained at the middle of the exponential growth phase, in contrast with other xylitol-producing strains, which produce this metabolite at the end of the exponential phase or even more in the stationary phase of growth (Mardawati *et al*., 2015; Lorliam *et al*., 2017). This characteristic could reduce fermentation time and costs in xylitol production.

Fermentative parameters, such as xylitol volumetric productivity (Q_P), cell mass yield coefficient ($Y_{X/S}$) and xylitol yield coefficient with respect to cell mass ($Y_{P/X}$), were calculated by linear regression of the values attained in the fermentative (Table 1).

Some studies have been reported xylose fermentation and xylitol production using OPEFB hydrolysate using *Candia tropicalis* and *Debaryomyces hansenii* ITB CC R85 (Mohamad *et al*., 2009; Kresnowati *et al*., 2016). The kinetic parameters of these studies showed low yield of fermentation with The Yp/s (g/g) values (0.098-0.24) of these studies were lower than those reported by our research (0.41)" However, it is difficult to compare kinetic parameters with other authors because the fermentation conditions were different. In our study *Candida tropicalis* ATCC 13803 was able to use xylose of OPEFB with a 86% conversion, while Kresnowati *et al*. (2016) have reported 58.4% with *Debaryomyceshansenii* ITB CC R85.

This study can be considered a starting point for future optimizations in terms of culture conditions, medium, pH, temperature, as well as in subsequent stages of scale up of the biotechnological production of xylitol using OPEFB hydrolysate and *Candida tropicalis* ATCC 13803.

CONCLUSION

The medium composition was important for biomass production, especially the source of nitrogen as the yeast extract, which take into account the xylitol production.

YPX is the best synthetic medium to be used for the production of biomass because showed the highest concentration in the shortest time.

Non-detoxified hydrolysate from OPFEB is a suitable media for xylitol production by non-adapted *Candida tropicalis* ATCC 13803 compared to the other synthetic media.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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