Research Article

Biotechnological Production of Xylitol from Oil Palm Empty Fruit Bunches Hydrolysate

1Manjarrés, Josefa K., 1Bravo, Alejandro, 1,2Arias, Juan P., 3Ortega, Isabel, 1Vásquez Andrés and 1Arias, Mario
1Facultad de Ciencias, Universidad Nacional de Colombia-Sede Medellín., Calle 59A #63 -02, 2Facultad de Ingeniería, Universidad de Antioquia., Calle 70 No. 52-21, 3Facultad de Minas, Universidad Nacional de Colombia-Sede Medellín., Calle 80 #65 -223, Colombia

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 valued-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 synthesized 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.
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$_2$SO$_4$ 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 transfer 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$_4$)$_2$SO$_4$ 0.5%, KH$_2$PO$_4$ 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$_2$SO$_4$ (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 was 17 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 13803 in non-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](image-url)
producing strains, which produce this metabolite at the end of the exponential phase or even more in the stationary phase of growth (Mardawati, 2016). The kinetic parameters of 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.

ACKNOWLEDGMENT

The authors would like to thank Palmar del Oriente, Colombia for provide the raw material and Colciencias for providing a grant to Katherine Manjarrés Pinzón.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES


