Optimization of xylitol production through *Candida Tropicalis* in xylose hydrolysate from rice husk

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Rice is the third primary crop most cultivated and harvested around the world with 8% and 0,8 billion tons of total primary crops in 2019 (FAO, 2021). It is cultivated in approximately 100 countries, making rice the stapple food for 3.5 billion people approximately (Lei & Yuan, 2019), this represents 70% of total world's population (Panesar & Kaur, 2016). Rice paddy is constituted by kernel 70%, husk 20%, bran 9%, and germ 1%, being rice husk the plentiest by-product of milling process (Fraterrigo Garofalo et al., 2020). In 2019, total production of rice paddy was 755.47 million tons (FAO, 2021) then annual production of rice husk was approximately 151 million tons, being an agricultural waste available in many rice producing countries.

Nowadays, in some regions, it's a common and fast practice to make combustion of rice husk to use it as fuel in milling process or field-burning as local fuel (Singh, 2018), it is estimated, 70% of rice husk is used through its burning. Nonetheless, hydrocarbons aromatics and dioxins emissions during burning can be toxic for human health (Abaide, et al., 2019), additionally it has been reported ash generated in combustion of rice husk, can have carcinogenic and bio-accumulative effects, resulting in expensive disposals to avoid its effects (Foo & Hameed, 2009). Another common practice is to dispose them in landfills, but, due to its high silica content and recalcitrant character, this material can be a source of contamination and diseases (Goodman, 2020). Despite its current uses, this by product is being economically underutilized, considering it is generated in massive quantities, its potential is being wasted.

Rice husk's lignocellulosic nature makes rice husks a source of carbohydrates, with an approximate composition of 40% cellulose, 19% hemicellulose and 16% lignin (Abaide, et al., 2019). The first two polymers promise economic benefits by converting them into fermentable sugars, because they would serve as platforms for different biotechnological processes and production of substances of higher economic value such as biofuels, food additives or biopolymers (Pedroso et al., 2019). Xylitol is one of them, it is an alcohol of five carbons used as sweetener, it has generated interest due to its properties as anticariogenic and its independent metabolism from insulin, so that, it is plenty used in food, pharmaceutical and dental industry (Venkateswar Rao et al., 2016), global market demand of xylitol in 2016 was of 190 thousands of tons and estimated to rise until 266 thousands of tons in 2022 (Trivedi et al., 2020), revealing a demand on growing of this compound. For that reason, xylitol is a suitable alternative of a high value metabolite to be produced through fermentation of rice husk hydrolysate. This study aims to produce xylose hydrolysate from rice husk hemicellulose by dilute acid hydrolysis, finding the best particle size for this pretreatment, thereupon, to optimize xylose fermentation in order to find the best local conditions to produce xylitol.

Methods and materials. Rice husk is milled to reduce particle size and then it is sieved to obtain two particle sizes, [0.25 0.6] mm, [0.6 1.2] mm and was evaluated unmilled rice husk; URH [0.2 - 0.8] mm, to obtain xylose as fermentable sugar and find which produces xylose the most through diluted acid hydrolysis. Conditions of hydrolysis were taken from (Cadavid et al., 2009) using sulphuric acid diluted at 4 % w/v. *Candida tropicalis* ATCC 1369 was adapted to xylose hydrolysate by a consecutive increase of hydrolysate to the medium for 48 hours each time, so that it was possible to avoid detoxification process, consequently, this would reduce costs making the process more sustainable, once inoculum was stablished with 150 rpm, pH of 5.5 and 30 °C, optimization of fermentation process was the next step to obtain xylitol, neutralizing and fortifying the hydrolysate. For dilute acid hydrolysis a randomized complete block design was used to evaluate the three particle sizes (one factor), and for optimization of xylitol production a central composite design (CCD) was executed, evaluating two factors: xylose initial concentration between 20 and 60 g/L and inoculum concentrations of 2 and 4 g/L, with xylitol concentration as response variable, 22 runs were completed and optimal point was found and verified by triplicate. Cell growth estimation was evaluated through cell dry weight correlating it with optical density at 600 nm (Manjarres pinzón et al., 2016). Xylose, glucose, xylitol, HMF and furfural concentrations were determined by High-performance liquid chromatography (HPLC).

Results and discussion. Cellulose content in rice husk is 36.66% ± 0.18% dw (dry weight) and hemicellulose content is 16.53% ± 0.81% dw. These represent more than 50 % of total biomass, being a good source of fermentable sugars once hydrolysis occurs.

For diluted acid hydrolysis, it was found there are significant differences in the treatments how figure 1 describes. Tuckey test was performed with a significance level of 0.05 to know which treatments are different, for xylose content with 0.6 mm -1.2 mm and URH does not have a significant difference, but 0.25 mm - 0.6 mm does. The best particle size is URH, because among the treatments it is the one who releases xylose the most, along with 0.6 mm -1.2 mm particle size. Nevertheless, URH doesn't require milling, generating operational ease and lower costs if the treatment is scaled. Moreover, for glucose, URH is the treatment that releases less glucose, allowing to conserve glucose in the biomass for later usage, creating the possibility of consecutive processes to produce other metabolites, the prospect of creating a biorefinery and highly valorizing this agricultural waste.

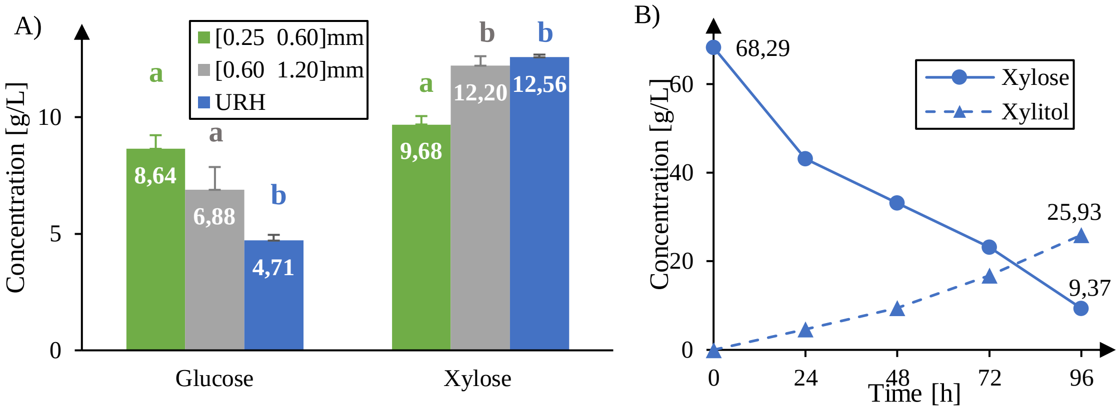

Figure 1. A. Concentration of glucose and xylose obtained in dilute acid hydrolysis with different particle sizes. Treatments with the same letter are not significantly different. B. Production of xylitol and consumption of xylose in optimal conditions.

After optimization process with CCD, the optimal production of xylitol occurs at 68 g/l of xylose and 4,32 g/L of inoculum, in figure 2 it is observed xylitol production in those conditions, while xylose is consumed. A product yield Yp/s of 0.440 g xylitol·g xylose^{-1} was obtained and a productivity Qp of 0.270 g xylitol·L^{-1}·h^{-1}, these results are higher than xylitol obtained by Rambo et al. (2013) with a yield of 0.4 g xylitol·g xylose^{-1} using *Candida guilliermondii* and 0.09 g xylitol·g xylose^{-1} using *Candida tropicalis*, in (Herazo et al., 2009) report a yield of 0.13 g xylitol·g xylose^{-1} with *Candida guilliermondii*.

Conclusion. Under the conditions studied and the tested yeast strain, an optimal production was found, demonstrating the potential of the strain as xylitol productor and its capacity of adaptation and tolerance in lignocellulosic hydrolysate, this allows to make evident the potential of rice husk as a source of fermentable sugars due to its composition and subsequent process of fermentation to produce high value metabolites, forming a possibility of valorization for rice husk.

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