

KINETIC BEHAVIOR OF ETHANOL PRODUCTION BY *Pichia stipitis* UNDER HIGH XYLOSE CONCENTRATION AND DIFFERENT K_La VALUES.

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Introduction. The pentose bioconversion to ethanol is a complex process influenced by several factors such as the cultivation conditions, fermentation medium, and the microorganism strain, among others. Two important aspects to be considered on ethanol production from xylose include the initial substrate concentration, which can affect the product yield; and the aeration, since its level determines the partitioning of the flux of carbon from substrate between growth and product formation (1). The present work evaluated the ethanol production by *Pichia stipitis* under high xylose concentration and

different oxygen transfer volumetric rates (K₁ a values).

Methodology. *Pichia stipitis* NRRL Y-7124 was the yeast employed for xylose conversion to ethanol. Inoculum was prepared in 500-mL Erlenmeyer flasks containing 100 mL of the following culture medium (g/L): xylose (30.0). glucose (5.0), arabinose (5.0); urea (2.3); MgSO₄.7H₂O (1.0); yeast extract (3.0). The flasks were incubated in rotary shaker at 30°C, 200 rpm, for 24 h. Fermentation medium was composed by (g/L): xylose (90.0), glucose (15.0), arabinose (15.0), yeast extract (3.0), MgSO₄.7H₂O (1.0) and urea (2.3). The assays were performed in a 1.6-L bioreactor, with 1.0 g/L initial cell concentration, at 30°C, under different oxygen conditions (0.5 to 1.5 vvm) and stirring rates (200 to 400 rpm) that when combined, resulted in K_La values: 2.3, 11.7, 18.7, 58.0, and 65.8 h⁻¹.

Results and discussion. The highest ethanol production was achieved when the fermentation assay performed with a K_La value of 2.3 h⁻¹. The K_La increase proportioned the yeast metabolism deviation from ethanol production to cell growth. As can be seen in Fig 1, the higher the oxygen availability to the medium, the higher the cell growth, and ethanol production was affected. Similar effect of the aeration was observed by Roberto et al. (2) during the xylose-to-ethanol conversion by Pichia stipitis CBS 5773 in stirred flasks. According to Taniguchi et al. (3) the xylose fermentation is strongly dependent of the oxygen level employed. Under anaerobic conditions. Pichia stipitis CBS 5773 consumed an insignificant xylose amount, being not able to produce ethanol. On the other hand, the excessive oxygen supply reduces the ethanol yield, both due to the product oxidation, as well as due to the high cellular growth.

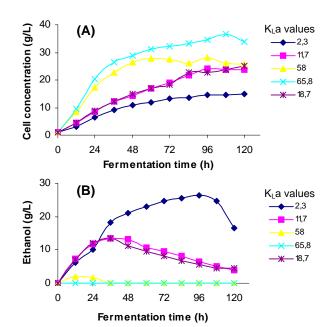


Fig. 1. Cellular growth (A) and ethanol production (B) by Pichia stipitis under high xylose concentration (90 g/L) and different K₁ a values.

Conclusions. The K_La importance on xylose-to-ethanol conversion by *Pichia stipitis* NRRL Y-7124 was demonstrated. Establishing a suitable aeration level is of great importance to achieve elevated conversion results.

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References.

- 1. Telli-Okur, M., Eken-Saraçoglu, N. (2008). Fermentation of sunflower seed hull hydrolysate to ethanol by *Pichia stipitis*. *Bioresource Technol*. 99: 2162-2169.
- 2. Roberto, I.C., Mancilha, I.M., Felipe, M.G.A., Silva, S.S., Sato, S. (1994). Influence of the aeration and pH on the xylose fermentation to ethanol by *Pichia stipitis*. *Arq. Biol. Tecnol.* 37: 55-63.
- 3. Taniguchi, M., Tohma, T., Itaya, T. Fujii, M. (1997). Ethanol production from a mixture of glucose and xylose by co-culture of *Pichia stipitis* and a respiratory-deficient mutant of *Saccharomyces cerevisiae*. *J. Ferment. Bioeng.* 83: 364-370.