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Kinetics of Xylitol Fermentation by *Candida guilliermondii* Grown on Rice Straw Hemicellulosic Hydrolysate

Inês C. Roberto,*[,1] Ismael M. de Mancilha,[1,3] and Sunao Sato[2]

[1]Department of Biotechnology, Faculty of Chemical Engineering of Lorena, 12600-000, Lorena, SP, Brazil, E-mail: ines@easygold.com.br; [2]Faculty of Pharmaceutical Sciences, University of São Paulo, Brazil; and [3]Food Science Department, Federal University of Viçosa, Brazil

Abstract

The fermentation kinetics for the conversion of rice straw hemicellulosic hydrolysate to xylitol by the yeast *Candida guilliermondii* was evaluated under batch conditions. The fermentation was accomplished in a 1 L working volume stirred-tank reactor with aeration of 1.3 vvm and agitation of 300 rpm ($k_L a$ = 15/h). The maximum specific rate of xylitol formation (0.12 g/g) was achieved when the specific growth rate was lowered to 1/5 of its highest value. From analysis of the fermentation kinetics, a linear correlation between specific growth rate (μ_x) and specific rate of xylitol formation (q_p) was evident. Based on the Gaden model, this bioprocess was classified as growth-associated production and the relationship between μ_x and q_p can be described by the equation $q_p = 6.31\ \mu_x$.

Index Entries: Rice straw; xylitol; fermentation kinetics; *Candida guilliermondii*.

Introduction

Xylitol is an economically important product used in the pharmaceutical, chemical, and food industries owing to its dietetic and anticariogenic properties. Traditionally, xylitol has been produced almost exclusively by chemical process, which involves an expensive step of purification of xylose and quite severe operational conditions. In recent years, attention has been focused on the biotechnological process, because it does not require initial xylose purification and is conducted under moderate temperature and atmospheric pressure. Studies on the utilization of hemicellulosic hydrolysates

*Author to whom all correspondence and reprint requests should be addressed.

of agricultural residues for this bioconversion have been carried out employing different yeast strains *(1–3)*. However, the viability of the biotechnological production of xylitol from lignocellulose hydrolysates is dependent on the type and concentration of inhibitors present in the medium *(4)*. Rice straw hemicellulosic hydrolysate, obtained by acid hydrolysis, has been effectively employed for this bioconversion owing to the low concentrations of toxic compounds generated throughout the chemical depolymerization *(5)*. Optimal conditions for xylitol production from this substrate, including pH value, medium composition, and inoculation parameters, have been previously investigated *(5–7)*. In the present work, we report the kinetic results of xylitol fermentation by *Candida guilliermondii* grown on rice straw hemicellulosic hydrolysate, under batch cultivation.

Materials and Methods

Microorganisms

C. guilliermondii FTI 20037, considered by Barbosa et al. *(8)* as a promising microbe for the conversion of xylose to xylitol, was used in the fermentation experiments. The stock culture was kept on malt-extract agar slants at 4°C.

Inoculum Preparation

The inoculum for the 1-L bioreactor was prepared from a suspension of cells cultivated in 125-mL Erlenmeyer flasks with 50 mL of synthetic medium composed of xylose (20.0 g/L), $(NH_4)_2SO_4$ (3.0 g/L), $CaCl_2$ $2H_2O$ (0.1 g/L) and rice-bran extract (20.0 g/L). The culture was incubated in a rotary shaker at 30°C for 24 h under agitation of 200 rpm. The cells were then collected by centrifugation (1500*g* for 10 min) and used to inoculate the fermentation medium to obtain an initial cell concentration of 1.0 g/L.

Medium and Fermentation Conditions

The fermentation runs were performed for 70 h in a 1-L Multigen stirred-tank reactor (New Brunswick, Scientific Co., Inc. Edison, NJ) containing 0.55 L of rice straw medium prepared according to Roberto et al. *(7)*. The fermenter was equipped with three six-bladed turbine impellers and operated under the following conditions: initial pH 5.4; air flow 1.3 vvm; agitation 300 rpm at 30°C. Aliquots of 3 mL were taken at different times for determination of sugars, xylitol, and biomass concentrations.

Kinetic Parameters

Specific growth rate (μ_x), sugar uptake rate (q_s) and specific rate of xylitol production (q_p) were determined by a computer program based on the method of Le Duy and Zajik *(9)*.

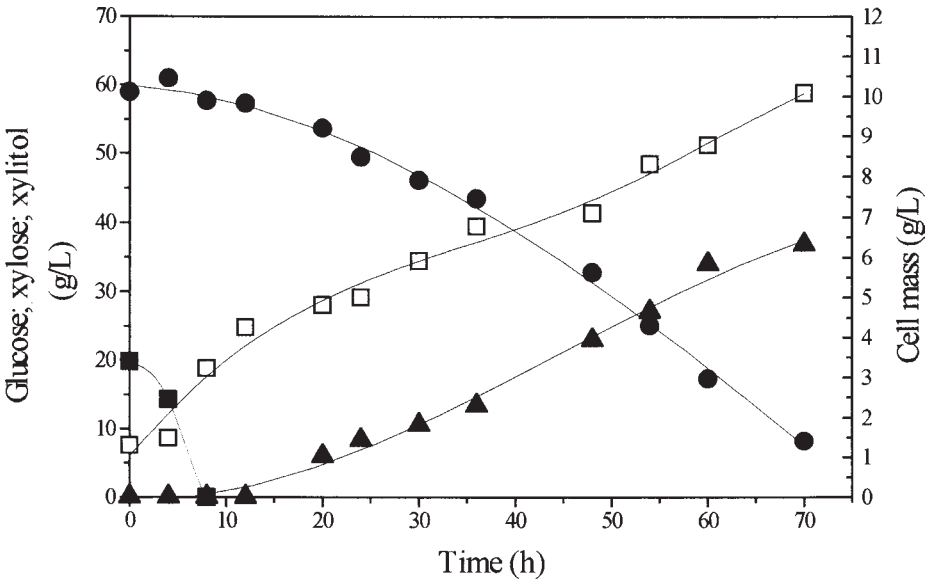

Fig. 1. Time of course of cell growth, xylitol production and sugar utilization during batch fermentation by *Candida guilliermondii* FTI 20037 in rice straw hemicellulose hydrolysate in a 1-L Multigen stirred-tank reactor (pH = 5.4; 30°C; 1.3 vvm and 300 rpm). ■, Glucose; ●, xylose; ▲, xylitol; and □, cell mass.

Analytical Methods

The sugar and xylitol concentrations were determined by high-performance liquid chromatography (HPLC) using a Hewlett-Packard HP 1082B chromatograph (Miami, FL) equipped with a Bio-Rad HPX-87H column (Hercules, CA) as previously described *(10)*. Cell concentration was estimated by means of a calibration curve (dry weight × optical density at 600 nm) prepared with cells grown on hydrolysate medium in a rotary shaker at 30°C for 48 h, under agitation of 200 rpm.

Results and Discussion

The kinetic behavior of the yeast *C. guilliermondii* grown on rice straw hemicellulosic hydrolysate was evaluated under batch cultivation. In Fig. 1, cell growth, xylitol production, and residual glucose and xylose concentrations are shown as a function of the cultivation time. *C. guilliermondii* preferentially metabolized glucose, which was completely depleted after 10 h of fermentation. During glucose consumption both xylose consumption and xylitol production were negligible. Cell growth rate was clearly higher for glucose than for xylose, indicating that glucose is a better substrate for cell growth than xylose. A similar pattern was found by Lee et al. *(11)* during the cultivation of *C. guilliermondii* in a medium containing glucose-xylose mixture. According to Preziosi-Belloy et al. *(1)*, the sequential sugar assimilation presents advantages over simultaneous assimilation, because

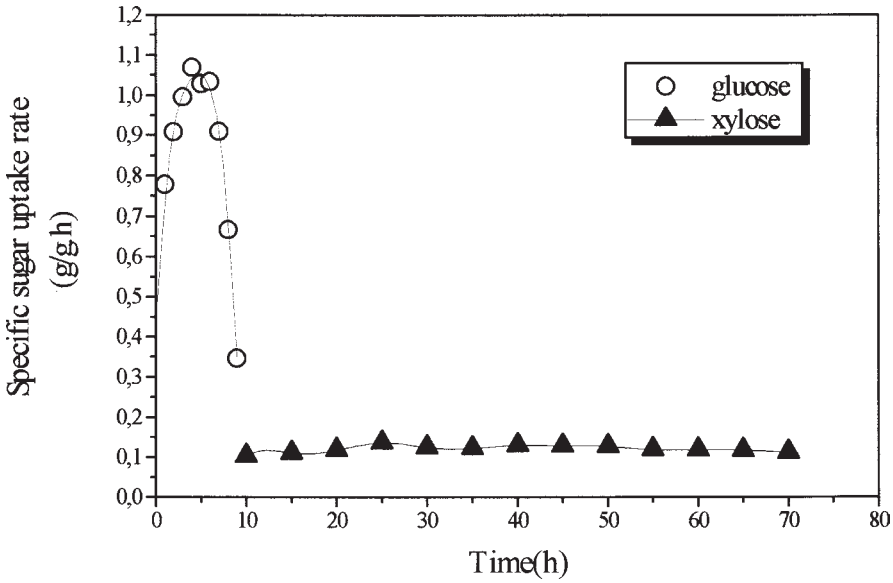

Fig. 2. Evolution of specific sugar-uptake rate in the course of batch fermentation of *Candida guilliermondii* FTI 20037 in rice straw hemicellulose hydrolysate.

by this way a greater part of xylose is available for xylitol production. These findings could partially explain the good performance exhibited by *C. guilliermondii* in fermentations conducted with rice straw hemicellulose hydrolysate, as observed in this work (xylitol yield of 0.73 g/g, corresponding to 80% of the theoretical yield).

The values of specific substrate-uptake rates in the course of the fermentation runs are presented in Fig. 2. The maximum specific xylose uptake rate (0.12 g/gh) was considerably lower than the maximum specific glucose-uptake rate (1.09 g/gh). These results are similar to those reported by Sugai et al. *(12)* using synthetic medium containing xylose or glucose as carbon sources. These authors obtained values of 1.07 g/gh and 0.10 g/gh for glucose and xylose, respectively. These results make it clear that *C. guilliermondii* can efficiently utilize a complex mixture of sugars derived from rice straw hydrolysate obtained by acid hydrolysis.

Figure 3 shows specific growth rates (μ_x) and specific rates of xylitol production (q_p) as a function of time. The maximum specific growth rate (μ_x = 0.15/h) was reached in the initial phase of the cultivation, owing to the fast use of glucose as a carbon source. When the xylose consumption began (after a period of 10 h), the specific growth rate gradually decreased. The maximum specific rate of xylitol production (0.12 g/gh) was achieved when the specific growth rate lowered to 1/5 of its highest value, after 15 h of fermentation. A similar value for maximum q_p (0.14 g/gh) was also reached at the beginning of xylose assimilation for *Candida parapsilosis* fermentation using semidefined medium *(1)*. However, in a wood hydrolysate fermentation by *C. guilliermondii*, a substantially lower specific rate of xylitol pro-

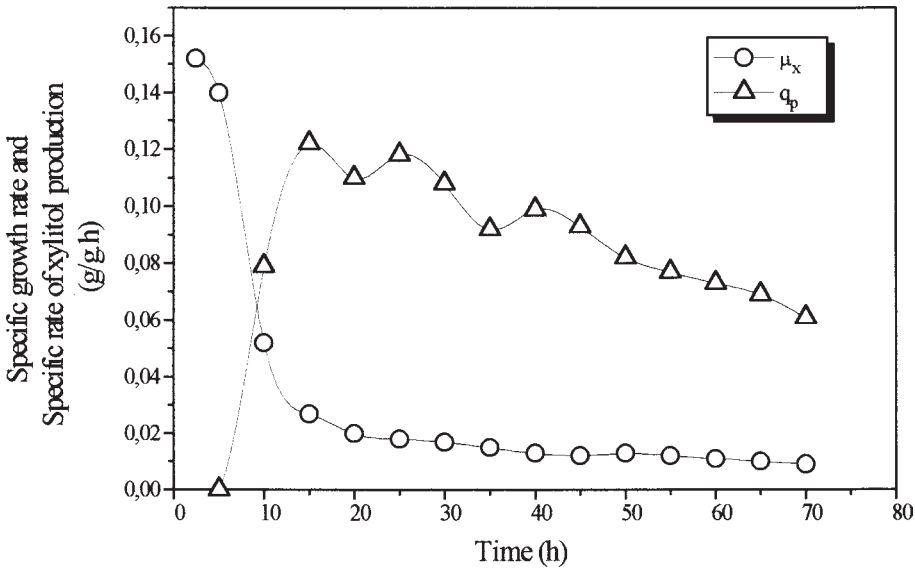

Fig. 3. Evolution of specific growth rate (μ_x) and specific rate of xylitol production (q_p) in the course of batch fermentation of *Candida guilliermondii* FTI 20037 in rice straw hemicellulose hydrolysate.

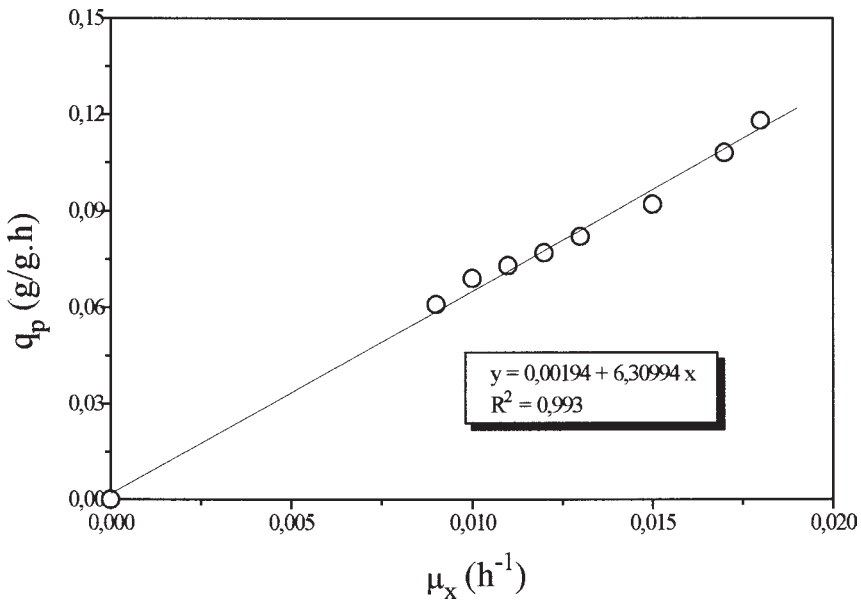

Fig. 4. Specific rates of xylitol production (q_p) as a function of specific growth rates (μ_x).

duction (0.01 g/gh) than that reported in the present work was attained *(13)*. The authors attributed these results to the presence of compounds such as furfural, acetic acid, and syringaldehyde in the hydroly-

sate, because they act as inhibitors of cell growth. This observation suggests that rice straw hemicellulose hydrolysate contains a lower level of toxicity.

Figure 3 also shows that, after 15 h of fermentation, the specific growth rates (μ_x) and the specific rates of xylitol production (q_p) decreased with time. This pattern indicates a dependence of xylitol production on cell growth, as confirmed by the linear relationship obtained between (μ_x) and (q_p) (Fig. 4). Because the linear coefficient of the equation was very low, this term was eliminated. Based on the Gaden model *(14)* this bioprocess can be classified as a growth-associated production and the relationship between μ_x and q_p can be described by the equation: $q_p = 6.31\ \mu_x$. This model is valid, because xylitol is an intermediary product of the xylose catabolism by yeasts. A similar kinetic behavior was also found for the xylitol production by *C. parapsilosis* yeast in synthetic medium *(15)*.

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References

1. Preziosi-Belloy, L., Nolleau, V., and Navarro, J. M. (1997), *Enzyme Microbial. Technol.* **21,** 124–129.
2. Parajó, J. C., Dominguez, H., and Dominguez, J. M. (1995), *Bioprocess Eng.* **13,** 125–131.
3. Dominguez, J. M., Gong, C. S., and Tsao, G. T. (1996), *Appl. Biochem. Biotechnol.* **57/58,** 49–56.
4. Parajó, J. C., Dominguez, H., and Dominguez, J. M. (1995), *Bioprocess Eng.* **16,** 39–43.
5. Roberto, I. C., Silva, S. S., Felipe, M. G. A., Sato, S., and Mancilha, I. M. (1996), *Appl. Biochem. Biotechnol.* **57/58,** 339–347.
6. Roberto, I. C., Sato, S., Mancilha, I. M., and Taqueda, M. E. S. (1995), *Biotechnol. Lett.* **17,** 1223–1228.
7. Roberto, I. C., Sato, S., and Mancilha, I. M. (1996), *J. Ind. Microbiol.* **16,** 348–350.
8. Barbosa, M. F. S., Medeiros, M. B., Mancilha, I. M., Schneider, H., and Lee, H., (1988), *J. Ind. Microbiol.* **3,** 241–251.
9. Le Duy, A. and Zajik, J. E. (1973), *Biotechnol. Bioeng.* **15,** 805–810.
10. Roberto, I. C., Felipe, M. G. A., Mancilha, I. M., Vitolo, M., Sato, S., and Silva, S. S. (1995), *Biores. Technol.* **51,** 255–257.
11. Lee, H., Sopher, C. R., and Yau, Y. F. (1996), *J. Chem. Technol. Biotechnol.* **66,** 375–379.
12. Sugai, J. K. and Delgenes, J. P. (1995), *J. Ind. Microbiol.* **14,** 46–51.
13. Nolleau, V., Preziosi-Belloy, L., Navarro, J. M., and Delgenes, J. P. (1995), *Proceedings of the 8th European Biomass Conference*. Chartier, P., Begnackers, C. M., and Grassi, G., eds., Pergamon, London, pp. 1411–1419.
14. Gaden, E. L., Jr. (1959), *J. Biochem. Microbiol. Technol. Eng.* **1,** 413–429.
15. Furlan, S. A., Bouilloud, P., and de Castro, H. F. (1994), *Process Biochem.* **29,** 657–662.