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Scientific Note

Effect of Culture Conditions on Xylitol Production by *Candida guilliermondii* FTI 20037

MARTIN J. PFEIFER,[2] SILVIO S. SILVA*,[1] MARIA G. A. FELIPE,[1] INÊS C. ROBERTO,[1] AND ISMAEL M. MANCILHA[1]

[1] *Faculdade de Engenharia Quìmica de Lorena-Centro de Biotecnologia e Quìmica-Rodovia Itajubá-Lorena, Lorena S.P. Brazil; and* [2] *Hochschule für Technik-Fachhochschule Mannheim-Fachbereich Biotechnologie, Mannheim, Germany*

Index Entries: *Candida guilliermondii*; fermentation; sugar cane bagasse; D-xylose, xylitol.

INTRODUCTION

Lignocellulosic materials like sugar cane bagasse include about 35% of hemicellulose *(1,2)*. Hemicelluloses consist of polymeric substances, such as xylans and glucomannans, which differ from cellulose in having shorter molecular chains, a homo- or heteropolymeric backbone structure, and branch molecules, like acetic acid and a variety of pentoses and hexoses *(3)*. The use of D-xylose, the main component of xylan, to obtain chemical products has been a challenge in wood chemistry for the last 10 years.

Much biotechnological research has already been carried out in order to achieve higher ethanol yields using hemicellulosic biomass *(4,5)*. However, these investigations have been disregarding the fact that xylitol, an economically more interesting product, can be obtained from D-xylose.

Xylitol is a five-carbon sugar alcohol that presents characteristics, such as sweetening power, comparable to sucrose and anticariogenic properties *(6)*. Furthermore, the direct human metabolism of xylitol is insulin-independent. Xylitol is therefore well suited as a substitute of sucrose in cases of diabetes *(7,8)*.

Current technologies in xylitol production are based on catalytic hydrogenation, with Raney-Nickel-catalysts *(9)*, and therefore demand a very pure xylose solution. The combination of dilute-acid hydrolysis that can be carried out efficiently at relatively low cost and a biotechnological hydrogenation process has significant advantages, since it does not require pure xylose solutions. Initially, xylose was regarded to be nonfermentable by yeasts *(10)*, but later xylose-fermenting yeasts were discovered *(5,11,12)*. The screening of various yeast strains by

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

Barbosa et al. *(13)* proved that the *Candida guilliermondii* FTI 20037 yeast has the ability to convert xylose to xylitol with high efficiency and the maximum theoretical yield of xylitol from D-xylose is 0.917 g/g *(13)*.

Even though the exact mechanism of xylitol formation in *C. guilliermondii* is not fully understood, fermentation parameters of the D-xylose-to-xylitol bioconversion need to be optimized in order to reach maximal xylitol productivity under microaerobic conditions.

This investigation was carried out to determine the effects of age of inoculum and carbon source in the growth medium for xylitol production. These are important factors that should help the development of a more efficient fermentation process.

MATERIALS AND METHODS

Microorganism

C. guilliermondii FTI 20037 was obtained from the Faculdade de Engenharia Quìmica de Lorena, Lorena, São Paulo, Brazil, and maintained at 4°C on malt extract agar slants.

Inocula and Culture Media

The growth inoculum and fermentation media had the following basic composition:

12 g/L yeast extract (Oxoid), 40–50 g/L of xylose, 10 g/L glucose, 2 g/L $(NH_4)_2SO_4$, 0.1 g/L $CaCl_2$.

The inoculum was prepared from one loopfull of cells from stock culture inoculated in 50 mL of inoculum medium of the above mentioned composition.

For the age of inoculum experiment, cultures were grown on mixed substrate medium (MS): glucose (20%) plus xylose (80%) with a total of 50 g/L of these sugars for 15, 24, 48, and 72 h.

For the carbon source experiment, cultures were grown on xylose, glucose, and MS medium. These media were also used in determining the specific growth rates.

For the fermentation of the sugar cane bagasse hemicellulosic hydrolysate, cultures were grown in two steps: first, in synthetic MS medium and then in a medium containing hydrolysate, which was prepared according to the method of Felipe et al. *(14)*.

The inoculum was grown in wool-stoppered 125-mL Erlenmeyer flasks at 200/min in an orbital shaker at 30°C for 24 h, unless otherwise stated.

Fermentation Conditions

The initial cell concentration in all fermentations was 1 g dry wt cells/L was used. The fermentations were carried out under the same conditions as the inoculations. The fermentation in the bioreactor was conducted in a 1-L fermenter (Multigen—New Brunswick Scientific Incorp, Edison, NJ) under the following conditions: 30°C, 300/min, using a flat-blade turbine and an oxygen supply of 0.8 vvm (volume of air per volume of medium per minute).

Analytical Methods

Xylose, glucose and xylitol were analyzed with a Shimadzu high-performance liquid chromatograph (HPLC, Kyoto, Japan), using a Bio-Rad (Hercules, Ca) Aminex

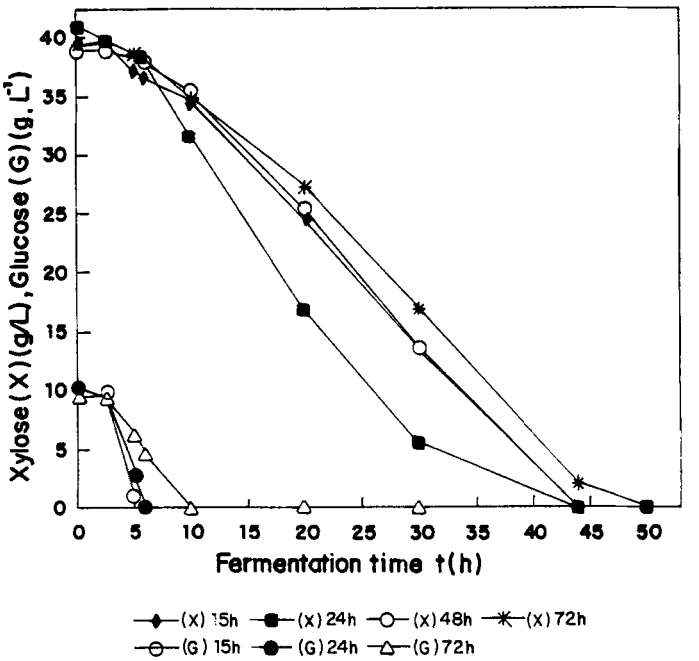

Fig. 1. Xylose (X) and glucose (G) consumption during batch fermentation with *C. guilliermondii* cultures of different ages.

HPX-87H column at 45°C and 0.02*N* H_2SO_4 as the eluent at a flowrate of 0.6 mL/min. Cell concentration was estimated by measuring absorbance at 600 nm. The relationship between absorbance and dry cell wt (g/L) was given by a standard curve (1 OD unit = 2.06 g dry wt cells/L).

RESULTS AND DISCUSSION

Effect of Age of the Inoculum on Xylitol Production

Substrates-to-product conversions can be characterized by the metabolic activity of the cells. In general, culture age is strongly related to this parameter. The results presented in Figs. 1 and 2 and Table 1 show to what extent age of inoculum influenced the fermentation of xylose to xylitol. According to Table 1, during the experiment, no substantial differences occurred in sugar (xylose and glucose) consumption or final xylitol concentration. The best results were achieved with a 24-h culture owing to more rapid fermentation, which ended after 30 h (Figs.1 and 2; Table 1). Probably, the 24-h culture used up most rapidly the NH_4^+ added to the medium, resulting in the liberation of H^+ in the medium. This may be related to the close relationship between growth and fermentation in this bioprocess.

An inoculum growth of 15 h appeared to be too short for effective fermentation (in the range of 48–72 h of growth) in comparison to 24 h of growth. A culture picked after that time still has not terminated all the metabolic changes necessary for the consumption of yeast extract and the fermentation of xylose.

The 72-h cultures in particular represented a stage of growth (Figs. 1 and 2; Table 1) in which the organisms do not have the same metabolic activity as in a

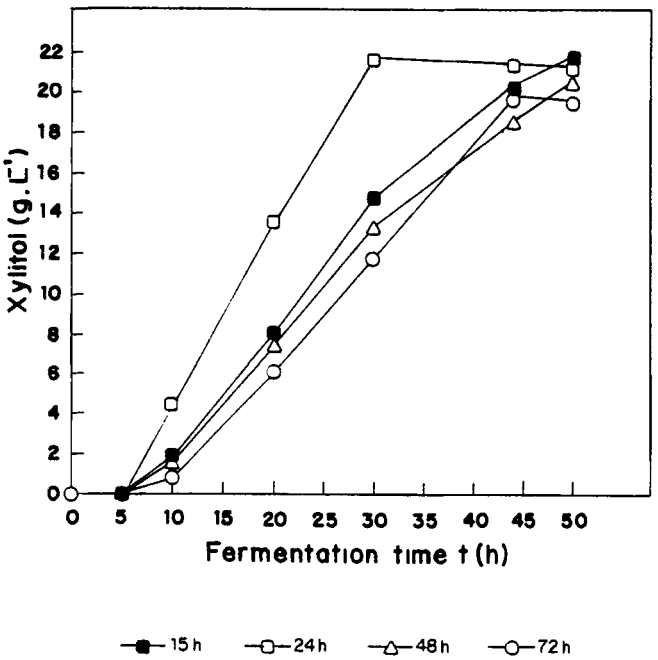

Fig. 2, Xylitol production during batch fermentation with *C. guilliermondii* FTI 20037 cultures of different ages.

Table 1
Effect of the Age of Inoculum on Xylitol Production

Age of inoculum h	[a]Final h	$Y_{P/S}$ $g \cdot g^{-1}$	δ $g \cdot L^{-1} \cdot h^{-1}$	η %	Final biomass $g \cdot L^{-1}$	ΔXOH $g \cdot L^{-1}$
15	44	0.56	0.50	61.0	17.53	21.92
24	30	0.53	0.66	57.7	18.56	21.50
48	44	0.53	0.49	58.2	18.30	20.60
72	50	0.50	0.39	55.2	17.01	19.80

[a]Final = time needed to consume at least 90% xylose, $Y_{P/S}$ = xylitol produced/xylose consumed, η = efficiency of fermentation, δ = volumetric productivity, ΔXOH = xylitol produced.

young culture. This may be the reason why the latter culture seemed to form xylitol later and was less productive, and consumed the substrate more slowly than the 24-h culture. Our observations agree with those of Sreenath et al. *(15)*, who reported that the metabolic activity of the cells changed appreciably as the cells aged.

Effect of Carbon Source in the Growth Inoculum on the Fermentation Performance

The choice of carbon source for the growth medium can be an important factor influencing the efficiency of a subsequent fermentation. Furthermore, mixed monosaccharide substrates can cause problems of biphase kinetics as reported by

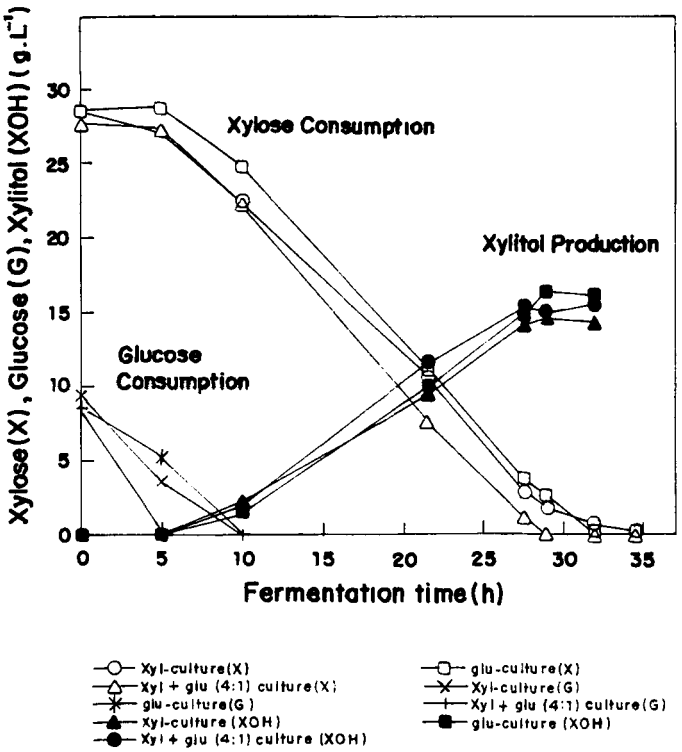

Fig. 3. Xylose (X) and glucose (G) consumption and xylitol (XOH) production during batch fermentation with *C. guilliermondii* cultures grown on xylose (xyl), glucose (glu), and mixed substrate (xyl + glu) medium.

Hsiao et al. *(16)*. According to Figs. 3 and 4 and Table 2, the results showed that the carbon source of the growth inoculum has an effect on the metabolism of *C. guilliermondii*. According to Fig. 3, the initial rate of xylose consumption was fastest in the medium with the xylose grown inoculum, followed by the MS medium grown inoculum and the glucose grown inoculum, which showed no initial xylose consumption at all. This suggests a catabolite repression *(17)* or catabolite inactivation *(18)* mechanism, similar to those reported by others *(16,19,20)*. Furthermore, the xylose consumption rate increases strongly when glucose enters low concentration ranges. This fact suggests that xylose reductase, the main enzyme in xylose metabolism, is reversibly inactivated by the presence of glucose at higher concentrations. This behavior has also been verified for *Pachysolen tannophilus* by Lee *(20)*.

As can be seen in Fig. 3, xylitol formation was hardly affected, since all the final concentrations were in the same range, althrough the glucose grown inoculum seemed to produce the highest final xylitol concentration. This is perhaps correlated to the problem of cofactor regeneration in a xylose grown inoculum, which uses xylitol for regeneration of NADPH under anaerobic conditions. Similar results have been obtained by Jeffries *(21)*, who found that the addition of glucose at low concentrations enhanced the product yield in xylose-to-ethanol fermentations. On the other hand, MS grown inoculum seemed to exhibit the highest xylitol productivity of 0.53 g/L/h, as shown in Table 2.

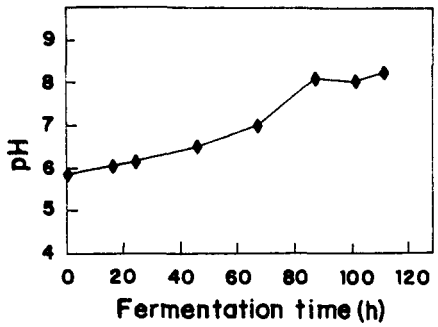

Fig. 4. Variation of pH during batch fermentation of sugar cane bagasse hemicellulosic hydrolysate by *C. guilliermondii* FTI 20037.

Table 2
Effect of Carbon Source in the Growth Inoculumon the Fermentation Performance

Composition of growth medium	[t]Final h	$Y_{P/S}$ $g \cdot g^{-1}$	δ $g \cdot L^{-1} \cdot h^{-1}$	η %	Final biomass $g \cdot L^{-1}$	ΔXOH $g \cdot L^{-1}$
Xylose	34	0.51	0.43	56.3	17.97	14.6
Glucose	32	0.57	0.51	62.9	18.49	16.4
MS medium	29	0.55	0.53	60.5	18.24	15.3

[t]Final = time needed to consume at least 90% xylose, $Y_{P/S}$ = xylitol produced/xylose consumed, η = efficiency of fermentation, δ = volumetric productivity, ΔXOH = xylitol produced.

The use of the carbon source (glucose) in the fermentation medium occurred without any adaptation time (Fig. 3), since all of the metabolic changes had already occurred during the growth phase in the growth inoculum of preliminary experiments. During this adaptation phase, no growth occurred but when the synthesis of all the necessary enzymes was completed, the growth seemed to enter the exponential phase directly.

The maximum specific growth rate (μ_{max}) was 0.04/h and 0.11/h in xylose and glucose medium, respectively. The different types of curves where perhaps owing to the rapid consumption of carbon source in the case of glucose and the slow, but constant consumption of xylose owing to the need for $NADPH_2$ as the reducer *(13)*.

Fermentation of Sugar Cane Bagasse Hemicellulosic Hydrolysate with *C. guilliermondii* FTI 20037

Fermentation in a complex medium, such as a hydrolysate, is always critical, since the hydrolysate still contains various substances that interfere with microbial metabolism. It is difficult to say in advance whether the organism will accept the mixture of nutrients, salts, and toxic substances, which are difficult to remove before conducting a fermentation. According to Figs. 4 and 5, the *C. guilliermondii* yeast was able to grow on a hydrolysate (initial pH: 5.86) still containing a variety of unknown substances and inhibitors, such as furfural, hydroxymethylfurfural, and acetic acid. The yeast fermented xylose at an uptake rate of 0.61 g/L/h, with productivity of 0.38 g/L/h and fermentation efficiency of 68.54%.

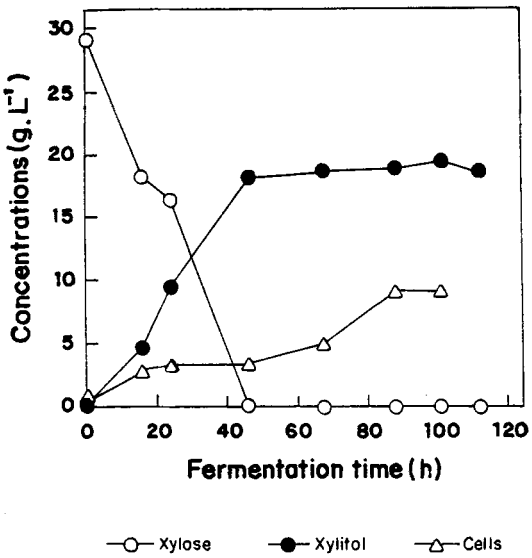

Fig. 5. Xylose consumption, xylitol production, and cell concentrations during batch fermentation of sugar cane bagasse hemicellulosic hydrolysate by *C. guilliermondii* FTI 20037.

The pH increased constantly, suggesting that the organism used the acetic acid present as a carbon source as well. According to the literature *(22,23)*, acetic acid at high concentrations strongly interferes with the energy metabolism of yeasts by reducing the H^+ gradient across the mitochondrial cell membrane used for ATP generation.

As shown by the results (Fig. 5), the hydrolysate did not inhibit the growth of *C. guilliermondii* significantly. Furthermore, the fermentation efficiency in this process was higher than in the foregoing fermentations. Probably, the organism uses carbon sources other than the sugars for growth and confirms the high adaptation ability of *C. guilliermondii* FTI 20037 to different media.

CONCLUSION

The demand for application of xylitol in food and pharmaceutical industries has created a strong market for low-cost xylitol production processes. The biotechnological approach for xylitol production appears to be efficient, and a high xylitol concentration can be obtained under controlled physiological conditions. The choice of a carbon source in the growth inoculum had only minor effects on the fermentation performance. Age of inoculum affected xylitol productivity considerably, but the final xylitol concentration was not influenced.

C. guilliermondii FTI 20037 yeast used in this bioprocess demonstrated high xylitol conversion rates, confirming its potential in sugar cane bagasse hemicellulosic hydrolysate fermentation.

Physiological parameters, such as age of inoculum and carbon source in the growth medium for xylitol formation by *Candida guilliermondii* FTI 20037, were investigated. Age of inoculum seemed to influence only the productivity and not the final xylitol concentration. The highest xylitol productivity (0.66 g/L/h) was reached using a 24-h culture. The specific growth rates (μ) in the different media

ranged from 0.04/h for xylose to 0.11/h for glucose, with intermediate values obtained for mixtures of these sugars. Cultivation of the inoculum using different carbon sources did not significantly influence fermentation performance. Batch fermentation was carried out in an 1-L fermenter using a sugar cane bagasse hydrolysate. The yeast was able to ferment this medium, producing 18.40 g/L xylitol from 29.50 g/L of xylose, at a production rate of 0.38 g/L/h. This lower value, compared to that of the synthetic medium, may be attributed to the various toxic substances that interfere with microbial metabolism (e.g., acetic acid).

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