0273-2289/99/77–79/0347/$12.00

Aspects of Xylitol Formation in Sugarcane Bagasse Hydrolysate by *Candida guilliermondii* in the Presence of Tetracycline

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Abstract

The bioconversion of xylose into xylitol using pH values of 4.0, 5.5, and 7.0 and tetracycline concentrations of 20 and 40 mg/L was carried out to verify the influence of these parameters on *Candida guilliermondii* metabolism for xylitol production. Experiments were performed with sugarcane bagasse hemicellulosic hydrolysate (48.5 g/L of xylose) in 125-mL Erlenmeyer flasks, at 30°C, 200 rpm, during 88 h. The results demostrated that the bioconversion of xylose into xylitol was significantly influenced by the pH. On the other hand, in media containing 20 or 40 mg/L of tetracycline, this bioconversion was not significantly affected. The best results of xylitol production were obtained in hemicellulosic hydrolysate without tetracycline, at pH 7.0. In these conditions, the maximum specific growth rate was 0.014/h and the yield factor of xylitol and volumetric productivity were 0.85 g/g and 0.70 g/L/h respectively. Xylitol and cell growth occurred simultaneously.

Index Entries: Hemicellulosic hydrolysate; sugarcane bagasse; xylose; xylitol; *Candida guilliermondii*; *Klebsiella pneumoniae*.

Introduction

Renewable resources in the form of agricultural residues have long been used as raw material by a wide variety of industries, such as pharmaceutical, food, odontological, pulping, and so on. Among the lignocellulosic materials, sugarcane bagasse have gained considerable attention as a

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alternative source for the production of many chemicals from economically and environmentally viable products. Xylose is present in the hemicellulosic fraction of the sugarcane bagasse, and through acid hydrolysis 80% of pentoses can be extracted, mainly xylose *(1)*. Xylose-xylitol bioconversion can be attained with different yeast strains, including *Candida tropicalis*, *Candida boidinii*, *Candida sp*., and *Kluyveromyces fragiles (2–6)*. One of these yeasts, *Candida guilliermondii* FTI 20037, has a high capacity to convert xylose into xylitol with an efficiency of 81% of the theoretical value in synthetic medium *(4)*.

Xylitol has received much attention for its applicability in pharmaceutical and food products as an alternative natural sweetener with anticariogenic properties *(7,8)*. Xylose-xylitol bioconversion can be carried out through batch, semi-continuous, and continuous fermentation processes *(9–11)*. Continuous cultivation usually provides a higher productivity than batch culture *(12)*. Nevertheless, the possibility of contamination is a disadvantage of the continuous fermentation. Bacterial contamination was observed during preliminary studies of continuous fermentation of sugarcane bagasse hemicellulosic hydrolysate using *C. guilliermondii.*

The aim of this investigation was to verify the influence of tetracycline concentration and pH on *C. guilliermondii* metabolism for xylitol production. Initially, tests were carried out to evaluate the sensitivity of the bacteria *Klebsiella pneumoniae* to antibiotics. Afterwards, a 2^2 factorial design was conducted with 0 and 40 mg/L tetracycline and pH 4.0 and 7.0 with a mid-point condition of 20 mg/L tetracycline and pH 5.5.

Materials and Methods

Microorganism

Candida guilliermondii FTI 20037 was obtained from Faculdade de Engenharia Química de Lorena, in Lorena, São Paulo, Brazil. *K. pneumoniae* NRRL B199 were provided by Northern Regional Research Laboratory (Peoria, IL). Both strains were maintained at 4°C on agar slants.

Testing Cultures for Antibiotic Sensitivity

The sensitivity of the cultures was determined by an agar diffusion method using the Kirby-Bauer method *(12)*. Tests were carried out with *K. pneumoniae* NRRL B-199 and *C. guilliermondii* FTI 20037, employing filter paper discs containing known concentrations of the following antibiotics: penicillin (10 μg), chloramphenicol (30 μg), tetracycline (30 μg), ampicillin (30 μg), and kanamycin (30 μg).

Inoculum Preparation

A stock culture of *C. guilliermondii* was transferred to 125-mL Erlenmeyer flasks containing 50 mL of medium (30.0 g/L xylose; 5.0 g/L ammonium sulphate; 0.1 g/L calcium chloride; 20 g/L rice bran) and incubated

under agitation of 200 rpm at 30°C for 24 h. The cells were then centrifuged at 2000*g* for 15 min and resuspended in sterile distilled water to reach a final concentration of 0.5 g/L.

Fermentation Conditions

Experiments were performed with sugarcane bagasse hemicellulosic hydrolysate (48.5 g/L of xylose) in 125-mL Erlenmeyer flasks closed with cotton-wool plugs, containing 50 mL of media, at 30°C, 200 rpm, for 88 h.

Analytical Methods

The fermentations were followed by measuring the consumption of glucose, xylose, and arabinose: production of xylitol and cell growth and acetic-acid concentration. The concentrations of glucose, xylose, arabinose, xylitol, and acetic acid were determined by high-performance liquid chromatography (HPLC; Shimadzu LC-10AD), under the following conditions: 0.01 *N* H_2SO_4 as eluent; 0.6 mL/min flow rate; column temperature 45°C; detector attenuation 16x, sample volume 20 µL. Cell concentration was determined using Beckman (Fullerton, CA) DU 640B spectrophotometer by comparing the optical density (OD) of a cell suspension against a standard curve (absorbance at 600 nm × dry cell weight).

Experimental Designs and Statistical Analysis

A 2^2 factorial design *(13)* was carried out in order to verify the influence of the tetracycline concentration and pH on *C. guilliermondii* metabolism for xylitol production. The levels of these factors were 0 and 40 mg/L and 4.0 and 7.0, respectively, with center condition of 20 mg/L and pH 5.5.

Results and Discussion

K. pneumoniae showed only sensitivity to tetracycline with an inhibition zone larger than 39 mm (Fig. 1A). On the other hand *C. guilliermondii* was resistant to all antibiotics (Fig. 1B).

In relation to fermentation process, Fig. 2 shows that the consumption of sugars and acetic acid as well as the production of xylitol changed when the tetracycline concentration and the fermentation pH were changed. After 20 h of fermentation, no glucose was detected, independent of the fermentation conditions. On the contrary, arabinose was slowly assimilated at the end of fermentation, and this coincided with the xylose exhaustion. A similar behavior has been reported by Meyrial et al. *(15).*

Xylose consumption was enhanced by increasing the pH of the fermentation. At pH 4.0, 98% of xylose was consumed after 72 h, whereas at pH 5.5 and 7.0, 100% was consumed after 48 h. Likewise, the yield factor of xylitol from xylose and volumetric productivity improved with the pH increase, its maximum values (0.85 g/g and 0.70 g/L/h, respectively) being achieved at pH 7.0 after 48 h. This response has been reported previously by Felipe et al. *(16)*.

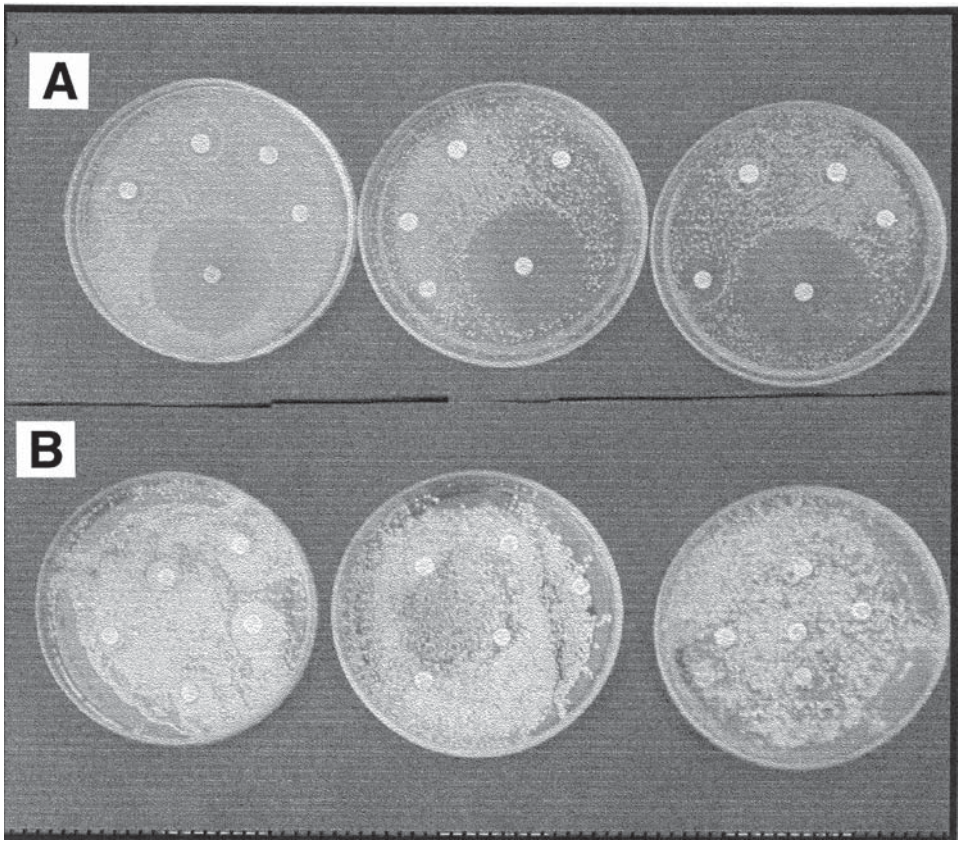

Fig. 1. Sensitivity of organisms to antibiotics penicillin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), ampicillin (30 µg), and kanamycin (30 µg). **(A)** Plates inoculated with *Klebsiella pneumoniae*, presence of inhibition zones around disc of tetracycline (39 mm). **(B)** Plates inoculated with *Candida guilliermondii*.

The experimental results presented in Table 1 were used to estimate the main effects of the variables and their interaction effects. Varying the tetracycline concentration did not have significant effect on the volumetric productivity or on the xylose consumption at 95% of confidence level with 5 freedom degrees (Table 2). On the other hand, increasing the pH level significantly affected the volumetric productivity and the xylose consumption, which enhanced from 0.21–0.70 g/L/h and from 50.5–100%, respectively.

The consumption of the acetic acid present in the hemicellulosic hydrolysate (4.4 g/L), was influenced both by the fermentation pH and by the tetracycline concentration. At pH 4.0 and with 40 mg/L of tetracycline, the acetic acid was completely consumed after 36 h, whereas without tetracycline or at pH 5.5 and 20 mg/L of tetracycline, the acetic acid was consumed only after 60 h. When the pH was increased to 7.0, a slow consumption of acetic acid occurred, independent of the tetracycline concentration, reaching 54% after 88 h. Acetic acid consumption by *C. guilliermondii* has also been observed in previous work with a semisynthetic medium *(17)*

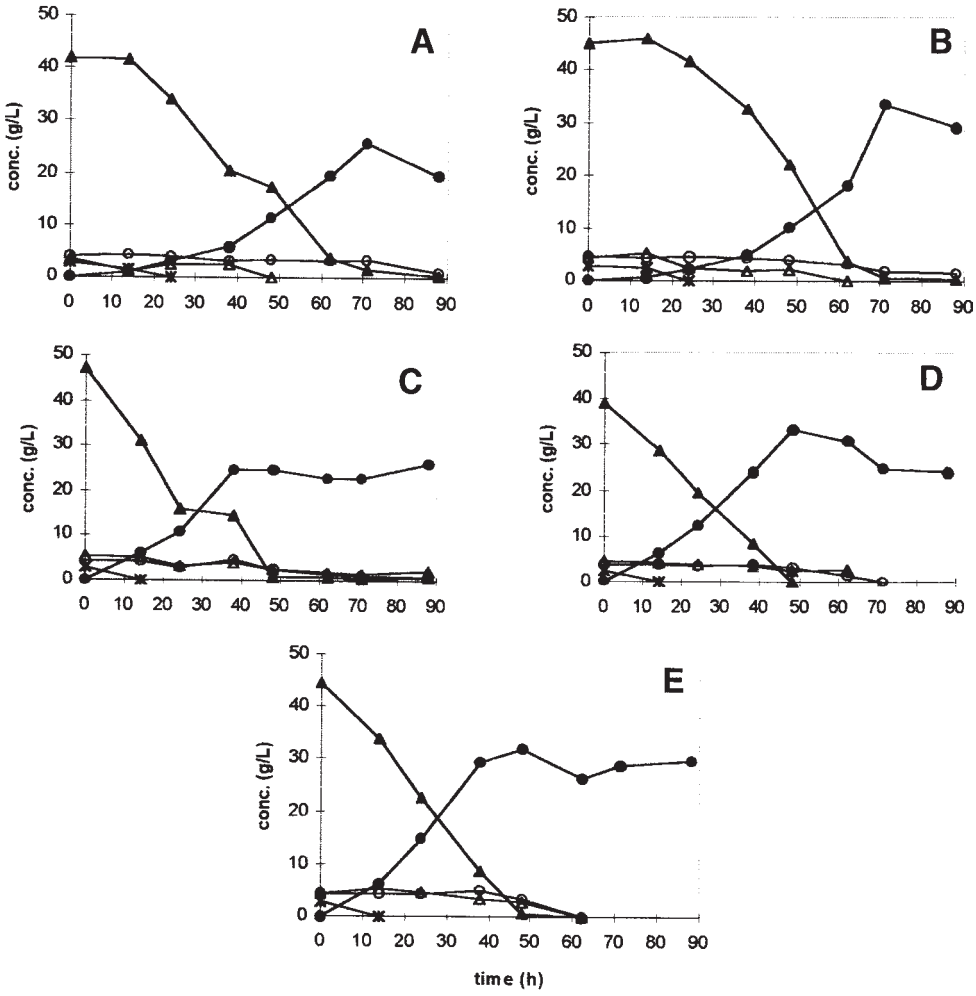

Fig. 2. Concentration profiles (g/L) of glucose (×), xylose (▲), arabinose (○), xylitol (●), and acetic acid (△) along the fermentation process. **(A)** pH 4.0, 40 mg/L of tetracycline; **(B)** pH 4.0, 0 mg/L of tetracycline; **(C)** pH 7.0, 40 mg/L of tetracycline; **(D)** pH 7.0, 0 mg/L of tetracycline; and **(E)** pH 5.5, 20 mg/L of tetracycline.

Table 1
Yield Factor of Xylitol from Xylose (Yp/s), Xylitol Volumetric Productivity (Qp), Maximum Specific Growth Rate (μ_{max}) and Xylose Consumption (xc) Obtained by Means of the Experimental Factorial Design

pH	Tetracycline (g/L)	Yp/s(g/g)	Qp (g/L/h)	μ_{max}(/h)	xc (%)
4.0	0	0.39	0.21	0.023	50.5
4.0	40	0.47	0.24	0.024	58.4
5.5	20	0.73	0.52	0.017	97.1
7.0	0	0.85	0.70	0.014	100.0
7.0	40	0.54	0.52	0.012	98.2

Table 2
Estimated Effects, Standard Errors, and Student's *t* Test
for Xylitol Volumetric Productivity (Qp)
and Xylose Consumption (xc) Using 2^2 Factorial Design

	Qp		xc	
Effects	Estimates ± s. errors	*t*	Estimates ± s. errors	*t*
Average	0.40440 ± 0.0379	10.67	82.034 ± 4.2507	19.32
pH	0.38920 ± 0.0847	4.59[a]	31.975 ± 9.5049	3.36[a]
Tetracycline	–0.00380 ± 0.0847	0.08	2.960 ± 9.5049	0.31
pH × Tetracycline	–0.00368 ± 0.0847	0.43	–4.165 ± 9.5049	0.44
Block	0.12920 ± 0.0758	1.70	–1.836 ± 8.5014	0.22

[a]Significant at 95% confidence level ($t \geq 2.57$).

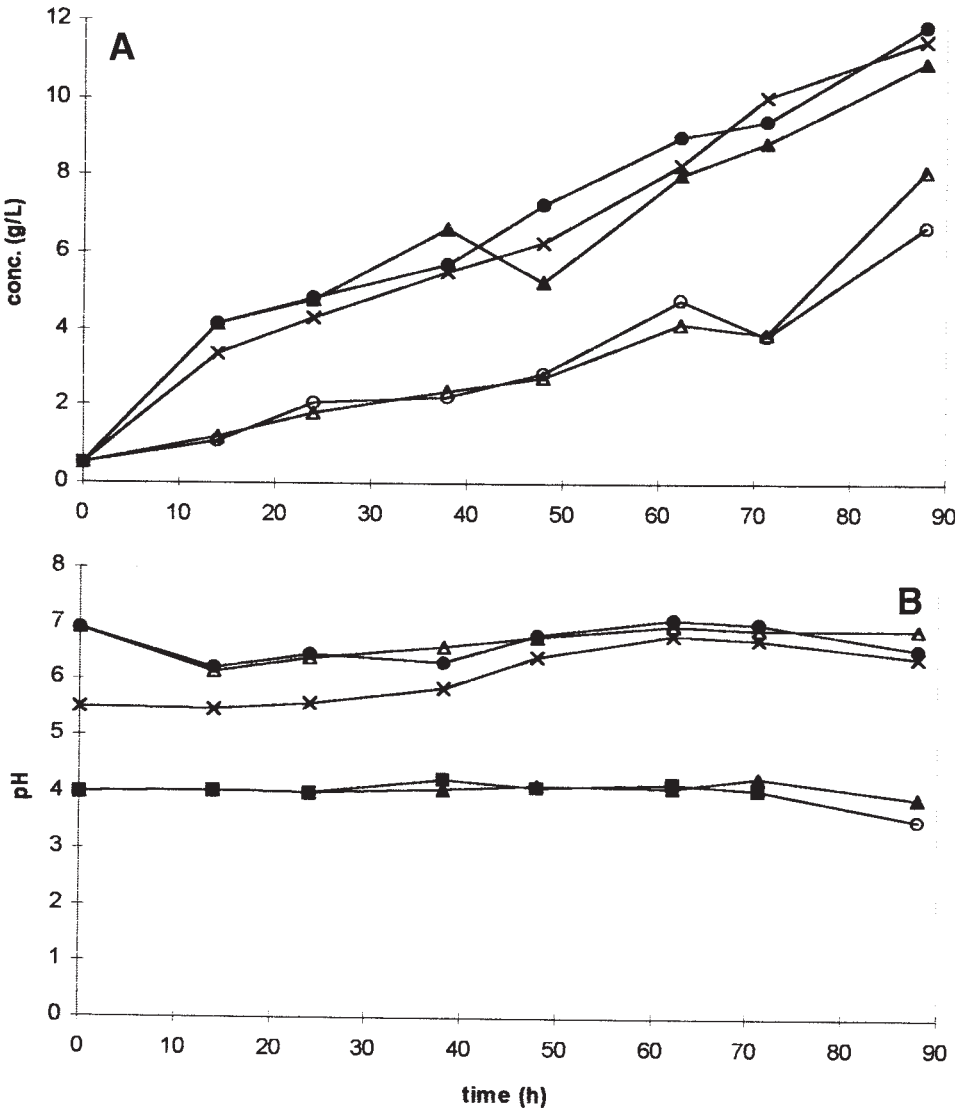

Fig. 3. Time profiles of cell growth **(A)**, and pH **(B)** during fermentation process. Symbols: ○, pH 4.0, 0 mg/L of tetracycline; △, pH 4.0, 40 mg/L of tetracycline; ▲, pH 5.5, 20 mg/L of tetracycline; ×, pH 7.0, 0 mg/L of tetracycline; and ●, pH 7.0, 40 mg/L of tetracycline.

and with sugarcane bagasse hydrolysate *(16)*. The ability of *C. guilliermondii* FTI 20037 to metabolize acetic acid is useful in xylose to xylitol conversion probably because these cells may act as medium-detoxifying agents, a fact that permits the microbiological process of xylitol production using xylose-rich lignocellulosic hydrolysates *(17)*. A possible explanation for the acetic-acid consumption is that this compound directly enters the Krebs cycle via acetyl-CoA and is used to produce energy necessary for the cells.

Cell growth and fermentation pH were also independent of tetracycline concentration (Fig. 3). On the other hand, increasing the pH of the fermentation led to an increase in cell growth. At pH 4.0, the cell growth was 7.8 g/L, whereas at pH 5.5 and 7.0, this value increased to 12 g/L. The fermentation pH values increased from 4.0–4.3 and from 5.5–7.3, possibly owing to the acetic-acid consumption by *C. guilliermondii*, as previously mentioned. When the initial pH value was 7.0, a decrease to 6.0 was observed in the first 20 h of fermentation, probably because of a smaller consumption of acetic acid.

Conclusions

The bioconversion of xylose into xylitol under the experimental conditions studied was influenced by the pH level. At pH 7.0 the yield and productivity were, respectively: 0.85 g xylitol/g xylose and 0.70 g/L/h. On the other hand, tetracycline concentration did not have a significant influence and did not affect the bioconversion process.

Acknowledgments

The authors acknowledge the financial support of Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and CNPq of Brazil. Thanks are also due to Maria Eunice M. Coelho for revising this paper.

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