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Effects of Initial pH on Biological Synthesis of Xylitol Using Xylose-Rich Hydrolysate

Tihany A. Morita, Silvio S. Silva,* and Maria G. A. Felipe

Biotechnology Department, Faculty of Chemical Engineering of Lorena, PO Box 116, Lorena, SP, Brazil, 12600-000, E-mail: silvio@debiq.faenquil.br

Abstract

Sugarcane bagasse, an agricultural residue plentiful in Brazil, was utilized for xylitol production by a biotechnological process. A medium fermentation prepared with this xylose-rich biomass at an oxygen transfer volumetric coefficient of $10/h^1$ and different initial pH values was inoculated with cells of *Candida guilliermondii* FTI 20037.

The maximum values of xylitol and cell volumetric productivities (Q_p = 0.56 g/[L·h] and Q_x = 0.11 g/[g·h]), xylitol yield factor ($Y_{P/S}$= 0.79 g/g), and xylose uptake rate (qs = 0.197 g/[g·h]) were attained at pH 7.0 without further pH control. The results show that the yeast performance was influenced by the pH, an important bioengineering parameter in this fermentation process.

Index Entries: Sugarcane bagasse hydrolysate; xylitol; detoxification method; pH; fermentative parameters.

Introduction

Bioconversion processes of xylose present in hemicellulosic hydrolysates have been extensively developed, utilizing lignocellulosic biomass for the production of chemicals, liquid fuels *(1)*, and feedstocks, such as ethanol and xylitol *(2,3)*. Xylitol, a five-carbon natural polyol *(4)* of high economic value, has aroused much interest in the food and pharmaceutical industries because it is anticariogenic *(5)*, has negative heat of dissolution *(6)*, and, according to preliminary studies, increases the lactoperoxidase activity, an enzyme that serves the function of defending the human body against pathogenic organisms *(7)*. The biological synthesis of xylitol is a promising alternative to the chemical process, because it uses microbial cells as catalysts and does not require initial xylose purification *(8)*.

Among the xylose-rich lignocellulosic materials is sugarcane bagasse, a residue whose production in Brazil reaches 295 million t/yr *(9)*. Most of this biomass is utilized in fuels, but new processes to use the bagasse

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

surplus, which ranges from 5 to 12 million t/yr *(10)*, still need to be developed. Sugarcane bagasse must be hydrolyzed to liberate sugars from the hemicellulosic fraction. Nevertheless, during hydrolysis several inhibitory compounds are formed. To reduce the concentration of these compounds and prevent inhibition of the cell metabolism, treatment of the hydrolysate is fundamental. Several detoxification methods have been proven effective for enhancing the hydrolysate fermentability *(11)*: extraction with organic solvents, ion exchange *(12)*, ion exclusion *(13)*, the use of molecular sieves *(14)*, overliming *(15)*, steam stripping *(16)*, the use of activated charcoal *(17)*, the use of polyelectrolytes *(18)*, and adaptation of microorganisms to the unfavorable environment *(19)*. One of these methods, overliming with calcium ions, has been widely used *(12,20,21)*, and promotes the removal of acids *(22)* and the precipitation of heavy metal ions *(23)*. Overliming can be combined with activated charcoal *(24)*, whose adsorption decreases the concentration of acetic acid and phenolics *(14)*, thereby clarifying the hydrolysates.

One of the most important parameters to be considered in fermentations for xylitol production is the pH, which influences the enzymatic activity *(25)*, yield, and productivity of xylitol *(26)*. Several studies have been published on the fermentation of hemicellulosic hydrolysates, especially wood hydrolysates *(14,23,27–29)*. However, only a few deal with sugarcane bagasse hydrolysate and the pH effect on its fermentation.

The present study describes the results of sugarcane bagasse hemicellulosic hydrolysate fermentation to xylitol at different initial pH values and at a fixed agitation/aeration rate (k_{La} = 10/h) and temperature in a bench-scale fermentor using *Candida guilliermondii* FTI 20037.

Materials and Methods

Hydrolysate Preparation, Concentration, and Treatment

Sugarcane bagasse hemicellulosic hydrolysate was obtained in a 350-L reactor (121°C, 10-min reaction time, 100 mg of H_2SO_4/g of dry matter) and concentrated under vacuum (70°C). The hydrolysate was overtitrated with CaO to pH 7.0, which was reduced to 5.5 with H_3PO_4. After the addition of activated charcoal, the hydrolysate was agitated at 200 rpm in a rotary shaker (New Brunswick Scientific, Edison, NJ) at 30°C for 1 h. At each pH alteration and after agitation, the hydrolysate was filtered to remove the resulting precipitate and to eliminate the activated charcoal. The treated hydrolysate was autoclaved for 15 min at 111°C to decrease sugar degradation.

Microorganism and Inoculum

C. guilliermondii FTI 20037, obtained from a stock culture of the Biotechnology Department, was maintained on malt-extract agar slant at 4°C. A preculture was conducted in a medium containing xylose (30 g/L), $(NH_4)_2SO_4$ (5 g/L), $CaCl_2$ (0.1 g/L), and rice bran extract (10 g/L), and incubated at 30°C for 24 h under shaking (200 rpm). The cells were har-

vested by centrifugation (1700*g*), washed with sterile water, and used in the suspension employed to inoculate the fermentation medium (initial cell concentration: 0.2 g/L).

Medium and Fermentation Conditions

A 1.25-L Bioflo III fermentor (New Brunswick Scientific, Edison, NJ) with a working volume of 1 L stirred at 300 rpm was employed for the batch fermentations. The treated hydrolysate supplemented with the same nutrients utilized in the inoculum preparation, except for xylose, was employed as the fermentation medium at 30°C, pH 4.0, 5.5, and 7.0; and at an oxygen transfer volumetric coefficient ($k_L a$) of 10/h.

Analytical Methods

Glucose, xylose, arabinose, xylitol, and acetic acid were quantified by high-performance liquid chromatography (HPLC) using a Shimadzu-LC10 AD apparatus (Kyoto, Japan), RID-6A index refraction detector, Bio-Rad Aminex HPX-87H (300 × 7.8 mm) column at 45°C, and 0.01 $\underline{N}$ H_2SO_4 eluent at a 0.6 mL/min flow rate. Furfural and hydroxymethylfurfural (HMF) were also detected by HPLC, but using an SPD- 10A UV-VIS detector, Hewlett-Packard RP 18 (200-mm) column at 25°C, and acetonitrile:water (1:8) with 1% acetic acid as the eluent at a 0.8 mL/min flow rate. Cell concentration was determined through the relation between the sample absorbance measurement at 600 nm (Beckman DU 640B spectrophotometer) and cell dry mass (g/L). The oxygen transfer volumetric coefficient was determined by the gassing-out methodology *(30)*.

Results and Discussion

Hemicellulosic hydrolysates are carbohydrate sources employed as fermentation media, together with other essential elements. Table 1 presents the partial composition and pH of sugarcane bagasse hydrolysate. It can be seen that this hydrolysate contains xylose as the predominant carbohydrate, as well as glucose, arabinose, acetic acid, furfural, and HMF. These components were also observed in acid hemicellulosic hydrolysates of rice straw *(31)* and eucalyptus *(27)*, at concentrations that varied according to the nature of the biomass and the hydrolysis conditions *(32)*.

Table 1 also shows that the amounts of sugar after the concentration step increased proportionally to the hydrolysate concentration factor ($f = 3$). However, this did not happen to the acetic acid owing to its volatility, which caused its partial evaporation *(33)*. HMF concentration also increased, whereas furfural concentration was 62.5% lower in the concentrated hydrolysate. This reduction can be attributed to the boiling point (54–55°C) at a reduced pressure *(34)*. A decrease in the pH of the concentrated hydrolysate (Table 1) was observed as a result of an increase in the concentration of H^+ ions proceeding from the H_2SO_4 utilized in sugarcane hydrolysis.

Table 1
Partial Composition of Sugarcane Bagasse Hemicellulosic Hydrolysate Before (A) and After (B) Concentration Step

Compound (g/L) and pH	Hydrolysate A	Hydrolysate B
Glucose	1.30	3.91
Xylose	18.92	53.62
Arabinose	1.94	5.40
Acetic acid	3.05	4.93
Furfural	0.16	0.06
HMF	0.04	0.17
pH	1.18	0.68

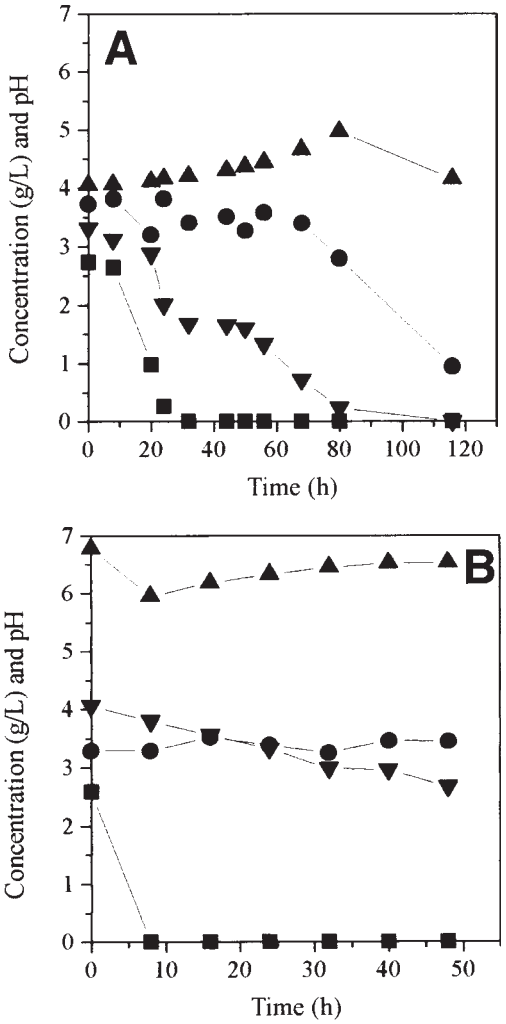

Fig. 1. Glucose (■), arabinose (●), acetic acid (▼) concentrations and pH (▲) of sugarcane bagasse hydrolysate fermentations conducted at $k_L a = 10/h$ and pH 4.0 **(A)**, 7.0 **(B)**, and 5.5 **(C)**.

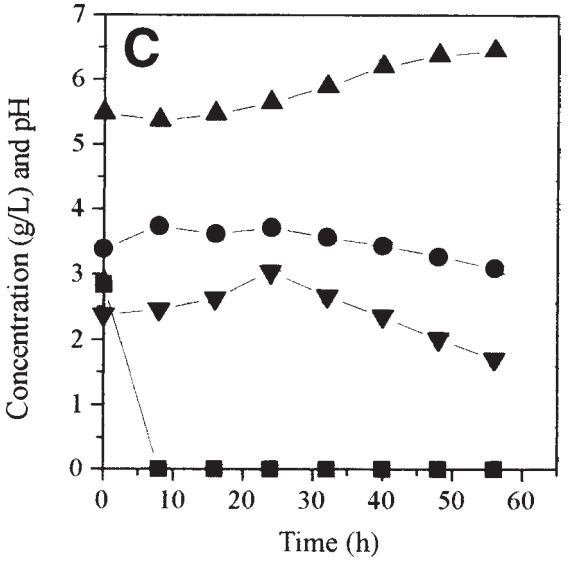

Fig. 1. *(continued)*.

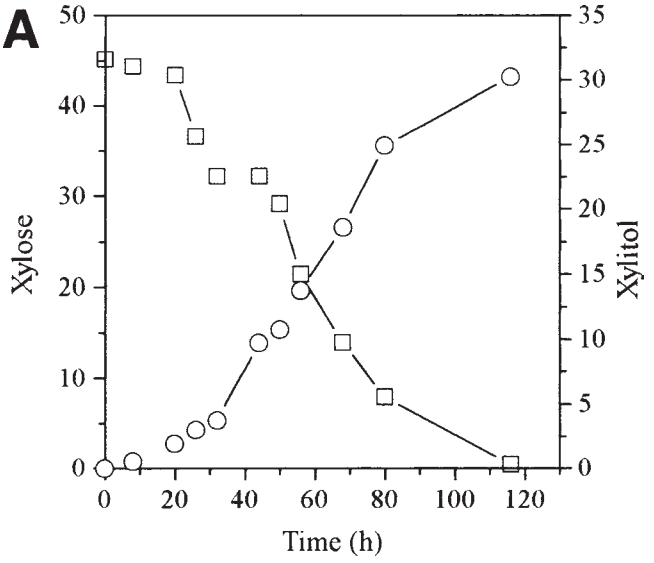

Fig. 2. Xylose (□) and xylitol (○) concentrations (g/L) obtained in sugarcane bagasse hydrolysate fermentations conducted at $k_L a$ = 10/h and pH 4.0 **(A)**, 7.0 **(B)**, and 5.5 **(C)**.

Figure 1A–C shows glucose, arabinose, and acetic acid uptake and pH profiles. Figure 2A–C presents xylose and xylitol uptake of the fermentation runs. Glucose was totally depleted in all the experiments. At pH 4.0, the depletion took 32 h of cultivation (Fig. 1A), whereas at pH 5.5 and 7.0, it

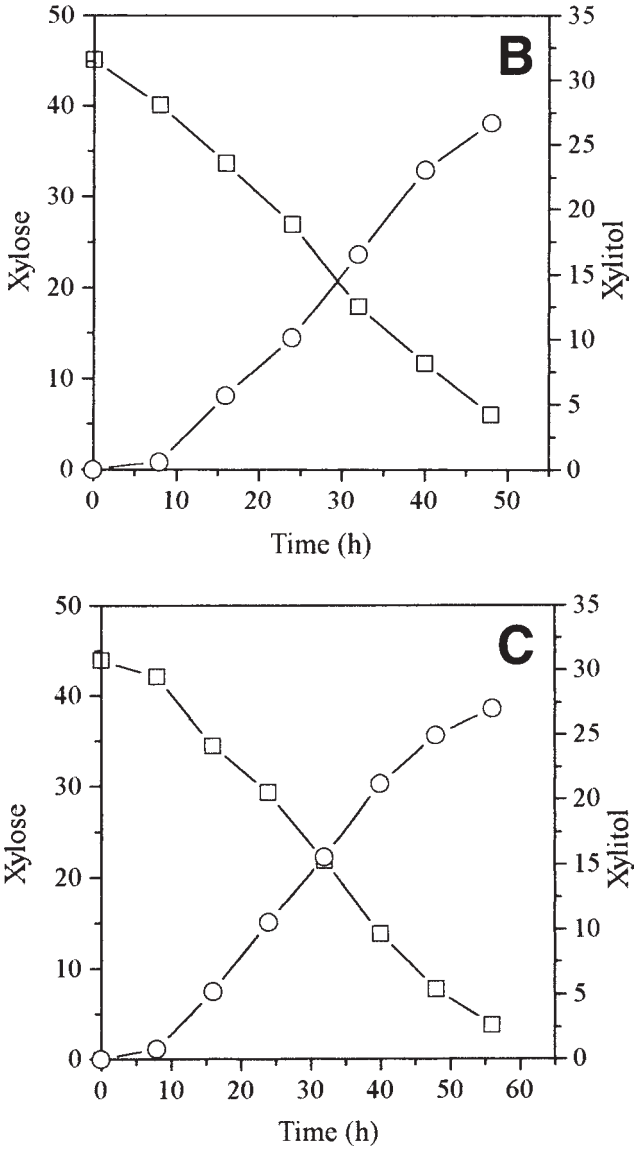

Fig. 2. *(continued)*.

took 8 h (Fig. 1 B,C). Roberto et al. *(31)* also observed that an increase in the initial pH value reduces the time required for the glucose uptake. Figure 1 shows that, like most xylose-metabolizing yeasts, *C. gulliermondii* was not able to ferment arabinose very strongly, except for fermentation conducted at pH 4.0 (Fig. 1A). At this condition, arabinose uptake occurred after the xylose depletion in the medium. The low arabinose assimilation rates were also evident in fermentations conducted with eucalyptus *(29)* and rice straw *(35)* hydrolysates. An increase in the pH of the fermentations (Fig. 1A–C) suggests the medium detoxification through the action of yeast cells. When

Table 2
Fermentative Parameters Obtained in Fermentations of Sugarcane Bagasse Hemicellulosic Hydrolysate at Different pH Values[a]

pH *i*	*P*	*X*	Q_p	Q_x	$Y_{P/S}$	*qS*
4.0	30.21	4.30	0.26	0.035	0.69	0.093
5.5	27.00	4.25	0.48	0.072	0.69	0.178
7.0	26.64	5.35	0.56	0.11	0.79	0.197

[a]pH *i*, initial pH of fermentation; *P*, final xylitol concentration (g/L); *X*, final cell concentration (g/L); Q_p, xylitol volumetric productivity (g/[L·h]); Q_x, cell volumetric productivity (g/[g·h]); $Y_{P/S}$, xylitol yield factor (g/g); *qS*, xylose uptake rate (g/[g·h]).

the initial pH value was 4.0, the pH increased constantly during the course of fermentation and decreased with the depletion of acetic acid (Fig. 1A). At initial pH 5.5 and 7.0 (Fig. 1B,C), the pH decreased in the first 8 h of cultivation, and then increased until the end of the fermentation. The pH decrease is quite common among yeasts, because of the high permeability of cell membrane to cations and hydrogen ions *(36)*.

Table 2 shows the fermentative parameters of sugarcane bagasse hydrolysate at different pH values. A faster cell mass production at pH 7.0 (Qx = 0.11 g/[g·h]) was probably the cause of the highest xylose uptake rate observed (qs = 0.197 g/[g·h]) and, consequently, of the increase in xylitol volumetric productivity at this pH (Qp = 0.56 g/[L·h]). It had been observed that for an effective xylitol production a rapid accumulation of microbial cells is needed *(37)*. It can also be verified that the final biomass concentrations were similar at all initial pH values evaluated, even though the cell volumetric productivity (Qx) values were very different from one another (Table 2). Although the highest xylitol concentration was obtained at pH 4.0 (Fig. 2A), the highest value of xylitol yield factor was obtained at pH 7.0. At pH 4.0, the values of the fermentative parameters were not significant, except for $Y_{P/S}$, which was identical to the one reached at pH 5.5, and for xylitol concentration (P = 30.21 g/L) (Table 2).

The efficiency and productivity of the fermentations are, to some extent, related to the activities of intracellular xylose reductase and xylitol dehydrogenase, enzymes responsible for the first steps of the xylose metabolism *(38)*. Rosa et al. *(39)* observed that at pH 3.0, the activity of xylose reductase decreased during the fermentation, but that the opposite occurred at pH 5.0. Even though enzymatic activities were not measured in the present work, one can believe that the results found by Rosa et al. *(39)* can explain the low values of xylose-xylitol bioconversion rates attained at pH 4.0.

The sugarcane bagasse hydrolysate was clarified after overliming and addition of activated charcoal, indicating that the phenolic compounds were removed or had their concentrations reduced. The removal of phenolics at a rate of 75% *(27)* and a faster xylose consumption on higher xylitol

productivity *(40)* were observed in hydrolysate subjected to overliming or treated with charcoal.

Conclusion

The maximum values of xylitol and cell volumetric productivities, xylitol yield factor, and xylose uptake rate were attained at pH 7.0, showing that biological synthesis of xylitol is influenced by the initial pH of the culture medium. Glucose uptake was more rapid with an increase in the initial pH value, whereas arabinose uptake only occurred at pH 4.0.

The treatment employed seemed to be efficient, because high values of fermentative parameters were reached, leading to the conclusion that the use of sugarcane bagasse hydrolysate as a substrate for xylitol production is possible through the hydrolysate treatment and by the use of appropriate physiological parameters, especially pH.

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