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Effect of the Oxygen Transfer Coefficient on Xylitol Production from Sugarcane Bagasse Hydrolysate by Continuous Stirred-Tank Reactor Fermentation

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Abstract

The effect of the oxygen transfer coefficient on the production of xylitol by bioconversion of xylose present in sugarcane bagasse hemicellulosic hydrolysate using the yeast *Candida guilliermondii* was investigated. Continuous cultivation was carried out in a 1.25-L fermentor at 30°C, pH 5.5, 300 rpm, and a dilution rate of 0.03/h, using oxygen transfer coefficients of 10, 20, and 30/h. The results showed that the microbial xylitol production (11 g/L) increased by 108% with the decrease in the oxygen volumetric transfer coefficient from 30 to 20/h. The maximum values of xylitol productivity (0.7 g/[L·h]) and yield (0.58 g/g) were obtained at k_La 20/h.

Index Entries: Hemicellulosic hydrolysate; sugarcane bagasse; xylose; xylitol; *Candida guilliermondii*; continuous fermentation.

Introduction

Each day the scientific community is becoming more and more concerned about the environmental problems caused by the accumulation of agroindustrial wastes in nature. Lignocellulosic residues gradually increase in quantity, and because they are cheap materials, several studies have been published on their use for the production of useful chemicals and feedstocks. Xylitol is a particular type of sugar-alcohol that can be produced from agricultural residues. Besides having anticariogenic properties,

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xylitol can be consumed by diabetics and employed in dietetic foods *(1,2)*. Its production by biotechnological reduction of xylose, obtained by means of acid hydrolysis of sugarcane bagasse, represents an alternative to the chemical production process. The biotechnological production of xylitol has been ultimately considered, and great efforts have been made to develop a low-cost technology. The microbial production of xylitol is more economical because it requires neither substrate purification nor high temperatures and pressures. Moreover, unlike the chemical process, it reduces pollution and avoids the concern for waste treatment.

Some studies on continuous fermentation of xylose to xylitol are cited in the literature *(3–5)*, but none of these studies used sugarcane bagasse hemicellulosic hydrolysate as the substrate. Among the environmental factors that exert influence on xylitol production by xylose-fermenting yeast, the dissolved oxygen concentration is a key parameter, because it determines whether xylose will be used for growth and/or fermentation *(6,7)*. Aeration conditions have an important role in xylitol production and in the activities of xylose reductase and xylitol dehydrogenase enzymes during xylose fermentation *(8–10)*. Consequently, the level of aeration must be carefully controlled to induce the formation of metabolic products rather than cell growth. The oxygen transfer volumetric coefficient (k_La) has influence on the oxygen transfer rate, since it is related to the agitation and aeration rates, as well as to the bioreactor design. This parameter can supply information for the process scale-up *(11)*.

This study deals with the effect of the oxygen transfer coefficient on the production of xylitol from xylose present in sugarcane bagasse hemicellulosic hydrolysate under controlled conditions in a continuous stirred-tank fermentor (CSTF).

Materials and Methods

Microorganism

A yeast strain of *Candida guilliermondii* FTI 20037, obtained from the Faculty of Chemical Engineering of Lorena, São Paulo, Brazil, was used. The cells were maintained at 4°C on malt extract agar slants.

Preparation of Sugarcane Bagasse Hemicellulosic Hydrolysate

The hemicellulosic hydrolysate was obtained by acid hydrolysis of sugarcane bagasse in a 250-L stainless steel reactor. The sugarcane bagasse was percolated with 100 mg of H_2SO_4 (98%) per gram of dry wt of bagasse for 10 min at 120°C. To reach a higher sugar concentration, the hydrolysate was concentrated under vacuum in a laboratory scale evaporator at 66 ± 4°C. The concentrated hydrolysate was treated prior to the fermentation for removing toxic components formed during acid hydrolysis. The hydrolysate was treated according to the method described by Alves et al. *(12)* using CaO and H_3PO_4 clarified with active charcoal (2.5%) under

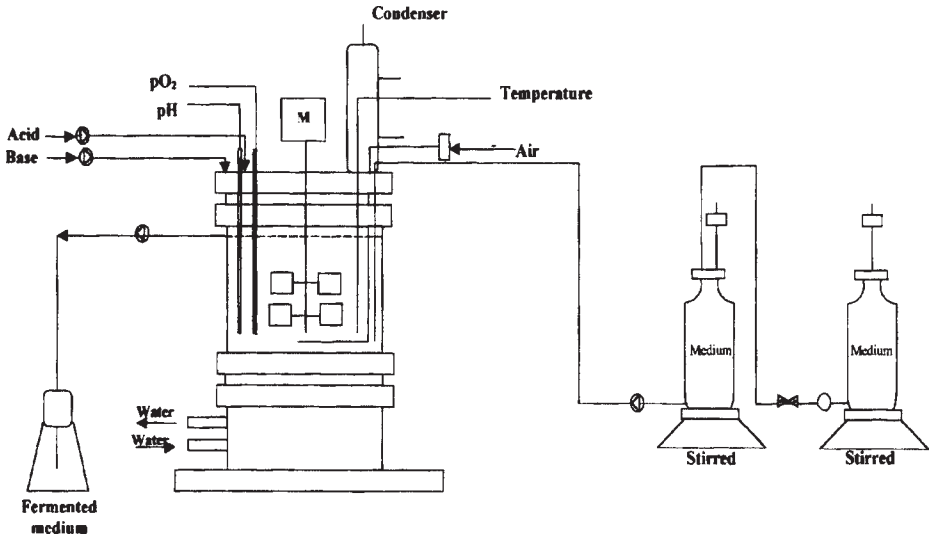

Fig. 1. CSTR fermentation of sugarcane bagasse hydrolysate.

agitation of 200 rpm, at 30°C for 1 h. The precipitate formed in each step was removed by filtration. The treated hydrolysate was then autoclaved at 100°C for 15 min, and aseptically supplemented with rice bran (20 g/L), $(NH_4)_2SO_4$ (3 g/L), and $CaCl_2 \cdot 2H_2O$ (0.1 g/L). This hydrolysate was used as a fermentation medium.

Preparation of Inoculum and Fermentation Medium

A stock culture of *C. guilliermondii* FTI 20037 was transferred to 125-mL Erlenmeyer flasks containing 50 mL of medium (30.0 g/L of xylose; 3.0 g/L of ammonium sulfate; 0.1 g/L of calcium chloride; 20 g/L of 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. All the experiments were performed with sugarcane bagasse hemicellulosic hydrolysate containing 3 g/L of glucose, 51 g/L of xylose, 4.3 g/L of arabinose, and 4.4 g/L of acetic acid.

Reactor Design and Experimental Start-Up

Fermentation runs were performed in a 1.25-L fermentor (BIOFLO III; New Brunswick Scientific, NJ). The pH, dissolved oxygen, agitation, and temperature were kept under automatic control. Agitation was performed by two flat-blade turbine impellers spaced 7.5 cm apart. MasterFlex (IL) and Watson Marlow 505S (Falmouth, England) peristaltic pumps were used to feed the substrate into the fermentor and to draw off the product, respectively. The bulk liquid in the Mariote flasks was continuously mixed by a Fisatom (Brazil) magnetic stirrer to maintain a homogenized feeding suspension. Figure 1 shows a schematic experimental setup.

A start-up procedure was required in order to establish a significant growth of the yeast cells in the reactor before starting steady-state operations. The experiments were carried out at 30°C, pH 5.5, 300 rpm, and a dilution rate (D) of 0.03/h, using oxygen transfer coefficients of 10, 20, and 30/h. Steady state in continuous culture was considered to be reached when three consecutive measurements of the cell and substrate concentrations were essentially similar (maximum of 10% deviation).

Analytical Methods

The fermentations were followed by measuring the consumption of glucose, xylose, and arabinose; production of xylitol; cell growth; and acetic acid concentration. The concentrations of sugars, xylitol, and acetic acid were determined by high-performance liquid chromatography on a Shimadzu LC-10AD column (Kyoto, Japan), under the following conditions: 0.01 N H_2SO_4 as the eluent; 0.6 mL/min flow rate; column Aminex HPX87H, 45°C; 16 X detector attenuation; 20-µL sample volume.

Cell concentration was determined by a Beckman DU 640B spectrophotometer (Beckman, Fullerton, CA), correlating optical density with dry cell weight. The methylene blue test for staining dead cells was performed to test viability, and the bacterial contamination was verified by microscopic observation of fixed sample colored with fucsina. The oxygen transfer volumetric coefficient (k_La) was determined under standard fermentation conditions by the gassing-out method as described by Pirt *(13)*.

Results and Discussion

Microbial xylitol production rates have been studied through different bioprocesses. The present study evaluated the effect of the oxygen transfer coefficient on xylitol production from sugarcane bagasse using a continuous culture of *C. guilliermondii*. Figures 2–4 present the concentration profiles of sugars, acetic acid, xylitol, and cells throughout the continuous fermentation processes at pH 5.5; D = 0.03/h; and k_La = 10, 20, and 30/h. Table 1 presents the results of the xylitol production rates.

In addition to xylose, a major component of hemicellulosic hydrolysates, small concentrations of sugars, such as glucose, are present in the hydrolysate. In agreement with these figures, no glucose was detected in the fermented medium, because it was consumed by the yeast. This suggests that, during xylose bioconversion to xylitol, the glucose was used as a cosubstrate preferentially to generate energy for cell maintenance and to regenerate NADPH, a fundamental cofactor in this bioconversion. Similar results were reported by Roca et al. *(5)* in a study on the continuous process of xylitol production using a mixture of xylose and glucose.

Xylose bioconversion into xylitol occurs as a function of the presence of xylose reductase enzyme in the xylose-fermenting yeast (Fig. 5). The dissolved oxygen concentration is a key factor in the flow of carbon for cell growth or xylitol biosynthesis. The increase in the process aeration rates,

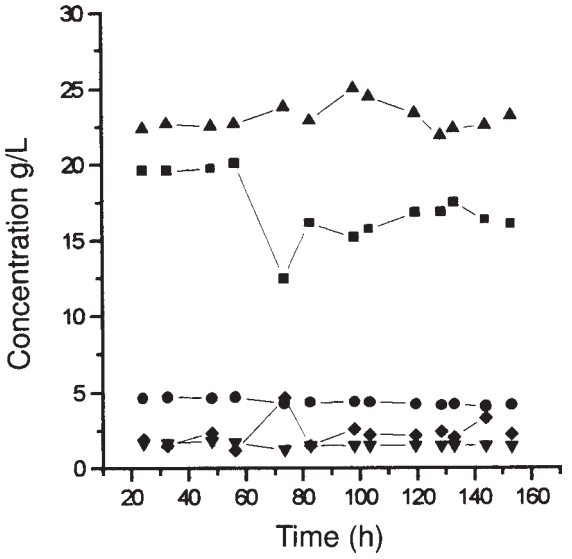

Fig. 2. Concentration profiles (g/L) of xylose (■), arabinose (●), xylitol (▲), acetic acid (▼), and cell growth (◆) throughout the continuous fermentation process of sugarcane bagasse hemicellulosic hydrolysate by *C. guilliermondii* at pH 5.5, $k_La = 10/h$, and $D = 0.03/h$.

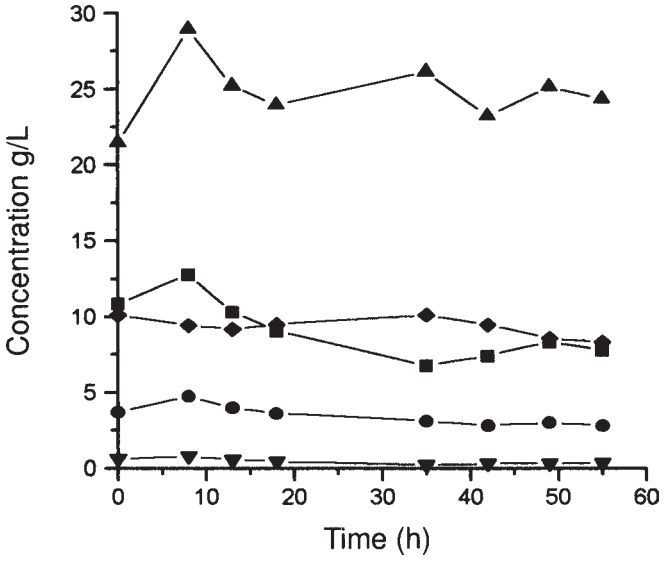

Fig. 3. Concentration profiles (g/L) of xylose (■), arabinose (●), xylitol (▲), acetic acid (▼), and cell growth (◆) throughout the continuous fermentation process of sugarcane bagasse hemicellulosic hydrolysate by *C. guilliermondii* at pH 5.5, $k_La = 20/h$, and $D = 0.03/h$.

corresponding to a k_La increase from 10 to 30/h, promoted an increase of approx 27% in the xylose assimilation rate and, consequently, a high cell growth (636%).

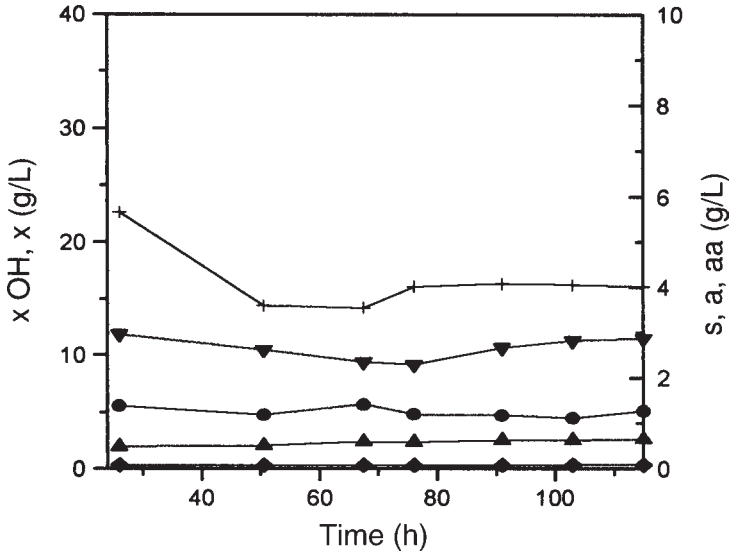

Fig. 4. Concentration profiles (g/L) of xylose (+), arabinose (●), xylitol (▲), acetic acid (▼), and cell growth (◆) throughout the continuous fermentation process of sugarcane bagasse hemicellulosic hydrolysate by *C. guilliermondii* at pH 5.5, $k_La = 30/h$, and $D = 0.03/h$. xOH, xylitol production; x, cell concentration; 5, xylose consumption; a, arabinose consumption; aa, acetic acid consumption.

Table 1
Effect of Oxygen Transfer Volumetric Coefficient on Xylitol Production Rates Obtained by Continuous Fermentation of Sugarcane Bagasse Hemicellulosic Hydrolysate[a]

k_La (h)	ΔS (%)	Δa (%)	Δaa (%)	xOH (g/L)	x (g/L)	$Y_{p/s}$ (g/g)	$Y_{x/s}$ (g/g)	Q_p (g/[L·h])	Q_e (g/[g·L])
10	71.4	3.22	65.9	22.7	2.2	0.54	0.05	0.68	0.305
20	81.3	0.92	93.2	23.1	10.6	0.58	0.22	0.70	0.080
30	90.9	43.80	93.0	11.1	16.2	0.24	0.35	0.33	0.020

[a]ΔS, xylose consumption; Δa, arabinose consumption; Δaa, acetic acid consumption; xOH, xylitol production; x, cell concentration; $Y_{p/s}$, xylitol yield; $Y_{x/s}$, cell yield; Q_p, xylitol volumetric productivity; Q_e, cell volumetric productivity.

Figure 6 depicts biomass and xylitol production rates. The xylitol production rates were not affected by k_La values between 10 and 20/h, but when these values increased from 20 to 30/h, xylitol production rates decreased from 0.58 to 0.24 g/g. A similar result of xylitol yield (0.60 g/g) was obtained by Silva and Afshar *(15)*, when studying continuous fermentation of xylose by *C. guilliermondii* in a fluidized-bed bioreactor. Biomass yield increased by 340–600% with the increase in k_La value; that is, the xylose metabolism is more directed toward cell growth than toward xylitol production when the oxygen supply increases. A probable explanation for this fact is that the oxygen supply activates the xylose transport system and

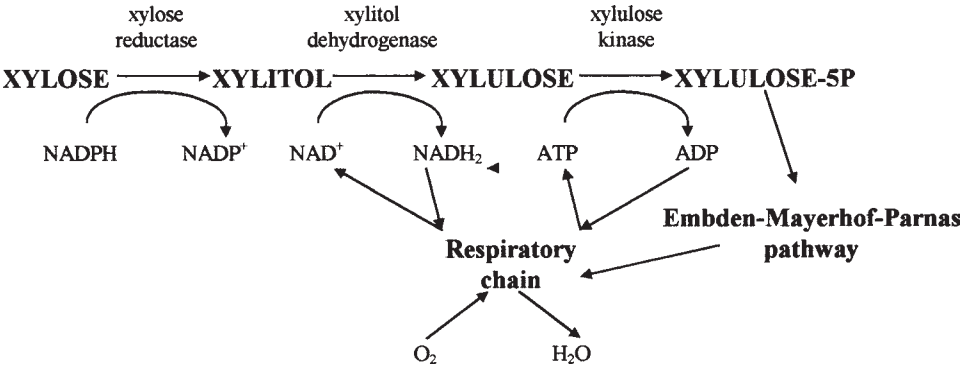

Fig. 5. Scheme for xylose fermentation by yeast *(14)*.

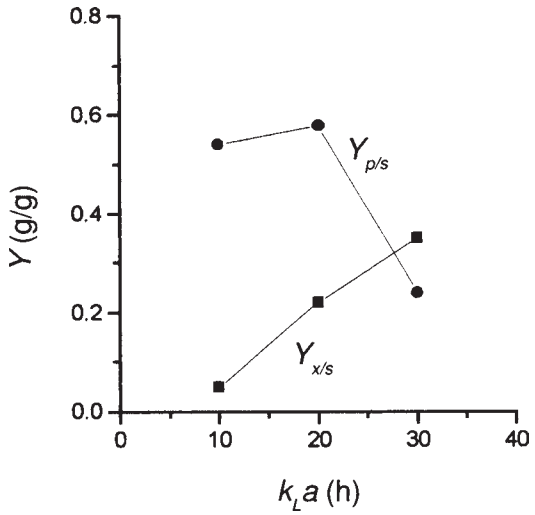

Fig. 6. Effect of oxygen transfer volumetric coefficient on global yields of biomass (■) and xylitol (●).

determines the partitioning of the carbon flux from xylose between cell growth and product formation. The excess of oxygen causes NADH to be oxidized to NAD^+, and the high NAD^+/NADH ratio leads to the oxidation of xylitol into xylulose. As a result, less xylitol and more cells are accumulated *(16–19)*. Similar results were reported by Furlan et al. *(3)* and Winkelhausen et al. *(4)* in studies of xylitol production by *Candida parapsilosis* and *Candida boidinii*, respectively, using synthetic medium and limited oxygen conditions. Since for all the aeration conditions tested, the dissolved oxygen concentration in the medium was relatively low, and the culture was in limited oxygen conditions, the oxygen consumption by the microorganism increased when the aeration rate was increased *(3,4)*.

Similarly to xylose, the consumption of arabinose increased with the increase in the aeration rate, since 93% of this pentose was assimilated with

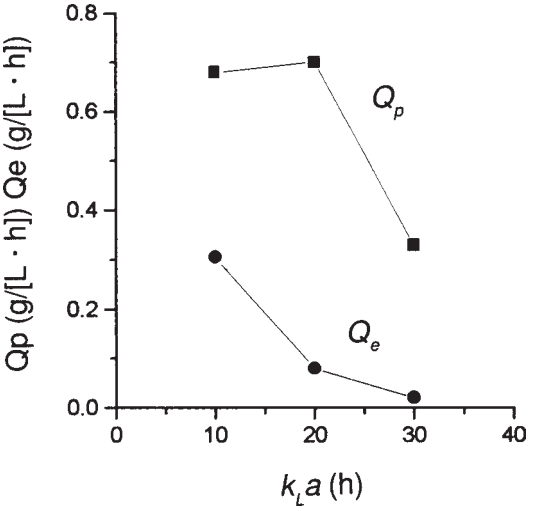

Fig. 7. Effect of oxygen transfer volumetric coefficient on volumetric (■) and specific (●) xylitol productivities.

higher k_La values (20 and 30/h). In the same manner, an increase in the consumption of acetic acid was observed, possibly because with high oxygen availability this acid may have been converted into acetyl-Co, the main intermediary of the tricarboxylic acid cycle, used for formation of reduced coenzymes that are oxidized in the respiratory chain. Acetic acid has been cited as an inhibitor of yeast metabolism, and its degree of inhibition depends on its concentration and on the oxygen availability. According to Parajó et al. *(20)* and van Zyl et al. *(21)*, no acetic acid is consumed in anaerobic conditions.

The maximum volumetric productivity of xylitol attained in the present study was 0.70 g/L·h. The influence of the broth aeration levels on the xylitol production was confirmed by the results of specific and volumetric productivities of xylitol, which were affected by the high aeration levels. Figure 7 shows that the increase in k_La from 20 to 30/h had a pronounced effect on these fermentative parameters, which were 53 and 75% lower, respectively, under the highest aeration rates. Furlan et al. *(3)* and Winkelhausen et al. *(4)* reported similar behavior.

Conclusion

The continuous system can be employed for xylitol production from sugarcane bagasse hemicellulosic hydrolysate by *C. guilliermondii*. Our results prove that low k_La values (10 and 20/h) are essential for maximal xylitol production rates and that the oxygen supply is an important parameter in this bioprocess. The xylitol yield and volumetric productivity remained high, averaging 0.58 g/g (63% of theoretical yield) and 0.69 g/L·h, as the oxygen transfer volumetric coefficient was increased from 10 to 20/h.

Meanwhile, increasing the k_La value to 30/h caused these fermentative parameters to decrease by 50% and cell growth and yield to increase by 52 and 59%, respectively. Therefore, owing to the oxygen supply, the flux of xylose used for xylitol production was directed toward cell growth.

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