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Maximizing the Xylitol Production from Sugar Cane Bagasse Hydrolysate by Controlling the Aeration Rate

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ABSTRACT

Batch fermentations of sugar cane bagasse hemicellulosic hydrolysate treated for removing the inhibitors of the fermentation were performed by *Candida guilliermondii* FTI 20037 for xylitol production. The fermentative parameters agitation and aeration rate were studied aiming the maximization of xylitol production from this agroindustrial residue. The maximal xylitol volumetric productivity (0.87 g/L · h) and yield (0.67 g/g) were attained at 400/min and 0.45 v.v.m. (K_La 27/h). According to the results, a suitable control of the oxygen input permitting the xylitol formation from sugar cane bagasse hydrolysate is required for the development of an efficient fermentation process for large-scale applications.

Index Entries: Sugar cane bagasse hemicellulose hydrolysate; xylitol; xylose; aeration; *Candida guilliermondii*.

INTRODUCTION

Lignocellulosic materials from forestry and agriculture residues such as rice husk, eucalyptus, and sugar cane bagasse are inexpensive and abundant sources of energy that can be used in several biotechno-

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logical processes for the obtention of products of high economic value. Sugar cane bagasse is the most important residue in Brazil and an amount of 5–12 millions t/yr of this biomass is generated by the Brazilian sugar-alcohol industries *(1,2)*. Every year large amounts of waste biomass are accumulated in nature, causing serious environmental pollution problems. It is necessary to find new technologies to use this renewable biomass in different processes to produce economically valuable products. The biotechnological approach is one way to use this biomass as a micro-organism substrate for production of several useful feedstocks.

Xylitol, a valuable product with high sweetening power, anticariogenic properties, and several clinical applications is a substance that can be produced by fermentation processes using sugar cane bagasse as the substrate *(3,4)*.

Currently, xylitol is produced by chemical hydrogenation using Nickel as the catalyst. However, this process is expensive since it requires several steps of xylose purification before the chemical reaction *(5,6)*. Microbial production of xylitol from agroindustrial residues is a simpler and more ecomomic process since it occurs at lower temperatures and pressures and does not require pure xylose.

The use of sugar cane bagasse as the substrate in fermentative processes for xylitol production consists initially in releasing sugars from the hemicellulose portion through a mild acid hydrolysis process. This process is accompanied by the formation of considerable amounts of hemicellulose decomposition products, such as furfural, hydroxymethylfurfural, acetic acid, and other products derived from lignin degradation. These chemical compounds interfere negatively with the yeast cell growth *(7)* and the additional xylitol fermentation *(8)*. Thus, the use of this biomass hydrolysate as a fermentation medium for micro-organism growth is critical, and several treatments are necessary for removing these products. The cell growth in this hydrolysate and the xylitol formation depend on the treatment and the fermentation conditions employed *(3,4)*. The oxygen transfer rate is the most significant of all parameters that affect the biological synthesis of xylitol by xylose-fermenting yeasts *(9–11)*. According to the literature the effect of oxygen on xylitol production is not fully understood and appears to be related to the initial steps of the xylose metabolism and to the NAD/NADH pool *(12)*.

In this communication the authors present a simple method of treatment of sugar cane bagasse hemicellulosic hydrolysate for removing toxic compounds and the xylitol production by *Candida guilliermondii* FTI 20037 from this biomass under different O_2 conditions. It is of fundamental importance to understand the influence of this factor on xylitol formation for the development of an efficient technology for large-scale xylitol production from sugar cane bagasse by the biotechnological process.

MATERIALS AND METHODS

Micro-organism

Candida guilliermondii FTI 20037 from the Biotechnology Department of the Faculty of Chemical Engineering of Lorena, FAENQUIL, Lorena, S.P.–Brazil, was used. The culture was maintained in malt extract agar slants at 4°C.

Preparation and Clarification of the Acid Sugar Cane Bagasse Hemicellulosic Hydrolysate

The hemicellulosic hydrolysate was obtained by acid hydrolysis of sugar cane bagasse in a 360 L stainless steel reactor. The sugar cane bagasse was percolated with 10% H_2SO_4 per dry weight of bagasse for 20 min at 120°C. To reach a higher sugar concentration the hydrolysate was concentrated under reduced pressure in a lab-scale evaporator at 70°C. The concentrated hydrolysate was treated prior to the fermentations for removing the toxic components formed by acid hydrolysis. The hydrolysate was treated according to Felipe et al. *(3)* and clarified with active charcoal (30 g/L) under 200 rpm stirring for 1 h at room temperature. In this step CaO and H_2SO_4 were used, mainly because of beneficial effects of the Ca^{++} ions on the possible removal of unknown compounds during precipitation. The precipitate formed was removed by centrifugation at 1000*g* for 20 min. The treated hydrolysate was then autoclaved with steam at 100°C for 20 min, and aseptically supplemented with rice bran (10 g/L) to provide vitamins and $(NH_4)_2SO_4$ (2 g/L). This treated hydrolysate was then used as a fermentation medium to evaluate the xylitol production by *C. guilliermondii* FTI 20037.

Inoculum Preparation

The inoculum was grown in the aforementioned treated hydrolysate containing the same nutrients that were used in the fermentation medium. A loopful of cells from stock culture was inoculated in 50 mL of this medium in 125 mL Erlenmeyer flasks and incubated for 24 h at 30°C and 200 rpm in a rotary shaker. The cells were harvested by centrifugation at 1000*g* for 20 min, washed twice with distilled water, and resuspended in 10 mL distilled water. The cell concentration was determined and a volume sufficient to give 0.5 g/L cell dry weight was used to inoculate the fermenter.

Fermentation Conditions

Batch fermentation runs were performed in a 1 L fermenter (MULTIGEN-New Brunswick Scientific, Edison, NJ) containing baffles and two sets of disk Rushton turbines with six flat-blades and a working volume of 0.55 L of medium prepared as described according to the preparation and clarification of the hydrolysate. The fermentation system was equipped with

Table 1
Concentration of Some Components in the Sugar Cane Bagasse Hemicelulosic Hydrolysate

Components	Hydrolysate Concentrations (g/L)	
	original	concentrated
Glucose	5.54	8.04
Xylose	26.38	62.13
Arabinose	2.07	5.11
Acetic acid	4.52	7.00
Furfural	< 0.5	< 0.1
Hydroximethylfurfural	< 0.1	< 0.1
Lignin degradation products	n.d	n.d

n.d = not determined.

pH, pO_2, temperature and aeration rate controllers. The temperature was maintained at 30°C and the agitation/aeration rates were set at different values according to a previous statistical factorial design. The agitation was set at 200, 300, and 400 min^{-1} and the oxygen supply was varied from 0.10 to 0.80 vvm (volume of air per volume of medium per minute). The oxygen volumetric transfer coefficient (K_La) in all conditions was determined.

Analytical Methods

Batch fermentation runs were monitored by periodic sampling to determine the sugar consumption and xylitol formation. Samples of appropriate dilutions were prepared by filtration through a 0.22 micron filter (Waters Set-pak Cartridge, Millipore, Bedford, MA)

Xylose, glucose, arabinose, acetic acid, and xylitol were analyzed in a Shimadzu high performance liquid chromatograph (HPLC), using a Bio-Rad Aminex HPX-87 H column at 45°C and 0.02 NH_2SO_4 as the eluent at a flow rate of 0.6 mL/min.

Growth was monitored by measuring the culture turbidity at 600 nm. The cell mass was estimated using a relationship between optical density and dry cell weight.

The volumetric oxygen transfer coefficient (K_La) was determined under standard fermentation conditions by the gassing-out method as described by Pirt *(13)*.

RESULTS AND DISCUSSION

The basic composition of the sugar cane bagasse hydrolysate used in this work is shown in Table 1. Under the conditions used, a mixture of sugars (pentoses and hexoses) was obtained. Xylose was the major pentose present in this hydrolysate representing about 70% of the total monosaccharides. The presence of acetic acid was a result of the de-*o*-acetylation of acetylated

Table 2
Xylitol Production Rates from Sugar Cane Bagasse Hydrolysate by *C. guilliermondii* FTI 20037 under Different Oxygen Conditions

Physical Parameters										
Agitation (min^{-1})	*Aeration (v.v.m)*	*$K_L a$ (h^{-1})*	*ΔS (%)*	*XOH (g/L)*	*Final biomass (g/L)*	*Final pH*	*Q_P (g/L.h)*	*Qx (g/L.h)*	*q_P (g/g.h)*	*$Y_{P/S}$ (g/g)*
200	0.10	2	7	1.44	0.71	5.85	0.02	0.06	0.09	0 36
200	0.45	7	19	5 76	0.92	5.35	0.08	0.15	0.22	0.52
200	0.80	11	35	4 32	0.80	5.33	0.06	0.30	0.19	0.20
300	0 10	2	15	5 76	0.56	5.19	0.08	0.13	0 80	0.61
300	0.45	9	82	34.75	2.00	5.62	0.58	0.82	0 38	0.62
300	0.80	18	100	40.20	2.19	6.61	0.67	1.00	0.39	0.67
400	0.10	6	12	2.88	0.75	5.70	0.04	0.10	0.17	0.45
400	0.45	27	100	41.76	5.19	7.70	**0.87**	1.30	0 18	**0.67**
400	0.80	38	80	25.20	10.29	6.83	0.70	1.43	0.07	0.49

ΔS = xylose consumed; XOH = final xylitol concentration; Qp = xylitol volumetric productivy, Qx = xylose uptake rate; qp = specific rate of xylitol production; Yp/s = xylitol produced/substrate consumed.

sugars from the hemicellulosic fraction. This acid has been described as toxic for *C. guilliermondii* at concentrations up to 6 g/L. Consequently it interferes with xylitol production *(3)*. After the hydrolysate concentration step the acetic acid concentration increased from 4.52 to 7.0. A parallel increase in the xylose:glucose ratio was also observed from 4.8 to 7.7. Hence, the concentration of the original hydrolysate corresponded to a second and convenient hydrolytic step (xylo-oligosaccharides to free xylose with simultaneous de-*o*-acetylation). Other compounds like furfural and hydroxymethyl furfural were also present in this hydrolysate at low concentrations (Table 1).

The results of Table 2 demonstrated that the yeast *Candida guilliermondii* FTI 20037 was able to produce xylitol at different rates during the fermentation of pretreated sugar cane bagasse hemicelullosic hydrolysate, under all aeration conditions employed. It is known that the hemicellulosic hydrolysate fermentation is complex and critical since this hydrolysate contains several chemical compounds that are toxic to the micro-organisms. By treating the sugar cane bagasse hydrolysate the toxicity of these compounds was significantly reduced and therefore cell growth, substrate uptake, and xylitol formation could be observed (Table 2). By increasing the original pH of the hydrolysate from 1.1 to 10 and in the presence of active charcoal, the Ca^{++} ions may bind and precipitate some of these toxic compounds and improve further fermentations. The real beneficial effect of Ca^{++} ions on the treatment of the hydrolysate is difficult to detect. According to Van Zyl et al. *(14)*, this may be attributed to the poor solubility of calcium salts formed during the neutralization step and possible removal of unknown toxic substances during precipitation.

According to Table 2 the highest level of final biomass (10.29 g/L) was attained with the highest O_2 levels, whereas the lowest growth occurred at a substantially low aeration level. This fact reflects the importance of oxygen in the utilization of xylose by xylose-fermenting yeasts. *C. guilliermondii* FTI 20037 is considered a good xylose-fermenting yeast with a great potential for large-scale xylitol production *(15,16)* since this strain contains xylose reductase and xylitol dehydrogenase, which are the key enzymes for the xylose metabolism *(17,18)*. It is known that yeast growth depends on the formation of xylulose in the initial steps of the xylose metabolism. Xylose is first reduced to xylitol through a NADPH-linked xylose reductase. Then the xylitol is oxidized to xylulose, through a NAD-linked xylulose dehydrogenase. This is followed by the formation of xylulose-5-phosphate through an ATP-dependent xylulose kinase and by the further entrance of xylulose-5-phosphate into the pentose phosphate pathway. The formation of ATP and the oxidation of NADH are carried out at the respiratory chain in the mitochondria and are strongly dependent on the oxygen availability. The oxygen transfer rate is fundamental for the regeneration of cofactors, which are essential for xylose metabolism, biomass formation, and xylitol excretion.

According to Shook and Hahn-Hägerdahl *(19)*, the available oxygen has a great influence on xylose fermentation. Under aerobic conditions the organism mainly produces cell mass, and under semiaerobic or anaerobic conditions by-products are formed. In our experiments, the xylitol formation was strongly dependent on the oxygen supply. By increasing the aeration rate and consequently the oxygen input, the xylitol formation increased until a suitable agitation/aeration rate relationship was reached. Similar results were found by Silva et al *(11)* using synthetic medium containing xylose as the major carbon source. Thus, for the maximum xylitol production, it is fundamental to control the oxygen transfer rate. In our experiments, the maximum xylitol production (41.76 g/L) from sugar cane bagasse and the maximum xylitol volumetric productivity (0.87 g/L.h) were attained under agitation set at 400 min^{-1} and aeration rate of 0.45 vvm (Table 2).

The K_La is an important parameter since it describes the aeration capacity of the fermentation system and supplies information for the process scale-up. Under the experimental conditions used, the K_La varied from 2 to 38 h^{-1} (Table 2). Few reports describe the influence of this parameter on xylitol production and many published data are contractitory. Under our conditions, by controlling the aeration rate, the K_La for maximum xylitol production is near 27 h^{-1}.

The fermentation pH was also affected by the aeration rate (Table 2). At low aeration rates its increase was not pronounced. However, increasing the oxygen the pH increased from 5.5 to near 7.7. These results are in agreement with those found by Van Zyl et. al. *(20)*. According to these authors, under aerobic conditions, the xylose and the acetic acid present in sugar cane bagasse hydrolysate, were consumed simultaneously, whereas under anaerobic conditions there was no acid consumption. The consumption of acetic

acid, as a carbon source, by *Candida guilliermondii* is a fact observed in previous work *(8)*, and the effect of this acid on cell growth is highly dependent on the fermentation pH. It is known that the acetic acid toxicity depends on its concentration and strongly interferes with the yeast energy metabolism by reducing the H^+ gradient across the mitochondrial membrane.

CONCLUSIONS

The demands for xylitol by the food and pharmaceutical industries have aroused great interest in the development of a low cost techonology for xylitol production. Biotechnological processes using agroindustrial residues as the substrate for xylitol production appear to be more efficient and more economically advantageous when compared to chemical processes. It can be inferred from our results that the pretreated sugar cane bagasse hemicellulosic hydrolysate is a valuable substrate for xylitol fermentation by *Candida guilliermondii* FTI 20037.

The maximum xylitol production rates can be attained at an adequate agitation/aeration rate relationship. A suitable control of the oxygen input permitting the xylitol formation from sugar cane bagasse hydrolysate is required for the development of an efficient fermentation process for large-scale applications.

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