Fermentation of Corncob Hydrolysate for Xylitol Production

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ABSTRACT

A yeast that produces a high concentration of xylitol was isolated and identified as *Candida guilliermondii-IB*. We studied the effect of concentration of $NH_4(SO_4)_2$ (0.5; 1.0; 2.0; 3.0; 4.0 and 5.0 g/l); pH (3.0; 4.0; 5.0; 5.5 and 6.0); temperature (20; 25; 30; 40 and 45 ºC) and agitation speed (100; 200; 250 and 300 rpm) on yeast growth and xylitol production. A production medium with corncob hydrolysate supplemented with 3.0 g/l $NH_4(SO_4)_2$ and without the addition of yeast extract, was formulated. In this medium *C. guilliermondii*-IB reaches a xylose to xylitol conversion of 49 % of the theoretical maximum, a volumetric productivity of 0.23 g/lh and yields of Yp/s 0.5 g/g and Yp/x 1.5 g/g. Xylitol production optimal conditions were pH 5; temperature 30º C and an agitation speed of 250 rpm, in a rotary shaker. Corncob was the selected substrate raw matter for xylitol production because it is a low cost industrial residue and its hydrolysates contains a high concentration of xylose.

Key words: Agroindustrial residues, biotechnology, yeast, xylitol

RESUMEN

Una levadura que produce alta concentración de xilitol fue aislada de jarabe de glucosa e identificada como *Candida guilliermondii-IB*. Se estudio el efecto de la concentración de $NH_4(SO_4)_2$ (0.5; 1.0; 2.0; 3.0; 4.0 y 5.0 g/l); pH (3.0; 4.0; 5.0; 5.5 y 6.0); temperatura (20; 25; 30; 40 y 45 ºC) **y** velocidad de agitación (100; 200; 250 y

300 rpm) sobre el crecimiento de la levadura y la producción de xilitol. Se formuló el medio de producción usando hidrolizado de marlo de maíz suplementado con 3.0 g/l $NH_4(SO_4)_2$ y sin extracto de levadura. En este medio, con *C. guilliermondii-IB* se obtuvo una conversión de xilosa a xilitol del 49 % respecto al máximo teórico, una productividad volumétrica de 0.23 g/lh y rendimientos: Yp/s 0.5 g/g y Yp/x 1.5 g/g. Las condiciones óptimas de producción de xilitol fueron: pH 5.0; temperatura 30º C y una velocidad de agitación de 250 rpm en un agitador rotatorio. El Marlo de maíz fue usado como sustrato para la producción de xilitol debido a que es un residuo industrial de bajo costo y su hidrolizado contiene alta concentración de xilosa.

Palabras clave: residuos agroindustriales, biotecnología, Levadura, xilitol

INTRODUCTION

Xylitol ($C_5H_{12}O_5$) is a commercially important sugar alcohol, especially for the pharmaceutical and food industries (Magalhães *et al*., 2005). It is used in products for diabetic patients since it does not require insulin for its metabolization (Makinen, 2000). It inhibits pathogen development, thus averting ear infection and reducing adherence of *Streptococcus pneumoniae* and *Haemophilus influenzae* to pharynx and nose cells (Murthy *et al*., 2005). Fruits and vegetables, which naturally contain xylitol, are not used for xylitol extraction because their low content (less than 9 mg /g) makes manufacturing expensive (Ooi *et al*., 2002). Xylitol is currently produced by catalytic hydrogenation from commercial xylose, but the process is expensive and with low yields (60%) due to the separation of xylitol from the chemical compounds formed during manufacturing. New and more economical technologies are thus being studied. Biotechnology provides an alternative through microorganisms such as bacteria, molds and yeasts that can convert xylose into xylitol, a highly specific and economic process since 80% of the sugar is transformed into sugar alcohol. The biotechnological alternative is even more appealing when using low cost raw matter such as cellulose from agricultural residues. Corn is the third largest crop Argentina. After the grain has been removed, the cob is left as a residue and accumulates in large piles where insects and rodents thrive.

Corncob is considered a environmental pollutant since per each ha of corn sown, 6000 kg of grain and 1 ton corncob are obtained. Large quantities of biomass in the form of agroindustrial residues rich in carbohydrates are annually produced in our country, causing environmental deterioration and loss of potentially valuable resources. Through hydrolysis they yield glucose, xylose and arabinose that are assimilated by microorganisms to produce fuels and valuable chemicals (Martínez *et al*., 2002; Sreenivas Rao *et al*., 2006; Villalba Cadavid *et al*., 2009). At present, corncob is commercialized as animal feed (Martín, 2009), fuel (3600 Kcal/Kg) (Keener *et al*., 1981) and is also used in the making of particleboards (Méndez & Sotelo, 2006).

The purpose of this paper was to use corncobs as a substrate for xylitol production. For that purpose, a producer microorganism was isolated of glucose syrup samples and identified, and the culture conditions such as pH, temperature and agitation speed for maximum xylitol production were determined**.**

MATERIALS AND METHODS

Isolation and identification

In order to isolate xylitol producer microorganisms, (10 g) soil and glucose syrup samples were dissolved in 30 ml physiological solution. Then, 0.5 ml of each sample was placed in flasks containing 15 ml culture medium composed of (g/l): 10 xylose, 20 malt extract, 5 yeast extract and 15 agar (MYXA), pH 5. The samples were incubated at 30 ºC for 160 h. The colonies developed on the agar medium were isolated by depletion on the surface in Petri dishes with MYXA medium. They were incubated at 30 ºC for fungi and yeasts, and at 37 ºC for bacteria, for 5 days. Isolated colonies were collected from the cultures that showed greatest development in the above medium and plated on slants with MYXA. They were incubated at the temperatures for each microorganism for 5 days, each constituting a pure culture. Yeasts were identified using a CANDIFAST test kit (International Microbio, USA) and confirmed with IMTECH (USA); filamentous fungi according to Piontelli & Toro (1997) and bacteria by Brock & Madigan (1991). The microorganisms were kept in MYXA medium at 4 ºC during the assayed period and were regrown monthly. The strains were

kept at -20 ºC with glycerol (15%) as a cryoprotectant and are deposited in the ceparium of the Institute of Biotechnology (UNT).

Inoculum preparation and microorganism selection

In all cases the inoculum was prepared from a 24 h incubation culture. Each microorganism was added to the fresh medium until an OD 0.30 at a wave length of 560 nm corresponding to 0.75 mg/ml (dry weight) of cells was obtained. Three ml of the inoculum (10% v/v) of each microorganism was separately placed in Erlenmeyer flasks (125-ml) containing 30 ml of the synthetic medium of the following composition, in g/l: 33 xylose (Fluka, >99 % HPLC); 5 yeast extract (Merck microbiological grade); 3 $(NH_4)_2SO_4$ and 0.1 $CaCl_2x\ 2H_2O$ (Panreac, >99,9%). The medium was adjusted to pH 5 with 20 % (v/v) NaOH solution and incubated in a rotary shaker at 250 rpm, at 30 ºC for 48 h.

Preparation of the corncob hydrolysate

The hydrolysate was prepared with 200 g of ground corncobs; it was adjusted to 1000 ml with 1% (v/v) H_2SO_4 solution and heated at 100 ºC for 3 h to extract the xylose. Then it was filtered and adjusted to pH 5 with 20% (v/v) NaOH solution (Cicarelli p.a).

Determination of culture conditions

The effect of the addition of 5.0 g/l yeast extract; $(NH_4)_2SO_4$: 0.5;1.0; 2.0; 3.0; 4.0 and 5.0 g/l and $CaCl_2x\ 2H_2O$: 0.1 g/l, to the corn cob hydrolysate with 33.0 g/l xylose was studied. These assays were carried out at 30 ºC, pH 5.0 and 250 rpm in a rotary shaker. The variation of the following parameters: pH (3.0, 4.0, 5.0, 5.5 and 6.0), temperature (20, 25, 30, 40 and 45°C) and agitation speed (100, 200, 250 and 300 rpm) was also studied on yeast growth and xylitol production. The samples were taken every 12 h for 96 h in all assays and run in a rotary shaker in duplicate and in separate experiments.

Analytical methods

The cell mass concentration was estimated using a standard curve, which correlated absorbance at 560 nm and dry cell weight. Xylose, xylitol, glucose and arabinose (Sigma, >99,9% HPLC) were determined qualitatively by descending chromatography in Nº 1 Whatman

paper and butanol-pyridine-water (52:33:15) was used as solvent. Sugars were detected with an $AgNO_3$ alkaline solution according to Trevelyan *et al.* (1950). They were quantified by high performance liquid chromatography (HPLC) with a Gilson 305 model using a chromatographic interaction column (300 x 6.5 mm) Icsep OHR-801 (Irvington, NE, USA) with a refractive index detector. The samples were eluted with a solution of sulphuric acid (0.01N) pH 2.1 at a flow rate of 0.5 ml/min at room temperature. Sample peak areas were compared with areas of standard sugars such as xylose, glucose, arabinose and xylitol (Sigma, >99,9% HPLC) in a Hewlet Packard integrator (3396 SII model). The acetic acid concentration was determined with a Boehringer Mannheim kit (UV-method for the determination of acetic acid in foodstuffs and other materials). Dissolved oxygen was determined with a Horiba meter U-22/23 with a DO sensor 5460.

Fermentation parameters

Yield coefficients: $Y_{p/s}$=g/g, where P is xylitol concentration (P=g/l) with respect to initial xylose concentration (S_i =g/l). Yield coefficient, $Y_{p/x}$=g/g, with respect to the biomass concentration in dry weight (X=g/l). $Y_{x/s}$=g/g, where X is biomass concentration (g/l) with respect to consumed xylose concentration (S_c=g/l). Specific rates of xylose consumption [q_s = dS/ Xdt] and of xylitol production [q_p = dP/ Xdt] where dS =(Substrate ^{t1}-Substrate ^{t2}) and dP=(Xylitol^{t1}- Xylitol^{t2}) with respect to time ($t_1 - t_2$). Volumetric productivity: Pv (g/l h), xylitol concentration per hour (h). Efficiency (Ef) according to: Ef (%)= (Rp/Rt) x 100, Rp being xylitol concentration obtained during the process and Rt, theoretical xylitol concentration calculated with respect to initial xylose concentration (Doran, 1998).

Statistical analyses

Arithmetic mean (ϖ), standard deviation (δ^2) and variance (s^2) were determined for all the values obtained from the experiments with a confidence interval of 95%.

RESULTS AND DISCUSSION

Screening of xylitol producers

A filamentous fungus, a bacterium and three yeast strains (Lev1; Any50 and MY50) were isolated from the soil and glucose syrup samples. The microorganisms were identified as belonging to the genera *Aspergillus*,

Bacillus and *Candida*. They were used in batch experiments to determine their ability to produce xylitol from D-xylose. The results showed that Lev 1 yeast had the maximum volumetric productivity (Pv, 0.30 g/ l h) and xylitol yield ($Y_{p/s}$, 0.63 g/g; Yx/s, 0.12 g/g) at 48 h of incubation.

This yeast was identified as *Candida guilliermondii*-IB and selected as the best xylitol producer for the following experiments. The low xylitol volumetric productivity (Pv) of the yeast strains Any_{50} (Pv, 0.16 g/ lh) and My_{50} (Pv, 0.15 g/ lh) was due to the fact that xylose is metabolized for the production of cellular mass rather than xylitol, as confirmed by the increase in cellular mass yield with respect to consumed substrate, $Y_{x/s}$, 0.52 and 0.84 g/g, respectively.

The results obtained with lev 1 (*C. guilliermodii-IB*) agree with the one found for *Debaryomyces hansenii* (Yp/s= 0.69 g/g; Pv=0.28 g/lh) (Prakash *et al.,* 2011). *Bacillus* sp. is the microorganism with lowest xylitol yield ($Y_{p/s}$, 0.01 g/g) and volumetric productivity (Pv, 0.03 g/ lh) which would be due to the lack of betaine, a growth factor that improves xylitol production and secretion, as reported by Nyyssölä *et al*. (2005). *Aspergillus* produced negligible amounts of xylitol in the conditions assayed (P, 0.01 g/l). This result agrees with the one obtained by Van de Vondervoort *et al*. (2006), who showed that some *Aspergillus* strains produce xylitol concentrations lower than 1.0 g/l in xylose media.

Corncob hydrolysate

The analysis of the corncob hydrolysate proved that it had 5 times more xylose (33 g/l) than glucose or arabinose (6.8 and 6.5 g/l, respectively). These values represent an advantage for the fermentation process, since high glucose concentrations (higher than 10 g/l) inhibit xylose metabolism by prolonging the time lapse for xylitol formation due to the fact that glucose represses the synthesis of xylose reductase, an indispensable enzyme for xylitol production (Martínez *et al*., 2002). The hydrolytic process was highly selective in order to free sugars from hemicellulose together with a low concentration of acetic acid **(**0.06 g/l) in the medium.

Determination of culture conditions

Culture medium composition and nature of the carbon source influence yeast when synthesizing polyols like xylitol. Thus, the effect of synthetic medium components was studied in a

corncob hydrolysate in order to formulate the production medium. When the yeast extract was added to the hydrolysate, the other components remaining constant, there was an increase in dry cell mass (X, 5.0g/l) and a decrease in xylitol yield ($Y_{p/s,}$ 0.02 g/g) with respect to the synthetic medium, X, 2.4 g/l and $Y_{p/s,}$ 0.70 g/g, at 72 h of incubation (Figure 1).

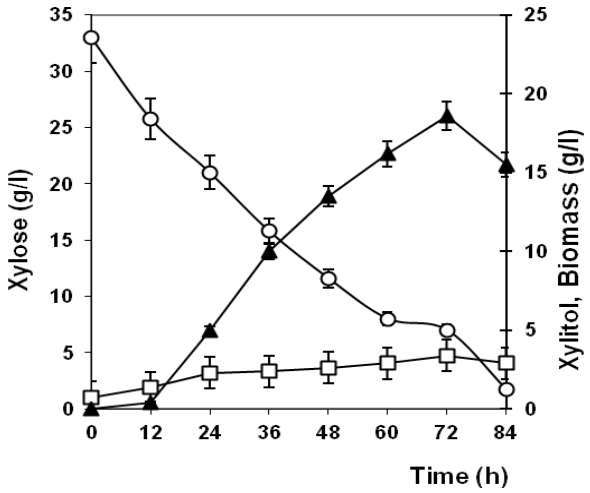

Fig. 1. Xylitol production by *Candida guilliermondii-IB* isolated in a synthetic medium, with xylose (33 g/l), supplemented with yeast extract (5 g/l); $(NH_4)_2SO_4$ (3 g/l) and $CaCl_2.2H_20$ (0.1 g/l). The assays were carried out at pH 5.0; 30 ºC and 250 rpm in a batch process. □, Biomass; ▲, xylitol; ○, xylose. The values given are means of three experiments done in duplicate. The error bars show standard deviations.

At 36 h, in the corncob hydrolysate medium with yeast extract, the specific rates of xylose consumption (q_s) and xylitol production (q_p) were as low as q_s, 0.11 g/g h and $q_{p,}$ 0.003 g/g h. After 48 h, the xylose concentration in this medium decreased to 0.5 g/l and xylitol was not detected in the medium. This suggests that under these conditions *C. guilliermondii-IB* metabolizes xylose and consequently produces less xylitol and increases cell mass. In contrast, Meyrial *et al*. (1991) reported a $Y_{p/s}$,0.59 g/g for *C. guilliermondii NRC* 5578 growing in

complex medium containing 10 g/l yeast extract.

In the corncob hydrolysate medium without yeast extract (Figure 2), maximum xylitol concentration (P, 16 g/l), volumetric productivity (Pv, 0.23 g/ l h), xylitol yield ($Y_{p/s}$, 0.51 g/g) and fermentation efficiency (E_f, 48%) reached values similar to those obtained in the synthetic medium at 72 h of incubation (P, 18 g/l; Pv , 0.26 g/l h; $Y_{p/s}$, 0.70 g/g and E_f , 54 %). This suggests that during the acid hydrolysis of the corncob there was no release of high concentrations of substances inhibitory of microbial growth (e.g. acetic acid, furfural). The concentration of acetic acid (0.06 g/l) detected in the corncob hydrolyzed medium had no toxic effect on the yeast.

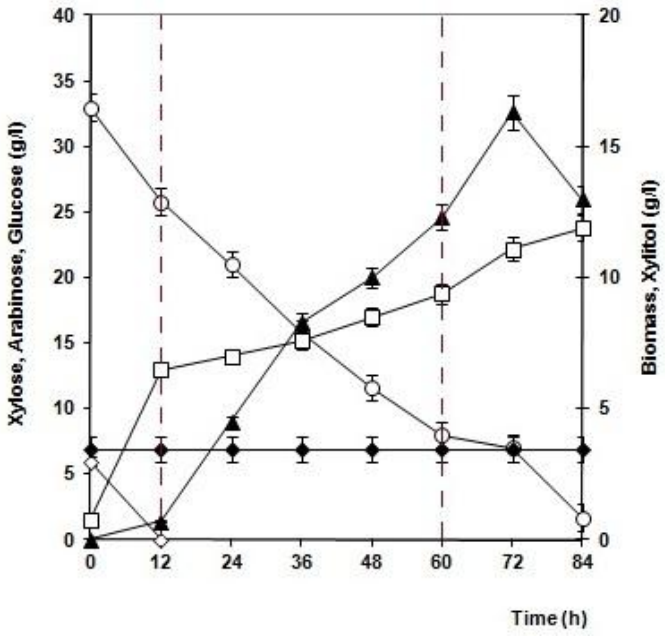

Fig. 2. Kinetics of xylitol production by isolated *Candida guilliermondii*-IB in corncob hydrolysate medium supplemented with $(NH_4)_2SO_4$ (3 g/l); $CaCl_2.2H_20$ (0.1 g/l) and without yeast extract. The assays were carried out at pH 5.0; 30ºC and 250 rpm in a batch process: □, biomass; ▲, xylitol; ○, xylose; ◆, arabinose; ◊, glucose. The values given are the means of three experiments done in duplicate. The error bars show standard deviations.

Figure 2 shows the xylitol production profiles compared to the growth curves of *C. guilliermondii*-IB in the corncob hydrolysate containing 33 g/l xylose. Three different phases can be observed. In the first phase (0 to 12h), the cellular mass increased rapidly with a high specific growth rate (μ, 0.1 h^{-1}) but low xylitol formation (P, 0.70 g/l).

At this stage, the increase in cell concentration was three times higher with respect to the synthetic medium (X, 1.3 g/l) since during the first 12 h yeast used all the glucose besides other carbon sources (mannose; galactose and arabinose) eventually present in the hydrolysate. At this time, low specific rates of xylose consumption (q_s, 0.03 g/ gh) and xylitol production (q_p, 0.01 g/g h) were obtained. During the second phase (12 to 60 h), rapid xylitol accumulation (P, 12.3 g/l) was associated with slower specific growth rate (μ, 0.02 h^{-1}) and maximum consumption of xylose (Sc, 21 g/l) was observed. In contrast, cell concentration increased slightly from 6.0 g/l (12h) to 9.0 g/l at 60 h of incubation. This indicates that during this phase a greater fraction of the xylose consumed was diverted to xylitol accumulation. The latest phase (60 to 84 h) was characterized by a slight increase in the average specific rate of cell growth (μ, 0.025 h^{-1}) and maximum xylitol concentration (P, 16.3 g/l at 72 h of incubation), associated with a slight increase of q_p (0.023 g/g h). After 72 h of incubation, when the xylose concentration decreased to 3.2 g/l, a 23.3 % of xylitol secreted in the medium was assimilated by the yeast (Figure 2). This suggests that the yeast, to fulfill its metabolic needs, consumes the initially produced xylitol when xylose levels do not meet minimum necessary requirements as observed in both media (synthetic and corncob hydrolysate). According to Girio *et al*. (1996), yeast usually uses xylitol first and then arabinose. In our case, the *C. guilliermondii*-IB isolated did not use arabinose (Figure 2) until the end of the fermentation (120 h, not shown); similar results were obtained with *D. hansenii* (Sampaio *et al*., 2005).

Effect of ammonium sulphate on xylitol production

The effects of different concentrations of $(NH_4)_2SO_4$ on the xylitol volumetric productivity and product conversion yields ($Y_{p/s}$ and $Y_{p/x}$) are shown in Figure 3.

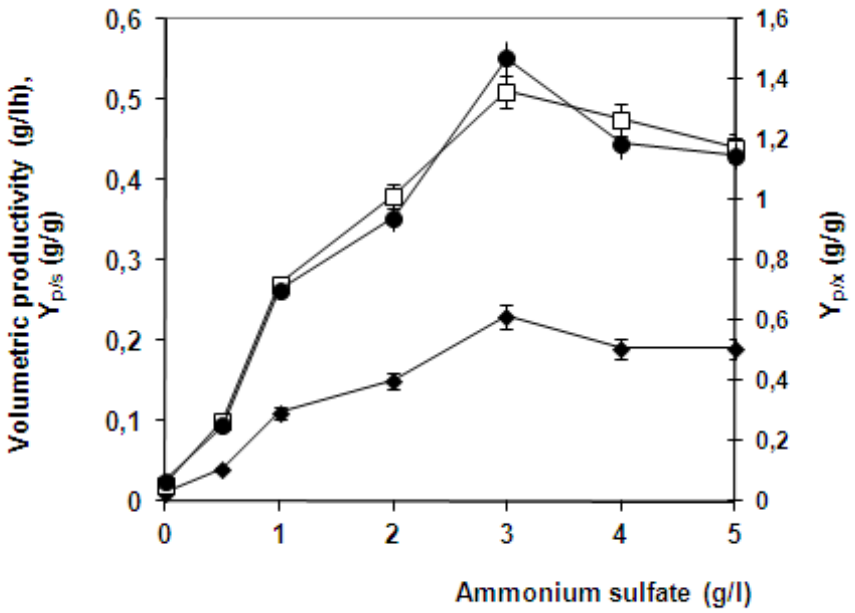

Fig. 3: Effect of $(NH_4)_2SO_4$ concentration on volumetric productivity (g/ lh) (◆); xylitol yields with respect to the substrate ($Y_{p/s}$) (□) and to biomass ($Y_{p/x}$) (●) by *Candida guilliermondii*-IB in the corncob hydrolysate medium. Table values were registered at 72 h of incubation and they are the means of three experiments done in duplicate. The error bars show standard deviations.

The xylitol volumetric productivity increased with the supplemented concentration of $(NH_4)_2SO_4$ within the range from 0 to 3 g/l (Figure 3), and product conversion yields ($Y_{p/x}$ and $Y_{p/s}$) were also increased. Maximun xylitol concentration (P,16.3 g/l), productivity (0.23 g/l h) and yields ($Y_{p/x}$ 1.5 g/g and $Y_{p/s}$ 0.51g/g) were obtained with 3.0 g/l of $(NH_4)_2SO_4$ at 72 h of incubation.

However, when $(NH_4)_2SO_4$ concentration was higher than 3.0 g/l a decrease of 22% in xylitol productivity was observed (Figure 3). When nitrogen concentration increases in the medium, biomass synthesis is favored; similar results were obtained with the addition of yeast extract to the corncob hydrolysate. Without $(NH_4)_2SO_4$ supplementation, xylitol concentration dropped to 95.6% of the theorical maximun (3.0 g/l of

$(NH_4)_2SO_4$). The addition of $CaCl_2$ x 2 H_2O to the corncob hydrolysate affected neither xylitol production nor yeast growth.

Effect of pH, temperature and agitation speed

The effect of the initial pH on the xylose/xylitol conversion by *C. guilliermondii*-IB grown in corncob hydrolysate medium is summarized in Table 1. The highest values of xylitol concentration and maximum yield ($Y_{p/s}$ 0.51 g/g), were observed at pH 5.0. This yield is 15.6% higher than to the one found for *D. hansenii* ($Y_{p/s}$ 0.43 g/g at pH 5.0; 30 ºC and 200 rpm) and it is similar for *Candida tropicalis* ($Y_{p/s}$ 0.57 g/g, at pH 5.0; 30 ºC and 250 rpm). In the latter, this value was reached at 48 h incubation with a previous adaptation to 20 yeast cycles in a sugar cane bagasse hydrolysate medium (Sampaio *et al*., 2005; Sreenivas *et al*., 2006). In contrast, a low yield ($Y_{p/s}$ 0.08 g/g) was found with *C. guilliermondii* in a rice bran medium at pH 5.0 and at 96 h of incubation (Villalba Cadavid *et al*., 2009).

Table 1: Effect of pH on growth and xylitol production by *C. guilliermondii*.-IB in corncob hydrolysate medium with 3 g/l $(NH_4)_2SO_4$. The assays were carried out at 30ºC and 250 rpm in a batch process. Table values were registered at 72 h of incubation.

pH	cell mass (g/l d.w[a])	Xylose [(c)] (g/l)	Xylitol (g/l)	$Y_{p/s}$ (g/g)	$Y_{p/x}$ (g/g)	Pd (g/ lh)
3.0	2.66 ±0.12	9.70 ±0.30	2.70 ±0.10	0.10 ±0.01	1.10 ±0.1	0.04±0.1
4.0	2.81 ±0.10	11.00 ±0.12	3.10 ±0.05	0.10 ±0.01	1.10 ±0.2	0.04±0.1
5.0	11.12 ±3.00	30.00 ±1.12	16.30 1.30	0.51 ±0.01	1.50 ±0.2	0.23±0.1
5.5	13.00 ±2..30	31.70 ±1.60	9.20 ±0.30	0.30 ±0.01	0.70 ±0.1	0.13±0.1
6.0	2.70 ±0.50	31.80 ±1.20	1.30 ±0.20	0.03 0.01	0.40 ±0.1	0.03±0.1

[a] dry weight; [c], xylose consumed; Yp/s and Yp/x product yields. Values in the table refer to mean; ± standard deviation

At pH 5.5, the cellular mass increased (X, 13 g/l), but xylitol production decreased 44% with respect to the value found at pH 5.0 (Table 1). These conditions favor the growth and fast assimilation of xylose ($S_{c,}$ 32 g/l) at the expense of a decrease in the specific production rate of xylitol (q_p, 0.01 g/g h). Later on, xylitol concentration in the medium decreased when it was used instead of arabinose for cell growth.

At pH 3.0 and 4.0, the low xylose consumption observed (Table 1) could be due to that at low pH there is a higher hydrogen-ion concentration. According to Brock & Madigan (1991), these ions enter the cell membrane diminish intracellular pH and influence enzymes responsible for xylose metabolism. At pH 6.0, xylitol concentration diminished 88% with respect to the maximum (pH 5.0) and there was low cell growth. These results differ from the ones obtained with *C. guilliermondii* FTI 20037*,* where higher xylitol yields were found at pH 8.0 (Martínez *et al*., 2003)

Optimal temperature for xylitol production by *C. guilliermondii*–IB was 30 ºC. At this temperature, maximum product yield with respect to biomass formed ($Y_{p/x}$ 1.50 g/g) and substrate utilized ($Y_{p/s}$ 0.51 g/g) were obtained at 72 h of incubation. In contrast, after incubation for 12 h, a high biomass yield ($Y_{x/s}$ 2.60 g/g) was obtained, associated with low xylitol concentration (P, 0.56 g/l). At this time, the consumption of the carbon source (glucose, 6.80 g/l and xylose, 2.40 g/l) is diverted to cell growth, as indicated by the low xylitol yield ($Y_{p/s}$ 0.10 g/g). Later, growth decreases and the substrate is consumed mainly for the production of xylitol, increasing its yield ($Y_{p/x}$). The values obtained agree with the results found for *Pachysolen tannophilus* (Sánchez *et al*., 2004). At 25 ºC, lower biomass ($Y_{x/s,}$ 0.4 g/g) and product ($Y_{p/x,}$ 0.6 g/g) yields were obtained, while at 35 ºC product yield ($Y_{p/x}$, 0.67 g/g) was 45% lower than at 30 °C. This is because a rapid disappearance of xylose from the medium (residual xylose, 0.30 g/l at 60 h of incubation) and the xylitol produced is used as a carbon source (from 7.30 g/l at 60 h to 5.60 g/l at 72 h of incubation). At 20 °C the lowest xylitol concentration (P, 0.70 g/l) and cell mass (X, 1.50 g/l) were obtained, while the yeast did not grow at 40 ºC to 45 ºC, unlike *Candida sp*, whose maximum xylitol production occurs at 40 ºC (Vanegas Córdoba *et al*., 2004).

Agitation is important in media growth since it increases oxygen dissolution, thus creating an aerobic environment.

Oxygen concentration in the medium influences xylitol production and this effect varies with yeast genus and species (Girio *et al*.,1996; Yun *et al*., 2010). The results concerning the effect of agitation speeds are shown in Table 2. Maximum values of xylitol productivity and concentration are achieved at 250 rpm.

Table 2: Effect of agitation speed on xylitol production, Productivity (Pv) and yield ($Y_{p/s}$) in a batch process. The agitation assays were performed in corncob hydrolysate medium with 3 g/l $(NH_4)_2SO_4$ at 30ºC and pH 5. Table values were registered at 72 h of incubation.

	Agitation speed (rpm)			
	100	200	250	300
Xylitol (g/l)	0.10 ±0.01	11.50 ±0.2	16.3 ±0.3	14.60 ±0.25
P_d (g/ lh)	0.01±0.001	0.16 ±0.01	0.23±0.01	0.20 ±0.01
$Y^a_{p/s}$ (g/g)	0.02 ±0.001	0.50 ±0.02	0.51±0.01	0.44 ±0.02

[a] Xylitol yield with respect to substrate consumed. Values in the table refer to mean; ± standard deviation

At 100 rpm, the culture is in micro aerobic conditions and the yeast growth and xylitol concentration were rather low, 1.0 g/l and 0.10 g/l, respectively. When agitation increased to 200 rpm, cell mass increased fivefold and xylitol production was 115 times higher in relation to 100 rpm. This indicates that aerobic conditions are beneficial for growth and xylitol production in our *C. guilliermondii-IB* isolate. However, at 300 rpm xylitol concentration decreased a 10.42% with reference to the maximum obtained (Table 2). At this speed, agitation increases oxygen dissolution (0.6 mmol/l) in the culture, favoring cell growth associated with fast xylose assimilation (S_c, 32.0 g/l at 72 h of incubation) to the detriment of xylitol formation; in this case, xylitol is oxidized to xylulose by xylitol dehydrogenase, thus diminishing metabolite excretion to the medium.

These results agree with those found in *Candida parapsilosis* which increased xylitol conversion into xylulose in aerobic conditions (Kim *et al*., 1997). The latter is an intermediate sugar in the xylose metabolism, indispensable for cell material production.

CONCLUSIONS

In corn producing countries, a residue is obtained such as corncob, with a high xylan content that is not effectively utilized. This highly available and low cost residue was used in this work as raw matter providing fermentable sugars such as xylose and glucose, to obtain a product with added value such as xylitol. Out of all the xylitol producer microorganisms isolated in this paper, *C. guilliermondii*-IB showed the best xylose growth and xylitol yield, $Y_{p/s}$ (0.70 g/g) in a synthetic medium with reference to the other microorganisms isolated. In the corncob hydrolysate, *C. guilliermondii*-IB achieved a xylose to xylitol conversion of 49% out of the theoretical maximum with an volumetric productivity of 0.23 g/ lh, yields: $Y_{p/s}$, 0.51 g/g and $Y_{p/x}$, 1.50 g/g, at pH 5.0, 30 ºC, 250 rpm and 72 h of incubation. These results were obtained with the corncob hydrolysate formulated without the addition of yeast extract and with the supplementation of 3.0 g/l $(NH_4)_2SO_4$, unlike the synthetic medium where the supplementation of growth factors is necessary. This makes corncob an inexpensive raw matter suitable for xylitol production.

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