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KINETICS OF LACTIC ACID FERMENTATION FROM SUGARCANE BAGASSE BY *Lactobacillus pentosus*

CINÉTICA DE FERMENTACIÓN DE ÁCIDO LÁCTICO A PARTIR DE BAGAZO DE CAÑA DE AZÚCAR POR *Lactobacillus pentosus*

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Abstract

Sugarcane bagasse is a waste product of the sugar industry and displays a high content of hemicelluloses, making it one of the most abundant and available agricultural residues in Mexico with a great potential for use in fermentations. The aim of this investigation was to develop fermentation kinetics to biotechnologically obtain lactic acid from sugarcane bagasse hydrolysates, concentrated and not concentrated with *L. pentosus*. The bagasse was characterized and chemically treated to obtain samples of hydrolysates, which were supplemented with nutrients and fermented with *Lactobacillus pentosus* to obtain lactic acid. The hydrolysates were evaporated in a vacuum to increase the initial concentration of sugars up to 7.77 gL^{-1} of glucose; 64.25 gL^{-1} of xylose and 3.67 gL^{-1} of arabinose were obtained. Under these conditions, lactic acid values reached 55.437 gL^{-1} (Q_P= 0.430 $gL^{-1}h^{-1}$, $Y_{P/S}$ = 0.724 gg^{-1}; $Y_{P/S}$ theoretical=81.43%) observing a clear inhibition of the product after 120 hours.
Keywords: Lignocellulosic biomass, hemicellulosic sugar, hydrolysates, lactic acid.

Resumen

El bagazo de caña de azúcar es un residuo de la industria azucarera, que presenta un alto contenido de hemicelulosas, siendo uno de los residuos agrícolas más abundantes y disponibles en México con gran potencial para ser utilizado en fermentaciones. El objetivo de esta investigación fue el desarrollo de cinéticas de fermentación para la obtención biotecnológica de ácido láctico a partir de hidrolizados de bagazo de caña de azúcar concentrados y sin concentrar con *L. pentosus*. El bagazo se caracterizó y se trató químicamente para obtener muestras de hidrolizados, los cuales se complementaron con nutrientes y se fermentaron con *Lactobacillus pentosus* para la obtención de ácido láctico. Los hidrolizados se evaporaron al vacío para aumentar la concentración inicial de azúcares hasta 7.77 gL^{-1} de glucosa, 64.25 gL^{-1} de xilosa y 3.67 gL^{-1} de arabinosa. En estas condiciones, se alcanzaron valores de ácido láctico de 55.437 gL^{-1} (Q_P= 0.430 $gL^{-1}h^{-1}$, $Y_{P/S}$ = 0.724 gg^{-1}; $Y_{P/S}$ teórico= 81.43%) observando una clara inhibición del producto después de 120 horas.
Palabras clave: Biomasa lignocelulósica, azúcares hemicelulósicos, hidrolizados, ácido láctico.

1 Introduction

Mexico is a privileged country for the sugarcane sector, because it integrates a large diversity of agricultural activities, from sugarcane growth, harvest, and transportation, to the production of standard and refined sugar, with other by-products like harvest residues, bagasse, molasse, cachaza, or mud filters and musts or cane vinasse; this is of great importance as a renewable source of energy and raw materials, as well as for the diversification and adaptation of production systems (Aguilar *et al*., 2011; Anaya-Reza and López-Arenas, 2018; Basanta *et al*., 2007).

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The lignocellulosic materials are renewable, low-cost, abundant, organic resources with a high capacity for biodegradability (Singh *et al*., 2009; Simas-Dias *et al*., 2018), currently subjects of study for many researchers for their use and application in the production of hydrocarbons (Suleiman, 2010; Saha *et al*., 2014; Faba *et al*., 2014), for the generation of energy (Cuervo *et al*., 2009; Simas-Dias *et al*., 2018), and this leads to a debate about its sustainability (Gorter *et al*., 2013). The main residue derived from the processing of sugarcane is bagasse, which is obtained in large quantities after extracting the juice from the cane (Pérez *et al*., 2011). Approximately 1.6 million tons of sugarcane from the sugar industry generates 493 million metric tons of bagasse (Khattab and Watanabe, 2019) annually.

The sugarcane bagasse (*Saccharum officinarum* L.) is a source of lignocellulosic biomass with a high potential for exploitation, since it is composed of approximately 50% cellulose, 25% hemicellulose, and 25% lignin (Martínez *et al*., 2002; Simas-Dias *et al*., 2018), which, when subjected to a chemical and/or enzymatic process that is fractioned to obtain the solutions of sugar that, when supplemented with nutrients, can be used as a fermentation medium in the production of food additives such as lactic acid, which increases the economy of the process. (Bustos *et al*., 2004a; Verde *et al*., 2006; Laopaiboon *et al*., 2010).

An interesting alternative is to develop an economically viable bioprocess without using modified bacteria strains. *Lactobacillus pentosus* can be considered a facultative heterofermentative organism, degrading hexoses (glucose) via the Embden-Meyerhoff-Parnas pathway and pentoses (xylose and arabinose) via the phosphoketolase pathway (Bustos *et al*., 2018).

Lactic acid or α-OH-propionic acid, with the formula $CH_3CHOHCOOH$, was isolated and identified in 1780 by Swedish chemist C.W Sheele in curdled milk and acknowledged as a product of fermentation by Blondeaur in 1847 (Parés and Juárez, 2002). Its industrial production started in the USA in 1881 and in Germany in 1895, with an approximate world production of 300,000 at 367,300 t/year (Abdel-Rahman *et al*., 2013; Blanco *et al*., 2016). Regarding the demand for this compound, it is estimated to grow at an annual rate of 16.2% from 2017 to 2025 (Espinel-Ríos *et al*., 2019).

Specialized bibliographic references mention that more than 50% of the lactic acid produced in the world is used in the food industry as an acidifier and food preservative, since it prevents the growth of *Salmonella* and *Staphylococcus aureus* (Salminen and Von Wright, 1993; Reyes *et al*., 2018), as well as in the production of emulsifying agents (Bustos *et al*., 2004a; Serna and Stouvenel, 2005; Bustos *et al*., 2005a; Moldes *et al*., 2007; García *et al*., 2010; Laopaiboon *et al*., 2010; Ghaffar *et al*., 2014; Wang *et al*., 2015; Espinel-Ríos *et al*., 2019). Approximately 20% is used in the production of Steaoryl-2-lactuylate, and the rest is used in the pharmaceutical industry (Narayanan *et al*., 2004; Bustos *et al*., 2005a), cosmetics (Vick Roy, 1985; Narayanan *et al*., 2004; Bustos *et al*., 2005b) and in polymerization into biodegradable polylactic acid (PLA) (Bustos *et al*., 2004b, Laopaiboon *et al*., 2010; Narayanan *et al*., 2014; Anaya and López, 2018), used in medical applications such as stitches, and clips for closing wounds or prosthetic devices. Its biotechnological production on an industrial scale represents potential advantages with the use of cost-competitive fermentation technologies with chemical synthesis (Moldes *et al*., 2007; Randhawa *et al*., 2012). Approximately 90% of the lactic acid in the world is produced using a biotechnological route (Hofvendahl and Hhan-Hägerdal, 2000; Espinel-Ríos *et al*., 2019). In addition, the tendency to replace the chemical process with biotechnology is currently predominant, since consumers prefer a natural product to a chemical.

Considering that most of the efforts are focused on the valuation of the fraction of the lignocellulosic material, the aim of this research was to take advantage of the hemicellulose sugars present in the liquid fraction obtained after the acid hydrolysis of the sugarcane bagasse, using *L. pentosus*, since it is a microorganism capable of fermenting both sugars via Embden-Meyerhof-Parnas, and the phosphoketolase pathway, respectively, to produce lactic acid. For this reason, in order to verify the ability to grow in the presence of these materials, the hemicellulosic sugars of the cane bagasse were fermented by adding different concentrations of hydrolysates.

2 Materials and methods

2.1 Material

The sugarcane bagasse was provided by Ingenio Aarón Sáenz Garza from Ciudad Mante, Tamaulipas, Mexico; it was dried at 105 ºC for 12 h, grinding, and sieving to a particle size below 1 mm. It was later, it was stored in closed containers at room temperature to avoid moisture fluctuations until use.

2.2 Analysis of sugarcane bagasse

The physical and chemical characterizations of sugarcane bagasse were carried out following the official Mexican standards NMX-F-083-1986 for the determination of humidity at 105 ºC for 24 h, the NMX-F-066-S-1978 for the determination of ashes at 550 ºC for 8 h and Browning's Quantitative Acid Hydrolysis (QAH) protocol (1967) to determine cellulose, hemicellulose, and Klason lignin (solid residue) contents; aliquots from the homogenized lots of raw materials and solid residue were subjected to moisture determination and to quantitative hydrolysis in a two-stage acid treatment (the first step with 72% sulfuric acid at 30 ºC for 1h, and the second one after dilution of the media to 4% sulfuric acid at 121 ºC for 1h). The solid residue after hydrolysis was found to be Klason lignin.

2.3 Acid hydrolysis

After the physical conditioning of the lignocellulosic biomass, the cane bagasse treatment was carried out, using a solid:liquid ratio of 1:8 (w/w) with sulfuric acid at 2% and at 122 °C with a reaction time of 24 min, previously developed by Aguilar *et al*., (2002).

The hemicellulosic hydrolysate obtained was separated from the fibrous material by vacuum Filtration in a roto-evaporator (Rotary Evaporator Mod. H5-2001NS. Nae-dong, South Korea) at 50 °C to reach final volumes of approximately 75% and 50% of the initial ones ([final volume]/[initial volume] ratios of 1/1.33 or 1/2 respectively), in order to increase the concentration of fermentable sugars, mainly xylose.

2.4 Microorganism

The *Lactobacillus pentosus* CECT-4023T (ATCC-8041) was obtained from the Spanish Type Culture Collection (CECT) (Valencia, Spain). Before the process of fermentation, the strain was kept at 35 °C for 24 h in plates using the complete medium proposed by Mercier *et al*., (1992) or the MRS (SIGMA ALDRICH 69964-500G) medium containing: (10 gL^{-1} peptone, 10 mL^{-1}, meat extract, 5 gL^{-1} yeast extract, 20 gL^{-1} glucose, 2 gL^{-1} $C_6H_{17}N_3O_7$, 5 gL^{-1} $C_2H_3NaO_2$, 2 gL^{-1} K_2HPO_4, 0.2 gL^{-1} $MgSO_4$-$7H_2O$, 0.2 gL^{-1} $MnSO_4{\cdot}4H_2O$ and 20 gL^{-1} of agar). The inoculum was prepared by solubilizing cells in plates with 5 mL of sterilized hydrolysate (final concentration in the inocula of 4.0 gL^{-1}). The biomass in the inoculum was measured by optical density at 600 nm using a spectrophotometer.

2.5 Fermentation of lactic acid

After the acid hydrolysis process, the bagasse hydrolysates were neutralized with $CaCO_3$, with a final pH of 6.5, and the precipitated $CaSO_4$ was separated from the supernatant by filtration. The neutralized hydrolysates were supplemented with 10 gL^{-1} of yeast extract and 10 gL^{-1} of sterilized Corn Steep Liquor, used directly as a fermentation medium (Vecino *et al*., 2012; Vecino *et al*., 2018). The experiments were carried out in 250 mL Erlenmeyer flasks with a final volume of 100 mL to the fermentation medium. $CaCO_3$ (30 gL^{-1}) was added to neutralize the lactic acid produced. The fermentation process was carried out in an orbital shaker (Mark MRC Model TU-400, Israel) at 150 rpm and 35 ºC, selecting 2 mL samples at different times of fermentation. The collected samples were centrifuged at 6000 rpm for 3 minutes. The experiments were performed in duplicate to increase the accuracy of the results.

2.6 Analytical methods

Glucose, xylose, arabinose, acetic acid and lactic acid were measured by HPLC using a Transgenomic ICSepICE-ION 300 column (mobile phase H_2SO_4 0.0025 N, flow of 0.5 mL/min, up to 37 °C, and an IR detector of 40 °C). Likewise, the content of inhibitor compounds released after hydrolysis was determined by a UV-Vis spectrophotometric analysis to determine furfural and hydroxymethylfurfural using a spectrophotometer (PerkinElmer lambda 35, Shelton, EUA) at wavelengths of 230 nm and 260 nm respectively.

2.7 Estimation of the lactic acid production and sugar consumption

Mercier's model was applied to determine the constants P, $Y_{P/S}$ and Q_S in the process of fermentation. The commercial software Microsoft Excel Solver 2013 was used to fit the experimental data to proposed models by nonlinear regression using the least-squares method. Lactic acid production was mathematically modeled following the equation proposed by Mercier *et al*. (1992).

$$P = \frac{P_0 P_m e^{P\gamma t}}{P_m - P_0 + P_0 e^{P\gamma t}} \quad (1)$$

where t is time, P is lactic acid concentration, P_m is maximum concentration of lactic acid, and P_γ is the ratio between the initial volumetric rate of product formation (r_p) and the initial product concentration is P_0. From the series of experimental data lactic acid concentration/time, the model parameters P_0, P_m, and P_γ can be calculated for each fermentation medium.

Glucose and Xylose consumption by *L. pentosus* strains can be interpreted from eq. (2)

$$S = S_0 - \frac{1}{Y_{P/S}}(P - P_0) \quad (2)$$

where $Y_{P/S}$ is the product yield, P_0 is the initial lactic acid concentration (gL^{-1}), P is the lactic acid concentration (gL^{-1}) for each time predicted for eq. (1), S_0 is the initial concentration of glucose (gL^{-1}), and S is the experimental glucose concentration (gL^{-1}) for each time. The model parameter $Y_{P/S}$ was calculated for each fermentation medium from the series of experimental data glucose concentration/time, P_0, and the P value for each time calculated from the regression parameters of Eq. (1).

3 Results and discussion

3.1 Characterization of sugarcane bagasse

As part of the chemical characterization of sugarcane bagasse, we determined the percentage of moisture and ashes, obtaining results of 9.36 ± 0.002 and 3.03 ± 0.1 respectively. It was possible to quantify its structural composition by reporting percentages of cellulose, hemicellulose, lignin, and other components present in the materials through a QAH (Table 1).

The results reached by QAH were higher to those reported by Torres *et al.* (2017), who obtained concentrations of 32.7, 21.3, 15.3, 1.3% of cellulose, hemicellulose, lignin, and other components respectively, also from sugarcane bagasse.

Table 1. Chemical composition of sugarcane bagasse.

Fraction	Content (%)
Cellulose	34.47 ± 0.50
Hemicelluloses	29.73 ± 0.98
Xylan	28.10 ± 0.88
Araban	1.63 ± 0.15
Acetyl groups	0.123 ± 0.02
Lignin	35.41 ± 0.37
Other	0.39 ± 0.91

Data indicate the mean values of three replications and their standard deviations.

Therefore, the high content of carbohydrates present in sugarcane bagasse makes it possible to state that this is an agro-industrial waste with a great potential for transformation into product with added value such as lactic acid. Valenzuela-Cobos *et al.* reported the chemical composition of the substrate (wheat straw and a mixture of oak sawdust) with a proximal analysis using acid detergent fiber (ADF) and neutral detergent fiber (NDF) using the methodology of (Gaitán-Hernández *et al.*, 2006).

3.2 Use of non-concentrated hydrolysates

To obtain hydrolysates rich in fermentable sugars, the bagasse is subjected to an acid hydrolysis with sulfuric acid (Aguilar *et al.*, 2002). The acid hydrolysis of this material lignocellulose leads to the production of the hemicellulosic sugar xylose, and glucose as the main components. At the same time, it leads to the formation of a complex mix of microbial toxins, (Pérez-Cadena *et al.*, 2018), being one of the mayor problems in relation with a mayor biotechnological transformation, showing a slow kinetics, limited productivity and performance in comparison with the fermentation media conducted from the commercial sugars or hydrolysates with less concentration of inhibitors (Bustos *et al.*, 2004a). Table 2 shows the composition of the hydrolysates at different levels of concentration.

Table 2. Composition of synthetic media and hydrolysates obtained at different concentration levels.

		Xylose (gL^{-1})	Glucose (gL^{-1})	Arabinose (gL^{-1})	Acetic acid (gL^{-1})	Furfural (gL^{-1})	HMF[b] (gL^{-1})
Medium 1	FV/IV[a] ratio: 1/1	30.77 ± 0.15	3.34 ± 0.17	0.20 ± 0.15	3.83 ± 0.13	1.71 ± 0.01	1.24 ± 0.09
Medium 2	FV/IV ratio: 1/1.33	48.22 ± 0.87	5.80 ± 0.13	0.26 ± 0.03	5.57 ± 0.19	2.51 ± 0.21	1.72 ± 0.32
Medium 3	FV/IV ratio: 1/2	64.25 ± 0.17	7.77 ± 0.27	3.67 ± 0.32	6.99 ± 0.26	1.56 ± 0.26	2.27 ± 0.20
Medium 4	Synthetic medium	65.20 ± 0.62	7.66 ± 0.16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

[a]Final volume/initial volume ratio, [b]Hidroximetilfurfural.

Table 3. Stoichiometric parameters productivities and yields for test of bioconversions for *L. pentosus*.

Parameter	FV/IV ratio: 1/1	FV/IV ratio: 1/1.33	FV/IV ratio: 1/2	Synthetic medium
Xylose$_{T=0}$ gL^{-1}	29.093 ± 0.849	47.052 ± 0.466	63.964 ± 1.027	65.200 ± 0.629
Xylose$_{TF}$ gL^{-1}	0.000 ± 0.13	1.191 ± 0.675	6.772 ± 0.109	0.501 ± 0.109
Glucose$_{T=0}$ gL^{-1}	3.745 ± 0.119	6.180 ± 0.248	8.134 ± 0.233	7.662 ± 0.166
Glucose$_{TF}$ gL^{-1}	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.000
Lactic acid$_{T=0}$ gL^{-1}	2.522 ± 0.584	2.150 ± 0.074	3.726 ± 0.143	2.281 ± 0.072
Lactic acid$_{TF}$ gL^{-1}	30.178 ± 0.689	44.866 ± 0.036	55.437 ± 1.629	60.963 ± 3.722
Acetic acid$_{T=0}$ gL^{-1}	4.113 ± 0.243	6.342 ± 0.010	6.219 ± 0.128	0.000 ± 0.000
Acetic acid$_{TF}$ gL^{-1}	11.588 ± 0.165	12.441 ± 0.274	17.241 ± 0.185	8.342 ± 0.167
Time (h)	72	102	120	96
Q_P (gL^{-1}h^{-1})	0.369	0.418	0.43	0.6112
Q_S (gL^{-1}h^{-1})	0.454	0.5102	0.594	0.753
$Y_{P/S}$ (gg^{-1})	0.811	0.82	0.724	0.81
Theoric yield (%)	93.172	88.352	81.437	87.97

Q_P=volumetric productivity of lactic acid; Q_S = sugar consumption; $Y_{P/S}$ = lactic acid yield (g lactic acid produced g glucose+xylose consumed); Theoretic yield= Lactic acid produced×100/ (xylose consumed×0.6+glucose consumed).

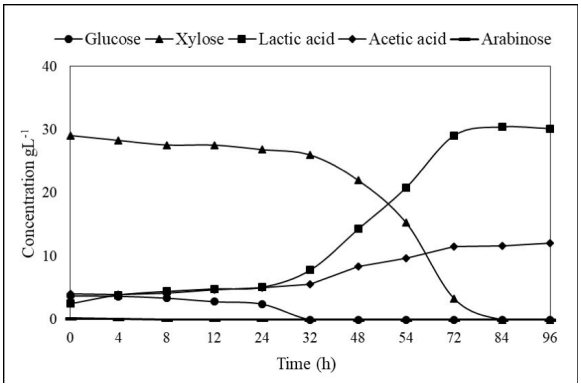

Fig. 1. Dynamics of the fermentation for experiments conducted with hydrolysates not concentrated and *L. pentosus*.

When the hydrolysates were not concentrated (medium 1), the media composition was of 30.77 g of xylose/L; 3.34 g of glucose/L and 0.20 g of arabinose/ L. Laopaiboon *et al*. (2010) reached concentrations of xylose, 22.59 gL^{-1} whereas González *et al*. (2017) obtained 24.79 gL^{-1} of xylose and Tizazu and Moholkar (2018) that reported values below the ones reached in this study with a concentration of xylose of 9.2 gL^{-1} with sugarcane bagasse.

Figure 1 shows the profile of the production of organic acid and the consumption of carbon sources by *L. pentosus*. As shown, the glucose was exhausted in the first 32 hours of fermentation, while the xylose was consumed more slowly, showing similar tendencies to the ones observed by Bustos *et al*., 2004b and 2005a with fermentation media in a sugar mixture from the trimmings of vine shoots.

The fermentation kinetics of Figure 1 shows that it can prepare suitable fermentation media from the hydrolysates of sugarcane bagasse, reaching a concentration of lactic acid of 30.178 gL^{-1} after 72 h of fermentation that represents a volumetric productivity (Q_P) of 0.369 gL^{-1}h^{-1}, a product yield ($Y_{P/S}$) of 0.811 gg^{-1} and a theorical yield ($Y_{P/S\,th}$) of 93.172%, taking into account the stoichiometry of the sugars consumed: glucose and xylose. These results are particularly interesting if you consider the absence of treatments of detoxification during the preparation of the hydrolysates.

These results also improve those obtained by Wischral *et al*. (2019), who assessed the optimal conditions utilizing hydrolysates of sugarcane bagasse for the production of lactic acid for *L. pentosus* producing 19.17 gL^{-1} of lactic acid in 48 h, with a performance of 0.80 gg^{-1}, corresponding to a volumetric productivity of 0.40 gL^{-1}h^{-1}. On the other hand, Espinel-Ríos *et al*. (2019) reached relatively lower concentrations: up to 4.2 g/L^{-1} with 67.6 g/L^{-1} initial sugars.

Table 3 presents the data related to the production of lactic acid in the fermentation of batches conducted in Erlenmeyer bottles, also indicating values of acetic acid reaching up to 11.588 gL^{-1}, which indicates that the main sub product obtained was acetic acid.

3.3 Use of concentrated hydrolysates

Once the fermentation of hemicellulosic hydrolysates was completed by *L. pentosus* with no inhibition observed, the effect of the initial hydrolyzate

concentration on the lactic acid production was studied. When the hydrolysates were concentrated 1.33 times, the initial concentration of hemicellulosic sugars increased to 54.28 gL^{-1} (Medium 2, Table 2). In this case, the time of fermentation was longer than in the non-concentrated hydrolysates. The sugars were consumed after 102 h of fermentation, observing a slight inhibition by the substrate in the media. The final concentration of lactic acid reached 44.866 gL^{-1}, Q_P= 0.419 $gL^{-1}h^{-1}$, $Y_{P/S}$= 0.820 gg^{-1} and a theoretical yield $Y_{P/S\,th}$= 88.352%). Figure 2 shows the temporary evolution of the sugar consumption and the product formation. Once again, it was observed that glucose was the first sugar to be consumed, followed by xylose, observing that the arabinose was not consumed by *L. pentosus*. The lactic acid was produced completely after 102 h, while the remaining xylose was used for the formation of acetic acid. The acetic acid began its production after the glucose was consumed (24 h) and increased continuously up to 96 h.

Based on the results obtained, the hydrolysates were concentrated twice, until reaching an initial concentration of sugars of 75.69 gL^{-1} (medium 3, Table 2). Figure 3 shows the kinetics achieved in this fermentation, observing a slight inhibition in the production of lactic acid around 55.437 gL^{-1}, because although the glucose was consumed completely after 32 hours, the xylose was consumed slowly for 120 h. The fermentation parameters indicated in Table 3 are almost equal to those reached in the previous case, with a final concentration of lactic acid of 55.437 gL^{-1}, Q_P= 0.430 $gL^{-1}h^{-1}$, $Y_{P/S}$ = 0.724 gg^{-1}; and a theoretical yield $Y_{P/S\,th}$= 81.437.

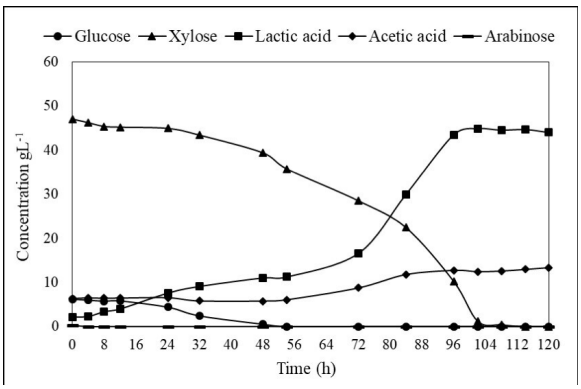

Fig. 2. Fermentation dynamics for experiments performed with concentrated hydrolysates at a FV/IV ratio: 1/1.33, using *L. pentosus*.

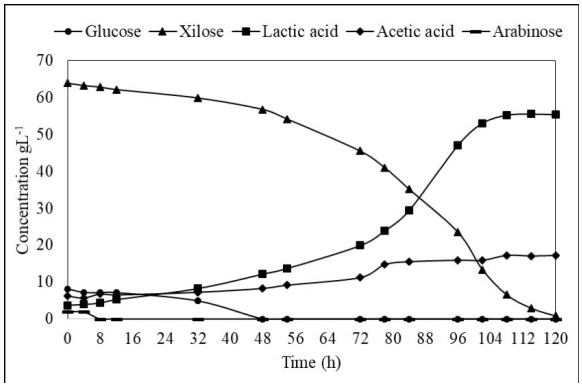

Fig. 3. Fermentation dynamics for experiments performed with concentrated hydrolysates at a FV/IV ratio: 1/2, using *L. pentosus*.

Although the yields reached are similar in both fermentations, the kinetics showed an inhibition in the production of lactic acid during the first hours, observing that increasing the concentration twice moderately increases the concentration of volatile inhibitors such as furfural and hydroxymethylfurfural (Table 2), although the increase in the concentration of non-volatile inhibitors derived from lignin and extractive fractions or the high concentration of acetic acid could exceed the limits needed to cause inhibition in the media.

Boguta *et al*. (2014) reported the capacity of several strains of L pentosus to grow in the presence of 0.15 and 2.70 gL^{-1} of furfural and acetic acid, respectively. However, in the present study, the concentration of these inhibitors (Table 2) was much higher. For example, the concentration of acetic acid varies from 3.83 to 6.99 gL^{-1} and the concentration of furfural from 1.56 to 2.51 gL^{-1} in the different tests with hydrolysates. This caused a slight inhibition in the growth and the production of lactic acid in the first hours but did not harm the yields in the formation of the product, so it was not necessary to detoxify the hydrolysates before fermentation.

Bustos *et al*. (2004a) investigated the use of other residues with *L. pentosus* CECT 4023T: residues of the pruning of vineyards to produce lactic acid without previous the detoxification producing stage (Q_P= 0.844 $gL^{-1}h^{-1}$, $Y_{P/S}$ = 0.77 gg^{-1}), representing a theoretical yield of 99.6%. In later works, Bustos *et al*. (2005a) concentrated the hydrolysates obtained from the residues of pruning to increase the initial concentration of sugars to 35.4 gL^{-1} glucose, 52.3 gL^{-1} xylose and 13.0 gL^{-1} arabinose. Under these conditions, the concentration of lactic acid reached 46.0 gL^{-1}

(Q_P = 0.933 $gL^{-1}h^{-1}$, $Y_{P/S}$ = 0.78 gg^{-1}, $Y_{P/S}$ theoretical= 91.7%), observing in this work a slight inhibition by the toxic compounds released after the fermentation of the material.

3.4 Use of a synthetic medium

In order to know if the inhibition should be distributed to the formation of the product during the heterolactic fermentation or the presence of toxins in the hydrolysates, a new fermentation MRS broth was produced (medium 4, Table 2) simulating the more concentrated hydrolysates. The mixture of synthetic glucose and xylose is visibly the same as medium 3. Figure 4 shows the evolution in time of the consumption of glucose and xylose, as well as the production of lactic and acetic acids.

The profiles are clearly like the ones observed by media 4, reaching a final concentration of lactic acid of 60.963 gL^{-1} in 96 h, which is similar to the 55.437 gL^{-1} reached after 120 hours for medium 3 (Table 3). The volumetric productivity (Q_P) of 0.6112 $gL^{-1}h^{-1}$ and a product yield ($Y_{P/S}$) of 0.810 gg^{-1} are also lower for the synthetic medium. The final concentration of acetic acid was only 8.342 gL^{-1} (Table 3) and therefore the acetic acid did not cause any type of inhibition.

The more relevant aspect of the comparison of Figures 3 and 4 is the confirmation of the production of lactic acid from the hydrolysates of the sugarcane bagasse following the heterofermentative route is limited in the first 24 h due to the presence of furfural or hydroxymethylfurfural. It is worth mentioning that hydrolysates present a remarkable concentration of phenolic compounds and acetic acid (Table 2) which affect the metabolism of *L. pentosus* (Bustos *et al.*, 2005a).

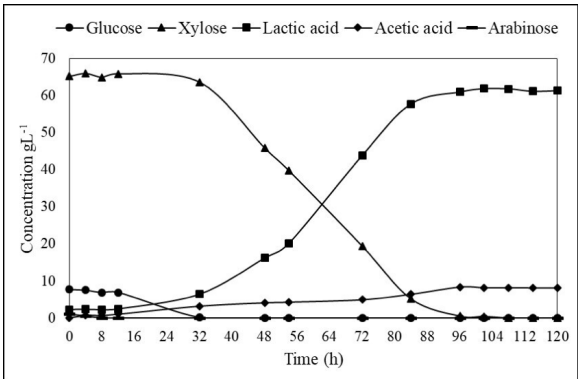

Fig. 4. Fermentation dynamics for experiments performed with a synthetic medium using the *L. pentosus* CECT 4023T.

Conclusions

The heterolactic bacterium *L. pentosus* CECT 4023T can grow in different concentrations of hemicellulosic sugars, tolerating the presence of inhibitory compounds and producing lactic acid as a fermentation product.

When the sugarcane bagasse was hydrolyzed, the hemicellulosic sugars were released correctly, especially the xylose, which was the predominant sugar of this liquid fraction. Although hydrolysates were obtained at different concentrations (FV/IV ratio: 1/1.33 and 1/2), the high increase of inhibitors produced in the latter slightly hindered the production of lactic acid. In contrast, the high yield of lactic acid (0.8208 gg^{-1}) was obtained from the hydrolysate rate FV/IV ratio: 1/1.33.

The bacterium *L. pentosus* presented good theoretical yields, but was affected in the first hours of fermentation by the inhibitory compounds released after the process of bagasse hydrolysis and its subsequent concentration, although it is noteworthy that the bacterium adapted to the media without applying a detoxification would increase the process production costs of lactic acid.

The results presented in this study not only corroborate the ability of the *L. pentosus* to grow using different concentrations of hemicellulose sugars, but also displayed the suitability of this strain to be applied as an efficient producer of lactic acid, using lignocellulosic hydrolysates from sugarcane bagasse in a biorefinery approach.

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