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ORIGINAL ARTICLE

Evaluation of fermentative potential of *Kluyveromyces marxianus* ATCC 36907 in cellulosic and hemicellulosic sugarcane bagasse hydrolysates on xylitol and ethanol production

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Abstract This paper evaluates the fermentative potential of *Kluyveromyces marxianus* grown in sugarcane bagasse cellulosic and hemicellulosic hydrolysates obtained by acid hydrolysis. Ethanol was obtained from a single glucose fermentation product, whereas xylose assimilation resulted in xylitol as the main product and ethanol as a by-product derived from the metabolism of this pentose. Fermentation performed in a simulated hydrolysate medium with a glucose concentration similar to that of the hydrolysate resulted in ethanol productivity (Qp=0.86 g L^{-1} h^{-1}) that was tenfold higher than the one observed in the cellulosic hydrolysate. However, the use of hemicellulosic hydrolysate favored xylose assimilation in comparison with simulated medium with xylose and glucose concentrations similar to those found in this hydrolysate, without toxic compounds such as acetic acid and phenols. Under this condition, xylitol yield was 53.8 % higher in relation to simulated medium. Thus, the total removal of toxic compounds from the hydrolysate is not necessary to obtain bioproducts from lignocellulosic hydrolysates.

Keywords Cellulosic hydrolysate · Hemicellulosic hydrolysate · Sugarcane bagasse · Ethanol · Xylitol · Kluyveromyces marxianus

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Introduction

Environmental preservation has been an increasing matter of concern, as has the interest to develop sustainable technologies for the optimized use of lignocellulosic biomass resulting from both agriculture and forest sectors, in order to obtain different products such as second-generation ethanol. Within such a context, biorefineries play an important role, as they offer sustainable economic growth and make it possible to transform residues, by-products and/or co-products of industrial segments into a diversity of bioproducts. In Brazil, sugarcane bagasse has been employed in distilleries as a source of steam and electricity, and the surplus is sold to distribution networks for the co-generation of electric power (UNICA 2012).

The chemical constitution of sugarcane bagasse is mainly represented by the fractions of cellulose, hemicellulose, and lignin (Fengel and Wegener 1984). The ability presented by a few microorganisms to ferment either C5 or C6 sugars has increased the interest in using this biomass in order to obtain biotechnological products such as xylitol and ethanol (Gírio et al. 2010). The solubilization of its sugars through hydrolytic processes, such as acid hydrolysis (Lenihan et al. 2010; Rodrigues et al. 2010; Rocha et al. 2012), is a critical stage for the bioprocess to succeed, since compounds that inhibit microbial activity are released along with sugars during this phase. These compounds include acetic acid, furfural, 5-hydroxymethylfurfural, and lignin-degrading products (phenols) (Palmqvist and Hahn-Hägerdal 2000; Nigam 2001; Lima et al. 2004; Silva et al. 2004; Alvira et al. 2010). The concentrations of toxic compounds in hydrolysates depend on the type of lignocellulosic material and also on the type of detoxification process used. The use of activated charcoal has been employed in hydrolysate detoxification (Marton et al. 2006), and more recently, vegetal polymer has

been used (Chaud et al. 2012). They act as adsorption and flocculation agents, respectively. The efficiency of detoxification depends on the type of toxic agent to be removed (Canilha et al. 2012). Residuals such as acetic acid, furfural, hydroxymethilfurfural and phenolic compounds are usually found in the hydrolysates (Marton et al. 2006; Canilha et al. 2010; Chaud et al. 2012), which is relevant not only to the C5 and C6 assimilation capacity by the microorganism in use, mainly due to the possibility that such microorganism is or is not able to resist to the action of the toxic compounds remaining in the hydrolysates.

The yeast *Kluyveromuces marxianus* is known for its ability to assimilate a mixture of sugars (Wilkins et al. 2008; Zhang et al. 2010), including xylose (Banat et al. 1998; Wilkins et al. 2008; Lane and Morrissey 2010; Matsuzaki et al. 2012). It is a thermotolerant yeast that shows considerable growth in the temperature range between 25 °C and 45 °C (Fonseca et al. 2008), and it has been studied in simultaneous saccharification and fermentation (SSF) of lignocellulosic biomass (Banat et al. 1998; Zhang et al. 2013). Besides, according to Lark et al. (1997), this yeast tolerates temperatures up to 42 °C but its growth is better at 30 °C, at which it also consumes glucose and produces ethanol. This versatility could be economically explored in a variety of applications, such as the production of ethanol, protein, bio-ingredients, and so forth (Fonseca et al. 2008; Gabardo et al. 2012; Kang et al. 2012), which requires further research towards a broader understanding of this yeast growth in hydrolysates derived from vegetal biomasses, to guarantee their use in industrial processes.

Under these circumstances, this paper evaluates the fermentative potential of the yeast *Kluyveromyces marxianus* ATCC 36907 during its cultivation in sugarcane bagasse hemicellulosic and cellulosic hydrolysates.

Materials and methods

Microorganisms and inoculum preparation

The experiments were performed with *K. marxianus* ATCC 36907 maintained on malt-extract agar slants at 4 °C. A loopful of cells grown on a malt-extract agar slant was transferred to the medium used for inoculum preparation containing xylose (30.0 g L^{-1}), rice bran extract (20.0 g L^{-1}), $(NH_4)_2SO_4$ (2.0 g L^{-1}) and $CaCl_2.2H_2O$ (0.1 g L^{-1}). Erlenmeyer flasks (125 mL) containing 50 mL medium were incubated on a rotary shaker (200 rpm) at 30 °C for 24 h. Afterwards, the cells were separated by centrifugation (2,000 g; 20 min), rinsed twice with distilled water, and then the cell pellet was once again suspended in an adequate volume of distilled water. The initial cell concentration for all experiments was around 1.0 g L^{-1}.

Preparation of the sugarcane bagasse hemicellulosic and cellulosic hydrolysates

The pre-treatment of sugarcane bagasse was performed employing H_2SO_4 1 % (w/v), 1:10 solid–liquid ratio, at the temperature of 121 °C for 20 min (Pessoa Júnior et al. 1997). The hemicellulosic hydrolysate obtained was filtered through a paper filter for solid mass separation (cellulignin). Cellulignin underwent alkaline hydrolysis for delignification purposes by using NaOH 1.5 % (w/v), 1:20 solid–liquid ratio, at a temperature of 100 °C for one hour. The hydrolysate obtained was filtered through a paper filter for solid mass separation (cellulose pulp). The resulting cellulose pulp underwent acid hydrolysis with H_2SO_4 2 % (v/v), 1:8 solid–liquid ratio, at a temperature of 155 °C for 10 min. The cellulosic hydrolysate obtained was filtered through a paper filter for solid mass separation. The hemicellulosic and cellulosic hydrolysates were concentrated at 70 °C under vacuum to obtain a fourfold increase in sugar content. Afterwards, both hydrolysates were submitted to detoxification procedure in order to reduce the concentration of toxic compounds. The hemicellulosic hydrolysate (59.10 g L^{-1} xylose, 5.17 g L^{-1} glucose; 9.40 g L^{-1} arabinose, 4.22 g L^{-1} acetic acid, 0.087 g L^{-1} furfural, 0.063 g L^{-1} hydroxymethylfurfural, and 7.17 g L^{-1} total phenolic compounds) was detoxified by adjusting initial pH to 8.0 with CaO (commercial grade), followed by the addition of 15.0 % (v/v) Acquapol WW® biopolymer (Acquaquimica), for 15 min under agitation (200 rpm, 25 °C). The precipitate formed as a result of this treatment was removed by centrifugation (2,000 g; 20 min) (Chaud et al. 2012). The cellulosic hydrolysate (70.0 g L^{-1} glucose, 0.032 g L^{-1} furfural, 0.468 g L^{-1} hydroxymethylfurfural, and 18.15 g L^{-1} total phenolic compounds) was treated by adjusting pH initially to 7.0 with CaO (commercial grade), and then to 2.5 with H_3PO_4, followed by the addition of 1.0 % (w/v) activated charcoal (refined powder), for 30 min under agitation (200 rpm, 60 °C). The precipitate formed as a result of this treatment was removed by vacuum filtration (Marton et al. 2006). Both hydrolysates were autoclaved at 110 °C, under 0.5 atm, in order to be used as fermentation medium.

Medium and fermentation conditions

For fermentation medium preparation, concentrated and treated sugarcane bagasse hemicellulosic hydrolysate (50.0 g L^{-1} xylose, 4.0 g L^{-1} glucose; 7.5 g L^{-1} arabinose, 3.7 g L^{-1} acetic acid, 0.0036 g L^{-1} furfural, 0.0013 g L^{-1} hydroxymethylfurfural, and 1.5 g L^{-1} total phenolic compounds) and cellulosic hydrolysate (69.2 g L^{-1} glucose, 0.0019 g L^{-1} furfural, 0.0542 g L^{-1} hydroxymethylfurfural, and 2.65 g L^{-1} total phenolic compounds) were supplemented with 2.0 g L^{-1} $(NH_4)_2SO_4$,

5.0 g L^{-1} peptone, 3.0 g L^{-1} yeast extract and 0.1 g L^{-1} $CaCl_2 \cdot 2H_2O$. Control experiments with semi-defined media simulating the concentrations of xylose and glucose in the hydrolysates were also performed. The media (50 mL) were placed in 125 mL Erlenmeyer flasks and fermented at 200 rpm at 30ºC for 96 h with initial pH adjusted to 5.5. Experiments were carried out in duplicate.

Analytical methods

Xylose, glucose, arabinose, xylitol, ethanol, glycerol and acetic acid concentrations were determined by HPLC (Waters, Milford, MA) with a refraction index detector on a Bio-Rad Aminex HPX-87H at 45ºC, with 0.01 N H_2SO_4 as the eluent at 0.6 mL min^{-1} flow rate. Stock solutions of 10 g L^{-1} of xylose, arabinose, glucose, xylitol, glycerol, acetic acid and ethanol were prepared separately in deionized water. From the stock solutions, suitably diluted mixed standard solutions were prepared to contain 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10 g L^{-1} of xylose and 0.5, 1.0, 2.0, 3.0, 4.0 and 5 g L^{-1} of arabinose, glucose, xylitol, glycerol, acetic acid and ethanol.

Furfural and hydroxymethylfurfural concentrations were determined with a Hewlett-Packard RP 18 column at 25 °C with acetonitrile: water (1:8) and 1 % acetic acid as the eluent, and a 0.8 mL min^{-1} flow rate in a visible ultraviolet-light detector (SPD-10^{A} UV–VIS). Stock solutions of 100 mg L^{-1} of furfural and 5-hidroxymetilfurfural were prepared separately in deionized water. From the stock solutions, suitably diluted mixed standard solutions were prepared to contain 5, 10, 20, 30, 40, 50, 60, 70, 90 and 100 mg L^{-1} of furfural and 5-hidroxymetilfurfural.

The total phenolic compound concentration was estimated by ultraviolet spectroscopy at 280 nm (Gouveia et al. 2009).

Cell growth was monitored by measuring the absorbance at 600 nm (Beckman-DU 640B spectrophotometer). Cell concentration was calculated based on the relationship between the optical density and cell dry weight through a calibration curve. Yeast cells were stained with methylene blue (1 %) and observed with a digital binocular light microscope (LABO) equipped with digital camera (× 100 objective) for morphology analysis.

Results

Sugar consumption and cell growth

The *K. marxianus* fermentative performance in sugarcane bagasse hemicellulosic and cellulosic hydrolysates can be observed in Fig. 1. Glucose and xylose co-fermentation was verified in the hemicellulosic hydrolysate, with full depletion of the glucose and assimilation of 47.37 % of xylose, whereas arabinose was not assimilated. Growing yeast in the cellulosic hydrolysate led to a partial glucose consumption (52.62 %), which resulted in a slower growth when compared with the one observed in the hemicellulosic hydrolysate (Fig. 1). The use of simulated hydrolysates favored the consumption of xylose and glucose at 44 % and 90 %, respectively, in comparison with the hemicellulosic and cellulosic hydrolysates. This higher sugar consumption favored cell growth, which was much higher when glucose was used, and the final cell concentration (12.25 g L^{-1}) was 195 % greater than the one found in the cellulosic hydrolysate (4.15 g L^{-1}) and 62.2 % greater than the one found in the hemicellulosic hydrolysate (7.55 g L^{-1}) (Fig. 1).

Ethanol and xylitol formation

In relation to the main metabolites produced (Fig. 2), it was verified that *K. marxianus* grown in the medium containing glucose produced only ethanol, while in medium containing xylose, xylitol was produced as the main product and ethanol as a by-product (Fig. 2).

Figure 2 also shows that ethanol production during yeast cultivation in the cellulosic hydrolysate started only after 72 h, which coincides with the maximum cell growth. A different behavior was observed in the semi-defined medium that simulated this hydrolysate, in which the maximum ethanol production occurred at the first 12 h and decreased from this time on.

The maximum values of yield ($Y_{P/S}$) and productivity (Q_P) during yeast cultivation in the hydrolysates and in the semi-defined media are found in Fig. 3. They confirm ethanol as the main product derived from glucose metabolism by the yeast and its prevalence during the culture in the semi-defined medium containing the same glucose concentration of the cellulosic hydrolysate ($Y_{P/S}$=0.33 g g^{-1} and Q_P= 0.86 g L^{-1} h^{-1}), which corresponded to increases of 1.5 and tenfold for $Y_{P/S}$ and Q_P, respectively, in relation to cultivation in the cellulosic hydrolysate ($Y_{P/S}$=0.22 g g^{-1} and Q_P= 0.08 g L^{-1} h^{-1}). Fig. 3 also shows xylitol as the main metabolite from yeast growth in hemicellulosic hydrolysate (9.35 g L^{-1}), and ethanol as a by-product (1.31 g L^{-1}). In addition, it was observed that the bioconversion of xylose into xylitol by *K. marxianus* in hemicellulosic hydrolysate was favored. The xylitol yield was 53.8 % greater in comparison with the semi-defined medium with glucose and xylose concentrations similar to those found in the hydrolysate.

Figure 4A shows the yeast capacity to assimilate acetic acid at the first 12 h of fermentation, with a consequent increase in pH values (Fig. 4B). However from this moment on, acetic acid concentration increased, which points to the fact that it was produced by the cells, resulting in pH reduction (Fig. 4B).

It was also noted that the toxic compounds such as furfural, hydroxymethylfurfural, and phenolic compounds, found in the hydrolysates as residual concentrations even after the

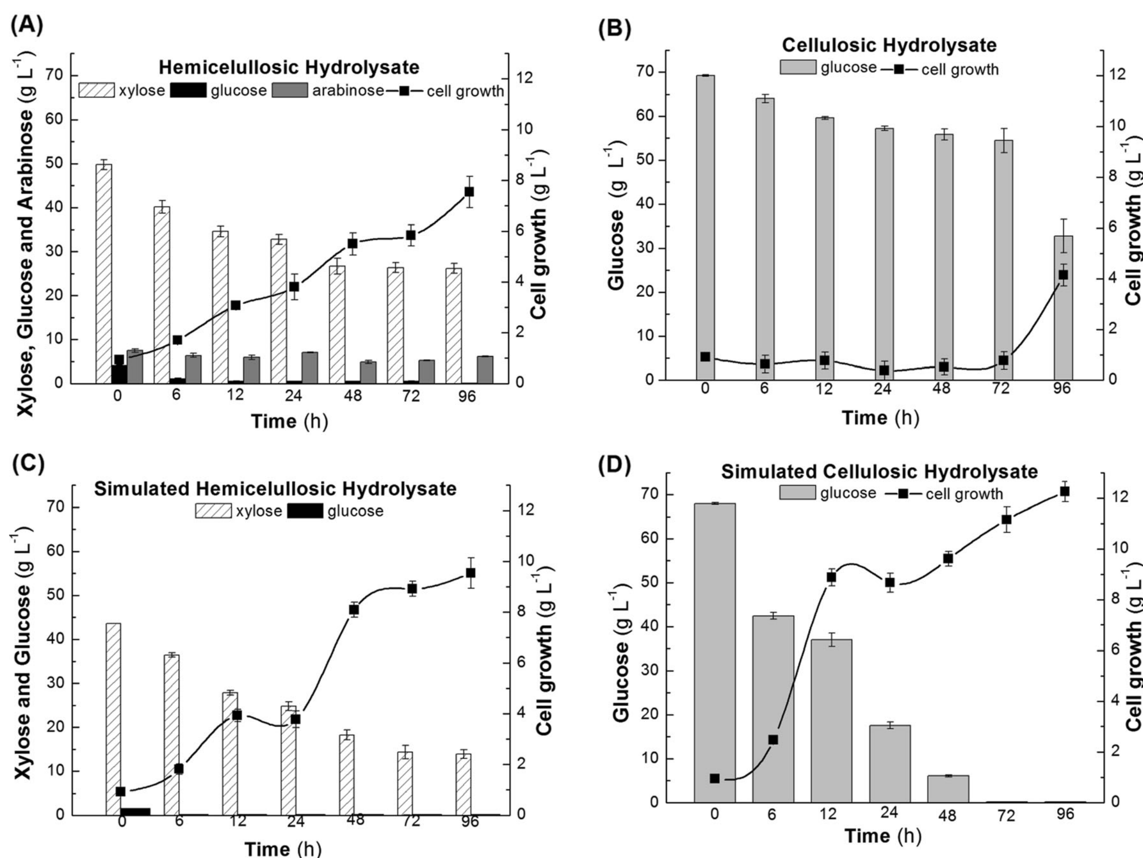

Fig. 1 Hexose and pentose consumption and *K. marxianus* cell growth during fermentation of sugarcane bagasse hemicellulosic hydrolysate (**A**), cellulosic hydrolysate (**B**), simulated hemicellulosic hydrolysate (**C**) and in simulated cellulosic hydrolysate (**D**)

detoxification procedure, were assimilated by *K. marxianus* at different rates due to the type of hydrolysate employed. Total assimilation of furfural and hydroxymethylfurfural was observed in the hemicellulosic hydrolysate, although phenolic consumption did not occur. On the other hand, when grown in cellulosic hydrolysate, 100 % assimilation of hydroxymethylfurfural and 12.45 % of phenolic compounds were observed at the end of fermentation, although furfural assimilation did not occur.

Discussion

The results presented in this paper allowed us to confirm the capacity of *K. marxianus* to co-ferment glucose and xylose, while the arabinose current in the hemicellulosic hydrolysate was not assimilated. Reports on the repression of xylose assimilation due to the presence of glucose are common for different yeasts (Lee et al. 2002; Silva et al. 2007). Specifically for *K. marxianus* UFV3, Santos et al. (2013) reported such

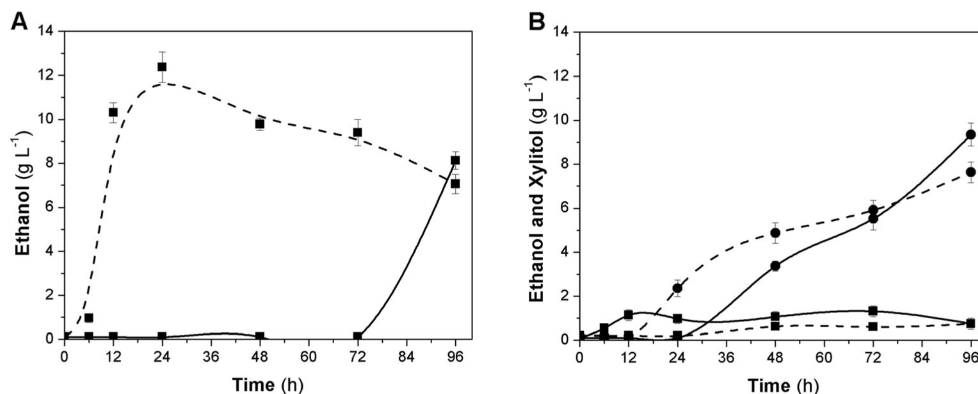

Fig. 2 Ethanol (*black square*) and xylitol (*black circle*) formation by *K. marxianus* during fermentation in: (A) cellulosic hydrolysate (*straight line*) and simulated hydrolysate containing glucose (*dashed line*) and (B) hemicellulosic hydrolysate (*straight line*) and simulated hydrolysate containing xylose and glucose (*dashed line*)

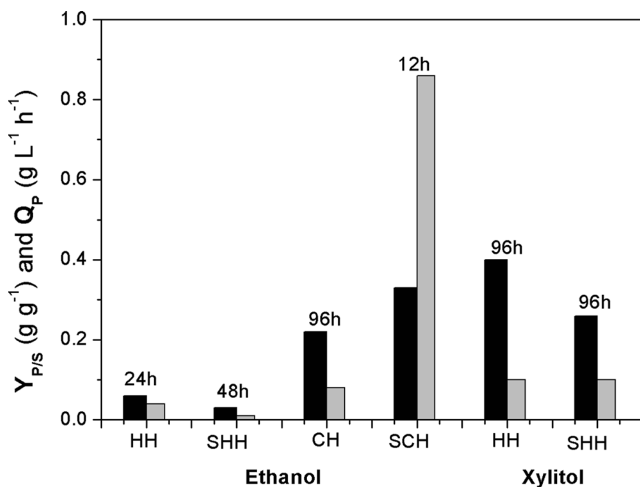

Fig. 3 Maximum yield (*black square*) and productivity (*grey square*) values for ethanol and xylitol by *K. marxianus* in [HH] hemicellulosic hydrolysate, [SHH] simulated hydrolysate containing xylose and glucose, [CH] cellulosic hydrolysate and [SCH] simulated hydrolysate containing glucose

catabolic repression, as xylose consumption began only after glucose exhaustion. This behavior differs from what has been observed in the present study, in which glucose and xylose consumption occurred simultaneously. On the other hand, arabinose is not usually assimilated by most microorganisms, and according to Mussatto et al. (2012), *Kluyveromyces fragilis* was not able to assimilate this pentose in experiments using coffee industry wastes hydrolysate. A 100 % consumption of glucose by *K. marxianus* NRRL Y-6860 was verified in the rice straw cellulosic hydrolysate obtained by enzymatic hydrolysis (Castro 2011). Another important aspect is that sugar consumption in the hydrolysates was lower than in hydrolysate-simulating media. The difficulty to assimilate these sugars in hydrolysates is possibly related to the concentration of the remaining toxic residues even after the detoxification procedures, since the residual phenolic concentrations were 1.50 g L^{-1} in the hemicellulosic hydrolysate and 2.65 g L^{-1} in the cellulosic hydrolysate.

Sugar assimilation difficulties also affected *K. marxianus* cell growth, mainly during cellulosic hydrolysate fermentations, in which a lag phase in glucose assimilation and in cell growth was observed (Fig. 1). It was also verified that, regardless of the carbon source (glucose or xylose), cell growth in hydrolysate-simulating media was greater than in hydrolysate-formulated media. These differences in yeast growth might be a consequence of two factors: 1) *K. marxianus* preference for glucose, since the consumption of this sugar and cell growth were greater in media that simulates cellulosic hydrolysate; 2) the effect caused by the presence of phenolic compounds on cell metabolism is due to a higher concentration of the compounds (2.65 g L^{-1}) in the cellulosic hydrolysate than in the hemicellulosic hydrolysate (1.50 g L^{-1}). The different physiological responses of the yeast when grown in hemicellulosic and cellulosic hydrolysates as well as in the semi-defined media can also be noticed in the cell morphology (data not shown). Robust cells presenting gemmule could be viewed during cultivation in the hemicellulosic hydrolysate and in its corresponding semi-defined medium in the first 12 h. With respect to the cellulosic hydrolysate, robustness and cell division were evident only in the simulated hydrolysate. In general terms, it can be considered that the yeast was able to adapt to the different media, with consequent growth and generation of ethanol and xylitol products, as previously shown in Fig. 2.

The ability of *K. marxianus* to drive its metabolism to xylitol production when grown in a medium containing xylose must be related to the type of co-factor required by the xylose reductase (XR) and xylitol dehydrogenase (XDH) enzymes (Zhang et al. 2011; Lulu et al. 2013). In pentose assimilating yeasts like *Candida guilliermondii* and *K. marxianus*, once inside the cell, xylose is reduced to xylitol in a reaction catalyzed by NADPH or NADH-linked xylose reductase. Xylitol is oxidized to xylulose by $NADP^+$ or NAD^+-linked xylitol dehydrogenase. Xylulose is phosphorylated into xylulose-5-phosphate, which can be converted into pyruvate through the connection of the phosphopentoses and the glycolytic pathway (Hahn-Hägerdal et al. 1994). The

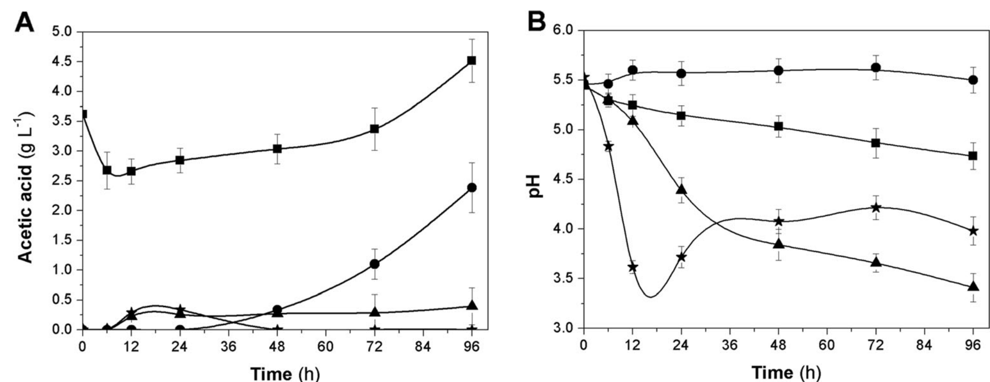

Fig. 4 Concentrations of acetic acid **a** and pH variation **b** during fermentation in hemicellulosic hydrolysate (*square*), cellulosic hydrolysate (*triangle*), simulated hydrolysate containing xylose and glucose (*circle*), and simulated hydrolysate containing glucose (*star*) by *K. marxianus*

type of co-factor dependence of XR may affect the proportion between the amounts of xylitol and xylulose produced. The intense xylulose formation is a necessary condition for ethanol production and for blocking the formation of xylulose due to XR dependence on NADPH, which leads to the production of xylitol as the main product of xylose fermentation (Sene et al. 2001; Yablochkova et al. 2003).

The values of fermentative parameters obtained during the cellulosic hydrolysate fermentation (Fig. 3) were similar to those found by Moreno et al. (2012) in experiments with *K. marxianus* CECT 10875 grown in rice straw cellulosic hydrolysate obtained by enzymatic hydrolysis ($Y_{P/S}$ ranging from 0.30–0.38 g g^{-1}), and lower than those reported by Castro (2011), when *K. marxianus* was grown in enzymatic rice straw cellulosic hydrolysate ($Y_{P/S}$=0.44 g g^{-1} and Q_P=2.89 g L^{-1} h^{-1}).

With regard to the values of fermentative parameters referring to xylitol production either in the hemicellulosic hydrolysate or in semi-defined medium containing xylose and glucose, the results obtained in this study were higher than the ones reported by Wilkins et al. (2008), who obtained $Y_{P/S}$=0.30 g g^{-1} and Q_P=0.04 g L^{-1} h^{-1} xylitol, when *K. marxianus* IMB 4 was grown in a medium containing 20 g L^{-1} glucose and 20 g L^{-1} xylose. Meanwhile, the values obtained in this study were lower than the xylitol yield (0.75 g g^{-1}) and productivity (0.48 g L^{-1} h^{-1}) obtained by *Candida guilliermondii*, a good and broadly studied xylitol producer, grown in sugarcane bagasse hemicellulosic hydrolysate (Chaud et al. 2012).

Another important issue to be highlighted is the fact that xylitol production was higher in hemicellulosic hydrolysate than the one obtained in the simulated hydrolysate medium with the same concentration of xylose and glucose in this hydrolysate, indicating the presence of a compound in the hydrolysate that might have favored the bioconversion of xylose into xylitol by *K. marxianus*, such as acetic acid, for example. According to Felipe et al. (1995), xylitol production by *C. guilliermondii* in a semi-defined medium was favored at a low concentration of this acid (1.0 g L^{-1}). According to these authors, the acid in low concentration would go straight to the Krebs cycle via acetyl-CoA. Diaz et al. (2009) also reported a facilitated fermentation by *Pichia stipitis* in the presence of acetic acid under concentrations considered to be non-inhibiting for cell growth (smaller than 3.0 g L^{-1}) in the hydrolysate from olive tree cuttings. The hydrolysate employed as fermentation medium in this study contained 3.7 g L^{-1} acetic acid, a concentration that is within the range considered not to be inhibitory to yeasts such as *C. guilliermondii* (Felipe et al. 1995; Lima et al. 2004); this could have favored the metabolism for xylitol production. Besides, during fermentation of hemicellulosic hydrolysate, a decrease in pH due to acetic acid formation was observed, but pH values remained higher than the pKa (4.75). Such behavior was favorable, since according to Lawford and Rousseau (1998), the acetic acid toxicity is related to the ability of undissociated (protonated) weak acid to act as a membrane protonophore, causing acidification of the cytoplasm.

The capacity of *K. marxianus* to assimilate the acetic acid present in the hemicellulosic hydrolysate is in accordance with these reports, in addition to its ability to form acetic acid along the fermentations (Fig. 4A). Wilkins et al. (2008) also reported the formation of about 2 g L^{-1} of acetic acid when *K. marxianus* IMB 4 was grown in a medium containing a glucose-xylose mixture under concentrations of 20 g L^{-1} of each sugar. The consumption of acetic acid was also noticed even before carbon source exhaustion during *K. marxianus* ATCC 26548 culture in a medium containing glucose (Fonseca et al. 2007).

In relation to the phenolic compounds, theye inhibit microbial growth as a result of changes in the plasmatic membrane, and the minimum inhibitory concentration verified for different bacteria and yeasts is around 1.5 g L^{-1} in media containing xylose as carbon source (Zaldivaar et al. 1999; Mills et al. 2009)—this is a concentration equal or smaller than the one found in the evaluated hydrolysates in this study. According to Mills et al. (2009), different microorganisms have already been described as presenting tolerance models for the phenolic compounds, mainly related to their conversion into carboxylic acids or alcohols due to the reduced toxicity of the functional groups, which could justify the consumption of these compounds by *K. marxianus* during the cellulosic hydrolysate fermentations.

Conclusions

The results reported in this paper improve our knowledge about *K. marxianus* physiology. This yeast showed an ability to ferment C5 and C6 sugars in sugarcane bagasse hemicellulosic and cellulosic hydrolysates in the presence of toxic compounds such as phenols and acetic acid. The metabolism of these sugars resulted in ethanol and xylitol production, depending on the hydrolysate employed. It was also observed that the use of cellulosic hydrolysate resulted in a long lag-phase for glucose assimilation when compared to simulate -hydrolysate medium. The behavior observed for sugar metabolism in hemicelullosic and cellulosic hydrolysates in comparison to their simulated media is related not only to the difference in the composition of carbohydrates, but also to the presence of toxic compounds liberated from the hydrolysis process. Indeed, the phenols concentration in cellulosic hydrolysates was 77 % higher than in the hemicellulosic hydrolysates. Probably this fact could contribute to the inhibition of glucose consumption and consequently low ethanol production. In the hemicellulosic hydrolysate, the toxics compounds did not inhibit sugar metabolism. On the

contrary, the presence of any other compound in this medium likely favored the xylose to xylitol bioconversion. Thus, the total removal of toxic compounds from the hydrolysate is not necessary to obtain bioproducts from lignocellulosic hydrolysates.

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