Glycerol use as strategy to improve xylitol production from sugarcane bagasse hemicellulosic hydrolysate

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Abstract

Biotechnological production of xylitol is a promising alternative for the use of the pentoses (mainly xylose) derived from the hemicellulosic fraction of the lignocellulosic biomass, such as sugarcane bagasse. Considering the increasing availability of glycerol as by-product of biodiesel production, the use of this polyolin association with reduction of oxygen availability after 12hof fermentation were evaluated as acombined strategy to improve the xylitol production by *Candida guilliermondii* FTI 20037 in sugarcane bagasse hemicellulosic hydrolysate. Before 12h, glucose and xylose consumption supported cell growth in detriment of xylitol production. With the reduction of oxygen availability, caused by diminution in K_La value from $20h^{-1}$ to $11h^{-1}$after 12h, xylose-to-xylitol bioconversion was promoted. Improvements on xylose consumption and xylitol production were evidenced in the medium with glycerol supplementation after reduction of oxygen availability. Such improvement was associated with glycerol uptake, suggesting that this compound was possibly used as co-substrate.

Keywords: Xylitol; glycerol; Sugarcane bagasse; hemicellulosic hydrolysate; oxygen availability; *Candida guilliermondii.*

1. INTRODUCTION

During the last decades large research efforts have been destined to the development of second-generation biofuels, in order to contribute to the diversification of the energetic matrix and reduction of fossil fuels dependency (Bozell, 2008; Menon & Rao, 2012). Lignocellulosic ethanol production is one the most promising technologies and sugarcane bagasse is one of the most used lignocellulosic biomasses (Soccol et al., 2010; Limayem & Ricke, 2012). An important challenge for this technology is the efficient use of the sugars derived from the hemicellulosic fraction, mainly xylose (Hahn-Hägerdal et al., 2006; Limayem & Ricke, 2012). Parallel to the options that are being developed for ethanol production from pentoses, alternative routes have been already proposed for a different use of the hemicellulosic fraction. Many of the bioprocesses that have been suggested represent the possibility to valorize the hemicellulose because of the high added value of the products, as well as to replace inefficient chemical processes with high environmental impact (Bozell, 2008; Menon &Rao, 2012).

Such is the case of biotechnological production of xylitol, which is a sugar-alcohol with important applications on food, odontological and pharmaceutical industries (Felipe, 2004; Silva & Felipe, 2012). It was already categorized as one of the top chemicals that can support economically and technically the production of low-value biofuels in an integrated biorefinery (Felipe, 2004; Werpy & Peterson, 2004). This bioprocess has been extensively studied as an alternative for the commercial chemical process, which is considered inefficient and expensive due to the complexity of the purification procedures of xylose (Parajó et al., 1998; Canilha et al., 2013).

This bioprocess is based on the natural ability of pentose-assimilating yeasts to reduce xylose to xylitol by NAD(P)H-depending Xylose Reductase (XR), as the first step in xylose metabolism (Yablochkova et al., 2004; Granström et al., 2007). Xylitol accumulation is induced by limitation on oxygen availability, which causes a NADH/NAD^+ redox imbalance that restricts the NAD^+-depending Xylitol Dehydrogenase (XDH) activity and, therefore, the xylitol-to-xylulose oxidation decreases (Yablochkova et al., 2004; Granström et al., 2007). In yeasts with high xylitol-producing potential, such as *Candida guilliermondii*, XR was characterized as exclusively dependent on NADPH (Silva et al., 1998; Yablochkova et al., 2004; Granström et al., 2007).

Several studies on biotechnological production of xylitol have been performed in order to increase its efficiency and productivity. They focused on many aspects, such as fermentation conditions -with particular interest in oxygen control-, process operation, use of recombinant yeasts and even enzymatic synthesis and biotransformation(Felipe, 2004; Silva & Chandel, 2012). Particularly, studies on the use of co-substrates can be highlighted, which are based on the fact that the xylitol accumulation led to a reduction of the carbon flux derived from xylose assimilation to the central metabolism (Kim et al., 1999; Kastner et al., 2001; Granström et al., 2007).It affects in general the cell growth, but in particular the adequate and constant supply of NADPH, which in turn limits the XR activity (Kim et al., 1999; Kastner et al., 2001; Granström et al., 2007). Therefore, a substrate simultaneously metabolized with xylose can maintain cell growth, metabolic intermediates formation and cofactor regeneration (Kim et al., 1999; Tamburini et al., 2012; Oh et al., 2013).

Glucose, which is already found in hemicellulosic hydrolysates, can be considered as the primary potential co-substrate for this bioprocess, since it is the substrate for the oxidative phase of Pentoses Phosphate Pathway, the main NADPH-producing pathway (Bruinenberg et al., 1983; Kim et al., 1999; Tamburini et al., 2012; Oh et al., 2013).Nevertheless, depending on the glucose: xylose proportion, this hexose can cause an effect of catabolite repression on xylose assimilation(Granström et al., 2007). In order to take advantage of this effect, a fermentation operated in two stages with different oxygen availability has been proposed (Kim et al., 1999; Ding & Xia, 2006). In the first stage, higher oxygen availability is

employed in order to allow the use of glucose as substrate for cell growth; in the second one, the oxygen availability is reduced to promote xylose-to-xylitol bioconversion (Kim et al., 1999; Ding & Xia, 2006). Although improvements have been shown in the process productivity due to the higher cell density reached in the first stage (Kim et al., 1999; Ding & Xia, 2006), within this strategy, glucose is not effectively used as co-substrate during xylitol production. In this sense, other strategies have been proposed, such as a fed-batch two-stage fermentation (Choi et al., 2000; Li et al., 2012), as well as studies on other compounds different from glucose.

Among the compounds that have been already evaluated as potential co-substrates, glycerol has been one of the most used ones, particularly in studies on synthetic medium with recombinant yeasts, whose XDH activity was disrupted or attenuated in order to increase xylitol yield without the need forprecise control of oxygen supply (Ko et al., 2006; Pal et al., 2013). In those studies it was suggested that glycerol assimilation in aerobic condition improved NADPH regeneration, increasing xylitol productivity (Ko et al., 2006; Pal et al., 2013). Regarding native yeasts, it was reported that glycerol addition in low concentrations improved the fermentative performance of *C. guilliermondii* FTI 20037 in semi-synthetic medium (Arruda & Felipe, 2009).

The use of glycerol as supplement in biotechnological production of xylitol results in an interesting strategy not only because its possible beneficial effects on the fermentative process, but also for its increasing availability as by-product of biodiesel production and its relative low cost (Dasari et al., 2005; Yazdani & González, 2007; Myint & El-Halwagi, 2009). The present work studies the use of glycerol in association with reduction of oxygen availability after 12h as acombined strategy to improve the fermentative performance of *C. guilliermondii* FTI 20036 in sugarcane bagasse hemicellulosic hydrolysate for xylitol production.

2. MATERIALS AND METHODS

2. 1 Microorganism and inoculum preparation

The yeast *Candida guilliermondii* FTI 20037 was maintained at 4ºC on malt-extract agar slants. The inoculum medium contained (g/L): xylose (30.0), rice bran extract (20.0), $(NH_4)_2SO_4$ (2.0), and $CaCl_2.2H_2O$ (0.1). The inoculum growth was carried out in Erlenmeyer flasks (125 mL) containing 50 mL of inoculum medium, and incubated in a rotary shaker (200 rpm) (model Co-Innova 4000; New Brunswick Scientific, Edison, NJ) at 30ºC for 24 h. The cells were separated by centrifugation (2000 x g for 20 min) and rinsed twice with distilled water. The cells pellet was re-suspended in distilled water and used as inoculum (1.0 g/L) in the fermentation medium.

2.2 Preparation of the sugarcane bagasse hemicellulosic hydrolysate

Sugarcane bagasse was submitted to dilute acid hydrolysis in a 100L steel reactor at 150ºC for 30 min with H_2SO_4 at 1.75:10 solid/liquid ratio (100 mg of H_2SO_4/ g dry matter). The hemicellulosic hydrolysate was filtered and vacuum evaporated at 70ºC to increase forth-fold the initial concentration of xylose. Afterwards, it was submitted to a treatment by pH adjustment to 7.0 with CaO (commercial grade) and to 2.5 with H_3PO_4, followed by the addition of 1.0 % (w/v) activated charcoal (refined powder) at 60ºC for 30 min, under agitation at 100 rpm in a rotary shaker (model Co-Innova 4000; New Brunswick Scientific, Edison, NJ). The formed precipitates were removed by vacuum filtration. Sugarcane bagasse hemicellulosic hydrolysate was autoclaved at 111 ºC, 0.5 atm for 15 min, after its vacuum evaporation and treatment.

2.3 Medium and batch fermentation conditions

Fermentation medium was composed by sugarcane bagasse hemicellulosic hydrolysate, with the following composition (g/L): xylose (55.9), glucose (4.72), arabinose (4.72), acetic acid (4.67), total phenols (3.25), furfural (0.01) and 5-hydroxymethylfurfural (0.02). It was supplemented with the same nutrients used during the inoculum preparation, except xylose. Initial pH was adjusted to 5.5 using sodium hydroxide solution (6 M). Batch fermentations were carried out in a 2.4L KLF 2000 bench-scale fermenter (Bioenginnering AG, Wald Switzerland) containing 1.6 L of fermentation medium at 30ºC. Air flow rate was kept constant at 0.8 vvm. Initially, agitation speed was kept at 500 rpm (k_La = 20h^{-1}) during 12 h and it was reduced to 400 rpm (k_La = 11 h^{-1}) till 96 h, in order to induce a reduction of oxygen availability. In order to evaluate the effect of glycerol on xylose-to-xylitol bioconversion by *C. guilliermondii*, fermentation medium was supplemented with glycerol (1.0 g/L). The used glycerol concentration (1.0 g/L) was based on preliminary studies in Erlenmeyer flasks[24, 28]. A medium without glycerol supplementation was used as control.

2.4 Analytical Methods

The concentrations of xylose, glucose, arabinose, acetic acid, xylitol, glycerol, and ethanol were determined by high-performance liquid chromatography (Shimadzu LC-10AD, Kyoto, Japan) using a refractive index detector and Bio Rad (Hercules, CA) Aminex HPX-87H column at 45 ºC, 0.01N H_2SO_4 as eluent, at flow rate of 0.6 mL/min. 5-hydroxymethylfurfural and furfural were determined in the same HPLC system, but using a visible ultraviolet light detector (SPD-10^A UV-VIS, Waters Corp.), a RP 18 column (Hewlett-Packard, Ramsey, MN, USA) at 25 ºC and acetonitrile: water (1:8) added with 10 % of acetic acid as eluent and flow rate of 0.8 mL/min. The injection volume was 20 µL. Cell growth was determined using a Beckman DU 640B spectrophotometer at 600 nm by the correlation of absorbance with cell dry weight. k_La was determined by gassing-out methodology (Pirt, 1975).

3. RESULTS AND DISCUSSION

In the present study, the use of glycerol in association with reduction of oxygen availability after 12h were evaluated as a combined strategy to improve the fermentative performance of *C. guilliermondii* FTI 20037 in sugarcane bagasse hemicellulosic hydrolysate for xylitol production. The profiles of xylose consumption, cell growth and xylitol production for both experiments with and without (control) glycerol supplementation are presented in Fig. 1, whereas in Fig. 2 are shown the profiles of glycerol and ethanol concentration. Table 1 summarizes the main fermentative parameters for both experiments.

Firstly, the reduction of oxygen availability after 12h, caused by a diminution in k_La value from 20$h^{-1}$to 11h^{-1}, led to a change in the fermentative performance of the yeast, as it was expected. Before 12h, complete consumption of glucose (in 6h, data not shown) and partial consumption of xylose supported an accelerated cell growth in detriment of xylitol formation, which was minimum in this stage, as response to the higher oxygen availability. Even though in the medium supplemented with glycerol the xylose consumption rate (1.01 g/L/h) was 42% lower than in the control (1.41 g/L/h), in the first condition the cell growth rate was 30% higher. Thus, before reduction of oxygen availability, in the medium supplemented with glycerol the xylose consumption (24%) was 50% lower, but the cell growth (3.12 g/L) was 28% higher than in the medium without glycerol addition (36% and 2.43 g/L, respectively).

Ethanol and glycerol production was evidenced before reduction of oxygen availability (Fig. 2), fact that could indicate that, despite the higher oxygen availability, the atmosphere was not completely aerobic, since it is known that the formation of these two compounds corresponds to alternative pathways to regenerate oxidized cofactor (NAD^+) if respiration metabolism is limited (Flores et al., 2000; Granström & Leisola, 2002). In the case of glycerol, it can also be produced as response to osmotic stress, in which case

this polyol would be intracellularly accumulated in order to avoid cell dehydration (Adler et al., 1985; Neivogt & Stahl, 1997). It is important to point out that in the medium without glycerol supplementation ethanol was rapidly produced in the first 6h, reaching a production rate (0.78 g/L/h) two-fold higher than that in the medium with glycerol addition (0.33 g/L/h). Nevertheless, after 6h, ethanol started to be consumed in the medium without glycerol supplementation, whereas in the medium with glycerol addition, ethanol production remained for 12h and reached a maximum concentration similar to that from the other condition (Fig. 2). Taking into account that ethanol formation could be supported not only by glucose but also by xylose assimilation, this result could be explained by the lower xylose uptake in the medium with glycerol supplementation during the first 6h of fermentation.

With the diminution in k_La, a change in the fermentative performance of the yeast was evident from 12h of fermentation, in which xylose uptake rate was highly reduced in comparison with that from the first stage (Fig. 1). The decrease on xylose uptake rate can be attributed to the reduction in oxygen availability, considering that in this condition the xylitol accumulation compromises the carbon flux to the central carbon metabolism, which in turn can reduce the flux through PPP, decreasing the availability of NADPH, cofactor that is essential for the XR activity(Kim et al., 1999; Granström et al., 2007). Interestingly, in the medium without glycerol addition (control), the xylose uptake rate decreased almost five-fold (from 1.44 to 0.29 g/L/h), whereas in the medium supplemented with glycerol the reduction was lower, only two-fold (from 1.01 to 0.44 g/Lh^{-1}). Thus, after reduction of oxygen availability, the xylose consumption in the medium supplemented with glycerol (72%) was 36% higher than that in the medium without glycerol addition (53%). Taking the global fermentation (96h) in consideration, the total xylose consumption was approximately similar in both conditions, with a difference of 8% between glycerol supplementation (96%) and without glycerol addition (89%).

The improvement of xylose consumption after reduction of oxygen availability can be associated with the profile of glycerol concentration. Whereas in the control experiment glycerol concentration increased in a low rate, in the medium supplemented with glycerol its concentration decreased in a similar rate (Fig. 2). Such fact could indicate that, in the medium supplemented with glycerol, this compound was mainly consumed after reduction of oxygen availability, which in turn leads to consider that glycerol was probably used as co-substrate in this particular fermentation stage. If this effectively happened, glycerol assimilation could support NADPH regeneration, contributing to keep a higher XR activity (Ko et al., 2006; Pal et al., 2013) and resulting in a beneficial effect on xylose assimilation.

An important issue to be discussed is why glycerol was consumed after reduction of oxygen availability in the medium supplemented with glycerol, differently from what happened in the medium without glycerol addition (control), in which uptake was not evidenced during the fermentation (Fig. 2). These results can be related to the fact that the glycerol concentration at 12h in the medium with glycerol supplementation (2.10 g/L) was approximately three-fold higher than that in the control (0.72 g/L). Hence, if the concentration in the medium supplemented with glycerol at that time was higher than the intracellular concentration, it is possible to consider that glycerol uptake could be stimulated by the concentration gradient, taking into account that glycerol uptake by facilitated diffusion was already demonstrated in yeasts, mainly *Saccharomyces cerevisiae* (Luyten et al., 1995; Oliveira et al., 2003).Therefore, although glycerol addition did not caused directly its consumption since the beginning of the fermentation, it possibly contributed to reach an extracellular concentration high enough to stimulate its uptake after reduction of oxygen availability, which in turn could have a beneficial effect on xylose assimilation.

The beneficial effect of glycerol supplementation on xylose consumption was also reported by other authors for native and recombinant yeasts in synthetic mediums (Ko et al., 2006; Arruda & Felipe, 2009; Pal et al., 2013). Arruda & Felipe (2006), who evaluated the effect of glycerol addition on xylitol production

with the same yeast used in the present study, but in semidefined medium and at laboratory scale, showed that glycerol addition in low concentrations (0.7 g/L) favored xylose consumption and xylitol production. Similar observation was made by Ko et al. (2006), who verified an improvement of xylose consumption during xylitol production when glycerol (20 g/L) was employed, but using a *C. tropicalis* recombinant strain (XDH disrupted) in aerobic conditions, 2.5l bioreactor and semi-synthetic medium containing xylose (50 g/L). In the same way, Pal et al.(2013)showed that simultaneous glycerol and xylose assimilation in a high aeration rate led to an improvement of xylitol productivity. These authors used a recombinant strain of *Debaryomyces hansenii*(XDH attenuated) and developed a similar two-stage fermentation strategy, but the stages were divided by xylose addition, instead of oxygen availability as it was done in the present work.

The reduction of oxygen availability after 12h stimulated the xylose-to-xylitol bioconversion, as it is evidenced by the increment in xylitol yield when comparing it to that before 12h. Interestingly, whereas before 12h the xylitol yield in the medium supplemented with glycerol (0.16 g/g) was 17% higher than that from the medium without addition (control, 0.11 g/g),after reduction of oxygen availability, it was 21% lower (0.65 g/g) than the control (0.79g/g). In spite of these differences in xylitol yield, xylitol production before reduction of oxygen availability was remarkably similar (1.96 g/L) in both mediums, whereas afterwards it was 18% higher in the medium supplemented with glycerol (23.94 g/L) than that in the medium without glycerol addition (20.21 g/L). Consequently, xylitol volumetric productivity was similar in both conditions (0.16 g/L/h) before reduction of oxygen availability, whereas, afterwards, it was 16% higher in the medium supplemented with glycerol (0.29 g/L/h) than in the control (0.25g/L/h).

The increase on xylitol production in the medium supplemented with glycerol can be mainly associated with the improvement of xylose consumption, which was discussed before. It can be also considered that glycerol uptake could have a direct benefic effect on xylitol accumulation and, consequently, in its production. It is known that one of the first steps in glycerol assimilation is its oxidation with the concomitant reduction of NAD^+ to NADH(Adler et al., 1985; Neivogt & Stahl, 1997), which in turn can contribute to the redox imbalance induced by limiting the oxygen availability and, therefore, to restrict the XDH activity even more. It is still under analysis how glycerol assimilation led to a lower xylitol yield.

It is worth highlighting that the improvement of xylitol volumetric productivity after reduction of oxygen availability in this work was similar to that found by Arruda & Felipe (2009)[24], who reported that the glycerol addition (0.7g/L) led to an increase of 25% on xylitol volumetric productivity(from 0.84 to 1.13g/L/h), in experiments at laboratory scale with the same yeast used in the present study.

Although with reduction of oxygen availability the xylitol yield in the medium with glycerol supplementation was not higher than that in the control medium, both values (0.65 and 0.79 g/g, respectively)obtained in the present work were higher than those already found in studies that evaluated other strategies, using the same yeast (*C. guilliermondii* FTI 20037), the same substrate (sugarcane bagasse hemicellulosic hydrolysate),at bioreactor scale, but in a single-stage fermentation (Martínez et al., 2000; Sene et al., 2001; Silva et al., 2005; Silva & Felipe, 2006). Silva et al.(2005) studied the effect of inoculum grown on xylose and/or glucose on the xylose-to-xylitol bioconversion in a 2.4L bioreactor with a k_La value of $17h^{-1}$. These authors reported a xylitol yield of 0.56g/g when inoculum was prepared with glucose as sole carbon source. In similar fermentation conditions, but with an inoculum prepared with xylose as sole carbon source and with an optimized glucose: xylose proportion of 1:5 for fermentation, Silva & Felipe (2006) verified a xylitol yield of 0.59g/g. Sene et al.(2001)obtained the same result, using a 5L bioreactor, k_La of $21h^{-1}$ and an inoculum prepared with progressive adaptation of the yeast to the hydrolysate. Martinez et al.(2000)evaluated the effect of the k_La on the xylitol production and found a maximum xylitol yield of 0.58g/g in continuous operation in a 1.25L bioreactor with k_La of $20h^{-1}$.

On the other hand, in the case of xylitol volumetric productivity, which was improved with reduction of oxygen availability and glycerol supplementation, as discussed above, the obtained value (0.29g/L/h) was lower than the values found in the studies previously cited (Martínez et al., 2000; Sene et al., 2001; Silva et al., 2005; Silva & Felipe, 2006). Silva et al.(2005) reported xylitol volumetric productivity of 0.46 g/L/h, whereas Silva &Felipe (2006) found a value of 0.53 g/L/h. Higher values were verified by Sene et al.(2001) and Martinez et al.(2000), in whose studies were reached maximum xylitol volumetric productivities of 0.60 and 0.70 g/L/h, respectively. Considering that the differences between the studies cited and the present work are mainly related to the strategies evaluated in each case, new studies are being done in order to establish new strategies to achieve improvements in this bioprocess through the use of glycerol.

Another issue to be discussed is the reduction of the cell growth (Fig. 1), due to the xylitol production and the consequent reduction of carbon flux derived from xylose metabolism after the decrease on oxygen availability. This result was evidenced by the diminishing on the cell growth rate, which was approximately five-fold lower than that from the first stage (Table 1). Despite the differences in this parameter before reduction of oxygen availability, which was discussed before, the cell growth rate was the same in the medium supplemented with glycerol and in the control (0.04 g/L/h) afterwards. Therefore, the slight difference of 11% between the total formed cell biomass in medium with glycerol supplementation (6.52 g/L) and without glycerol addition (5.88 g/L) can be attributed mainly to the higher cell growth in the former before reduction of oxygen availability, which cannot be directly related to glycerol assimilation.

It was also observed reductions on ethanol and acetic acid concentrations (Figs.2 and 3), which were already verified in other studies and can be assumed as consumption (Flores et al., 2000; Lima et al., 2004; Silva et al., 2004). Interestingly, whereas before reduction of oxygen availability the rate of acetic acid consumption was higher in the medium with glycerol supplementation (0.05 g/L/h) than that in the medium with glycerol addition (control, 0.01 g/L/h);after reduction of oxygen availability not only the rate of acetic acid consumption but also of ethanol were both higher in the control (0.03 g/L/h in both cases) than those in the medium supplemented with glycerol (0.01 and 0.02 g/L/h, respectively). As a result, in the medium without glycerol addition, total acetic acid consumption (67.66%) was approximately two-fold higher than that in the medium with glycerol supplementation (38.54%). It is important to point out that the decrease on acetic acid concentration after reduction of oxygen availability was related to an increment on pH in both experiments (Fig. 3), as already reported in other studies (Lima et al., 2004; Silva et al., 2004).

Based on the diminution in ethanol and acetic acid concentrations, it can be considered that these two compounds could be potential co-substrates, since the carbon flux derived from their assimilation can be integrated to the central metabolism (Flores et al., 2000). Nonetheless, it is interesting that in the medium with glycerol supplementation, in which glycerol could be acting as co-substrate, as discussed above, there was lower ethanol and acetic acid consumption, indicating that glycerol uptake possibly interfered on ethanol and acetic acid consumption.

Table 1. Main fermentative parameters of glycerol use in association with reduction of oxygen availability after 12h in sugarcane bagasse hemicellulosic hydrolysates with *C. guilliermondii* FTI 20037.

	Before reduction of oxygen availability (0 – 12h)		After reduction of oxygen availability (12 – 96h)		Global Fermentation	
Parameter	**Glycerol supplementation**	**Control**	**Glycerol supplementation**	**Control**	**Glycerol supplementation**	**Control**
Xylose consumption (%)	24	36	72	53	96	89
Xylose uptake rate (g/L/h)	1.01	1.44	0.44	0.29		
Cell biomass (g/L)	3.12	2.43	3.40	3.45	6.52	5.88
Cell growth rate (g/L/h)	0.26	0.20	0.04	0.04		
Xylitol produced (g/L)	1.92	1.96	23.94	20.21	25.86	22.17
Xylitol volumetric productivity (g/L/h)	0.16	0.16	0.29	0.25	0.27	0.23
Xylitol yield (g/g)	0.16	0.11	0.65	0.79	0.53	0.51

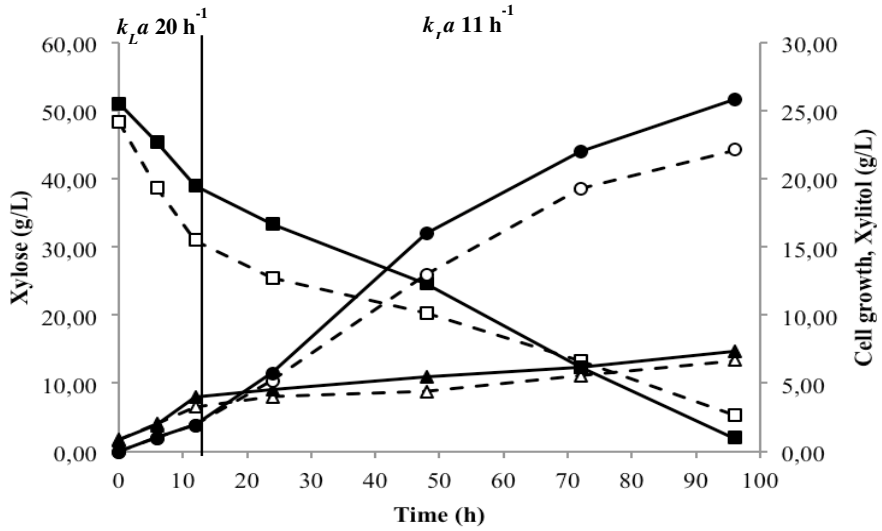

Fig. 1. Profiles of xylose (square), xylitol (circle) and cell biomass (triangle) in fermentation with reduction of oxygen availability in sugarcane bagasse hemicellulosic hydrolysates with *C. guilliermondii* FTI 20037. Dashed line (---) and open style: Without glycerol supplementation (control).
Complete line (—) and solid style: With glycerol supplementation.

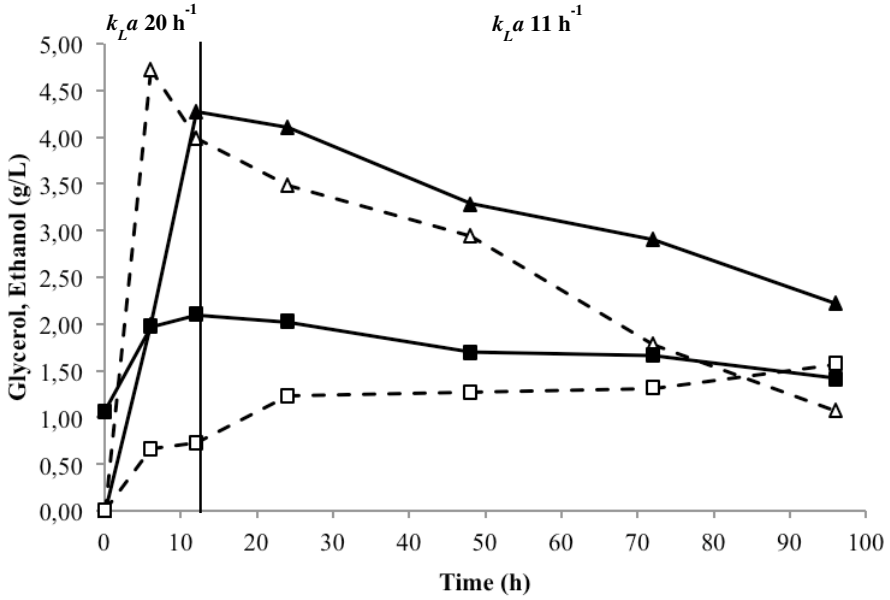

Fig. 2. Profiles of glycerol (square) and ethanol (triangle) in fermentation with reduction of oxygen availability in sugarcane bagasse hemicellulosic hydrolysates with *C. guilliermondii* FTI 20037.
Dashed line (---) and open style: Without glycerol supplementation (control).
Complete line (—) and solid style: With glycerol supplementation.

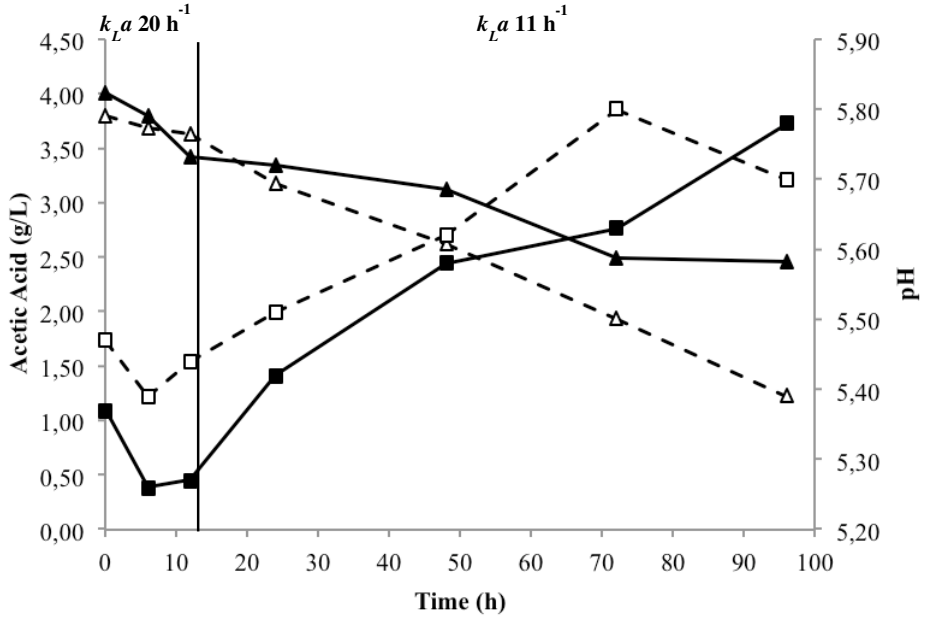

Fig. 3. Profiles of acetic acid (triangle) and pH (square) in fermentation with reduction of oxygen availabilityin sugarcane bagasse hemicellulosic hydrolysates with *C. guilliermondii* FTI 20037.
Dashed line (---) and open style: Without glycerol supplementation (control).
Complete line (—) and solid style: With glycerol supplementation.

4. CONCLUSION

It was implemented a combined strategy of glycerol use in association with reduction of oxygen availability after 12h in order to improve the fermentative performance of *C. guilliermondii* FTI 20037 in sugarcane bagasse hemicellulosic hydrolysate and, consequently, to increase efficiency and productivity of xylitol production bioprocess. In spite of the slight differences in the parameters of the global fermentation between the medium with glycerol supplementation and the control experiment (without glycerol supplementation), improvement scan be evidenced on xylose consumption and xylitol production with reduction of oxygen availability, which were associated with glycerol uptake, indicating that this compound was probably used as co-substrate. Considering these results, new studies are being done in order to establish new strategies to achieve improvements in this bioprocess through the use of glycerol.

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