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PVA-Hydrogel Entrapped *Candida Guilliermondii* for Xylitol Production from Sugarcane Hemicellulose Hydrolysate

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Abstract Viable cells of *Candida guilliermondii* were immobilized by inclusion into polyvinyl alcohol (PVA) hydrogel using the freezing–thawing method. Entrapment experiments were planned according to a 2^3 full factorial design, using the PVA concentration (80, 100, and 120 g L^{-1}), the freezing temperature (−10, −15, and −20 °C), and the number of freezing-thawing cycles (one, three, and five) as the independent variables, integrated with three additional tests to estimate the errors. The effectiveness of the immobilization procedure was checked in Erlenmeyer flasks as the pellet capability to catalyze the xylose-to-xylitol bioconversion of a medium based on sugarcane bagasse hemicellulosic hydrolysate. To this purpose, the yield of xylitol on consumed xylose, xylitol volumetric productivity, and cell retention yield were selected as the response variables. Cell pellets were then used to perform the same bioconversion in a stirred tank reactor operated at 400 rpm, 30 °C, and 1.04 vvm air flowrate. At the end of fermentation, a maximum xylitol concentration of 28.7 g L^{-1}, a xylitol yield on consumed xylose of 0.49 g g^{-1} and a xylitol volumetric productivity of 0.24 g L^{-1} h^{-1} were obtained.

Keywords *C. guilliermondii* · Immobilization · PVA-hydrogel · Sugarcane bagasse · Hydrolysate

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Introduction

Xylitol is a pentitol as sweet as sucrose and sweeter than sorbitol that exhibits anticariogenic and many other biomedical properties [1]. It is industrially produced by the expensive chemical hydrogenation of D-xylose, deriving from hemicellulosic hydrolysate, using Ni/Al_2O_3 as a catalyst [2]. Therefore, its production from agricultural residues, such as xylose-rich sugarcane hemicellulosic hydrolysates, is a promising and attractive alternative.

Although xylose-to-xylitol bioconversion could potentially be carried out by bacteria, filamentous fungi, and yeasts, the last microorganisms are the best xylitol producers, with special emphasis on the genera *Candida* and *Debaryomyces* [3]. The use of metabolically engineered *Saccharomyces cerevisiae* or *Candida* spp. has also been investigated as a promising alternative for industrial production of xylitol [4], however some doubts arise about their long-term stability and use in the production of a food additive like xylitol.

Xylose-fermenting yeasts reduce xylose into xylitol by NADPH- and/or NADH-dependent xylose reductase, which is then oxidized to xylulose mainly by NAD^+-dependent xylitol dehydrogenase. Afterwards, xylulose is phosphorylated by xylulose kinase to xylulose 5-phosphate that enters the pentose phosphate pathway and is metabolized [5]. However, under oxygen-limited conditions, NADH produced by xylitol dehydrogenation is not completely re-oxidized by the respiratory chain, hence xylitol accumulates as the main product [6].

Immobilized cell bioprocesses have been gaining increasing attention because they allow to protect the entrapped biocatalyst, prevent washout, and increase productivity in continuous processes and lower recovery and recycling costs [7–9]. Cell immobilization via gel entrapment is widely used in bench-scale tests, and many gel-like materials are used as carriers, which may be based on natural (alginate, κ-carrageenan, agarose, agar, chitosan, etc.) or synthetic (polyacrylamide, polyacrylate, polyurethane, etc.) polymers or precursors [10]. Contrary to synthetic polymers, the natural ones show poor mechanical strength and durability [11]. Among the supports employed by our research group for yeast cell immobilization to perform xylose-to-xylitol bioconversion, namely Ca-alginate [12], porous glass, zeolite [13], polyvinyl alcohol (PVA) [14], and sugarcane bagasse [15], PVA is of particular concern because it is cheap, non-toxic, and widely used in biomedical and food industries [16, 17].

The use of lignocellulosics as raw materials for high value chemical production is very attractive and promising, since they are an abundant and renewable source of carbohydrates. However, to utilize their sugar content, these materials have to be submitted to hydrolysis treatments, which generally are performed by chemical means [18]. The hemicellulosic fraction of lignocellulosics can be easily hydrolyzed by dilute acids, but the degradation of pentoses, hexoses, and lignin leads to several by-products toxic to the microorganisms [19]; therefore, detoxification procedures are required [19, 20].

The aim of this study was to select a protocol for *Candida guilliermondii* cells immobilization in PVA-hydrogel and to investigate the ability of the resulting pellets to catalyze xylitol production from sugarcane bagasse hemicellulosic hydrolysate in a stirred tank reactor (STR).

Materials and Methods

Chemicals

Polyvinyl alcohol with molecular weight of 89,000–98,000 g mol^{-1} and purity of 99.0% and sodium alginate (S1100) were acquired from Sigma-Aldrich (St. Luis, MO, USA) and Degussa Texturant Systems (Paris-la-Défense, France), respectively.

Preparation and Treatment of the Sugarcane Bagasse Hydrolysate

The composition of sugarcane bagasse used in this study was already reported by Pessoa et al. [21]. It contained 47.8% *w*/*w* of moisture, and the remaining dry matter was made up (in % *w*/*w*) of 70.9 total reducing sugars, of which 25.2 xylose and 41.0 glucose, 23.0 lignin and 1.1 ash. The sugarcane bagasse hemicellulosic hydrolysate was prepared by acid hydrolysis of sugarcane bagasse in a 250-L stainless steel reactor at 121 °C for 10 min, using 100 mg of sulphuric acid g^{-1} of bagasse (dry wt) at a solid–liquid ratio of 1:10 g g^{-1}. The liquid fraction was separated by centrifugation and fivefold concentrated at 70 °C under vacuum. To minimize the amounts of the main fermentation inhibitors, the concentrated hydrolysate was treated according to the method proposed by Alves et al. [20]. The pH was raised to 7.0 with calcium oxide and then lowered to 5.5 with phosphoric acid. Subsequently, 2.4% (*w*/*v*) activated charcoal was added to the hydrolysate, which was then left under agitation (200 rpm) at 30 °C for 1 h. The precipitates resulting from all the stages of the treatment were removed by vacuum filtration. The average composition of the hydrolysate was 64.7±0.4 g L^{-1} xylose, 3.08±0.04 g L^{-1} glucose, 4.23±0.08 g L^{-1} arabinose, and 1.84±0.05 g L^{-1} acetic acid.

Microorganism and Inoculum Cultivation

Cells of the yeast *C. guilliermondii* FTI 20037, described by Barbosa et al. [22], were maintained on malt-extract agar slants at 4 °C.

A loopful of cells was transferred to 125-mL Erlenmeyer flasks containing 50 mL of medium consisting of xylose (30 g L^{-1}), ammonium sulphate (3 g L^{-1}), calcium chloride (0.1 g L^{-1}) and rice bran extract (10% *v*/*v*). The rice bran extract was previously prepared by heating a 200 g L^{-1} aqueous suspension of locally available rice bran at 121 °C for 20 min. The inoculum was cultivated in a rotary shaker at 200 rpm and 30 °C for 24 h. Afterwards, the cells were collected by centrifugation (2000×*g*, 15 min), rinsed with sterile distilled water, centrifuged, and re-suspended in sterile distilled water to give a suspension with a cell concentration of about 50 g L^{-1} (dry weight).

Cell Immobilization

The yeast cells were immobilized by entrapment in hydrogel based on polyvinyl alcohol by using the freezing–thawing method.

A polyvinyl alcohol aqueous solution with 80, 100 or 120 g L^{-1} (according to the experimental design) and 2% sodium alginate (autoclaved at 121 °C for 20 min) were mixed with the thick cell suspension so as to reach a cell concentration of 6 g L^{-1} (dry weight). The mixture was then dropped into a gently stirred solution of calcium chloride (40 g L^{-1}). A 3.8-cm-long 19-gage needle and a peristaltic pump were used to make the beads. The beads were maintained in the calcium chloride solution at 4 °C for 3 h. Afterwards, they were washed with sterile deionized and distilled water and submitted to the freezing–thawing cycles.

A 2^3 full factorial design [23] was employed to select the most suited ranges of immobilization conditions for this support. The selected three independent variables were controlled at two levels (−, +), namely 80 and 120 g L^{-1} for the PVA concentration (PC), −10 and −20 °C for the freezing temperature (FT), and one and five for the number of freezing–thawing cycles (CN). These variables and levels were suggested by previous results obtained with *C. guilliermondii* cells immobilized onto other supports, namely

porous glass [13, 24, 25], calcium alginate [26], zeolite [13], as well as on the same hydrogel under different conditions [14]. Moreover, three additional tests were carried out at a central point out of the plan (0), using PC=100 g L^{-1}, FT=−15 °C, and CN=3, to make the analysis of errors as well as to explore an intermediate dominion.

Medium and Fermentations Conditions

The concentrated and detoxified hydrolysate was heated at 111 °C for 15 min and supplemented with ammonium sulphate (3.0 g L^{-1}), calcium chloride (0.1 g L^{-1}) and rice bran extract (10% *v*/*v*), to be used as a fermentation medium.

Batch fermentations were carried out in duplicate in 125-ml Erlenmeyer flasks containing 40 ml of fermentation medium and 10 g of beads containing cells (pellets), prepared according to the above experimental design. The flasks were maintained in a rotary shaker at 200 rpm and 30 °C for 72 h. The effects of the immobilization variables on the xylose-to-xylitol bioconversion as well as on the cell retention capacity by the support were investigated through statistical concepts using the Statgraphics program (version 6.0). To this purpose, the yield of xylitol on consumed xylose ($Y_{P/S}$), the xylitol volumetric productivity (Q_P), and the cell retention yield (Y_R) were selected as the responses.

Afterwards, a bioconversion was performed in duplicate in a 2.5 L-bench-scale stirred tank reactor BioFlo III (New Brunswick Scientific Co, NJ, USA) equipped with propeller-type turbine for less shearing. For the bioconversion, 960 mL of medium and 240 g of beads were introduced into the reactor up to an initial cell concentration of 1.4 g L^{-1} (dry weight), which was operated at 400 rpm, 30 °C, and 1.04 vvm (volumetric oxygen transfer coefficient (k_La) of 10 h^{-1}, determined in the absence of beads).

For entrapment tests, $Y_{P/S}$ was calculated as the amount of xylitol produced on consumed xylose at the end of fermentation (about 72 h), Q_P as the ratio of the maximum xylitol concentration to the same fermentation time and Y_R as the ratio of cell concentration in the beads after the same time to the sum of this and the corresponding free cell concentration. The same parameters were calculated in the same way for fermentations in STR but after 120 h instead of 72 h. Moreover, $Y_{X/S}$ was calculated as the amount of dry biomass produced on consumed xylose after 120 h.

The maximum percentage errors with respect to the mean values of duplicate experiments were only 1.6%, 3.8%, 2.7%, 1.3%, 4.0%, 3.0%, and 1.8% for the concentrations of xylose, arabinose, xylitol, glucose, acetic acid, immobilized, and free cells respectively, therefore no further statistical analysis of the experimental errors was needed.

Analytical Methods

The concentrations of sugars (glucose, xylose, and arabinose), xylitol, acetic acid, ethanol, and glycerol were determined by HPLC with a refractive index detector and Bio-Rad HPX-87-H (300×7.8 mm) column at 45 °C, using 0.005 M sulphuric acid as the eluent, flow rate of 0.6 ml min^{-1} and sample volume of 20 μL.

Free cell concentration was determined by a UV-VIS spectrophotometer model DU 640 B (Beckmam, Fullerton, CA, USA) at 600 nm using a calibration curve obtained through correlation between cell dry weight and optical density at 600 nm. The immobilized cell concentration was estimated by the same method after dissolution of lenses by heating at 70 °C.

The volumetric oxygen transfer coefficient was determined by dynamic gassing-out technique according to the method of Pirt [27].

Results and Discussion

The use of hydrogel as immobilization matrix can protect the biocatalyst against unfavorable environmental conditions. PVA-based gels as immobilizing supports have been frequently investigated because they are non-toxic to living organisms, besides exhibiting high mechanical strength, elasticity, and macroporosity. Previous efforts were made to evaluate the potential of different immobilizing supports for xylitol bioproduction from sugarcane bagasse, seeking to develop a feasible bioprocess alternative to chemical synthesis [12–15, 28–30].

In this study, yeast immobilization conditions in PVA-hydrogel were established through the factorial design described in the "Materials and Methods" section. The initial part of the work was addressed to identification of the influence of PVA-concentration, number of freezing–thawing cycles (CN) and freezing temperature on xylitol yield ($Y_{P/S}$), volumetric productivity (Q_P) and cell retention (Y_R) by the support, while the second part was focused on fermentations performed using pellets prepared according to the optimal protocol.

Pellets Preparation

The results of bioconversions performed with pellets prepared according to the factorial design (Table 1) demonstrate that all the biocatalysts were effective to produce xylitol, the xylitol yield, volumetric productivity, and yield of cell retained within the pellet varying in the ranges 0.49–0.63 g g^{-1}, 0.43–0.50 g L^{-1} h^{-1}, and 0.187–0.325, respectively. Comparable results in flasks were reported by Santos et al. [13] for cells immobilized in porous glass spheres and zeolite ($Y_{P/S}$=0.52–0.53 g g^{-1}, Q_P=0.32–0.33 g L^{-1} h^{-1}).

Table 2 shows the Student's *t*-test, the estimated effects, and the standard errors collected for the above responses following the experimental schedule reported in Table 1. It is noteworthy that $Y_{P/S}$ was significantly influenced only by PVA-concentration at 95% confidence level. The highest values of all responses as well as of maximum xylitol concentration were obtained using the lowest PC level (80 g L^{-1}) to prepare the biocatalyst; therefore, any subsequent evaluation dealing with the remaining responses will be restricted to such a condition.

Table 1 Values of xylitol yield ($Y_{P/S}$), volumetric productivity (Q_P) and yield of cell retention in the pellet (Y_R), after 72-h fermentation according to 2^3 full factorial design.

Test	Coded variable			Response		
	PC	CN	FT	$Y_{P/S}$ (g g^{-1})	Q_P (g L^{-1} h^{-1})	Y_R (–)
1	+	+	+	0.55	0.49	0.255
2	+	+	−	0.49	0.43	0.209
3	+	−	+	0.52	0.45	0.212
4	+	−	−	0.56	0.46	0.187
5	−	+	+	0.63	0.49	0.300
6	−	+	−	0.59	0.50	0.325
7	−	−	+	0.52	0.43	0.264
8	−	−	−	0.59	0.49	0.297
9	0	0	0	0.52	0.45	0.209
10	0	0	0	0.52	0.44	0.207
11	0	0	0	0.52	0.44	0.206

PC PVA concentration, *CN* number of freezing-thawing cycles, *FT* freezing temperature

Table 2 Estimate of effects, standard errors, student's *t*-test for xylitol yield ($Y_{P/S}$), volumetric productivity (Q_P), and cell retention (Y_R) after 72 h of fermentation.

Variable	$Y_{P/S}$ (g g^{-1})			Q_P (g L^{-1} h^{-1})			Y_R (–)		
	Effects	Standard error	*t* value	Effects	Standard error	*t* value	Effects	Standard error	*t* value
Average	+0.546	±0.008	–	+0.461	±0.006	–	+24.281	±0.011	–
PC	−0.053	±0.018	−2.767[b]	−0.020	±0.013	−1.517[d]	−8.075	±0.026	−3.161[b]
CN	+0.018	±0.018	0.922[c]	+0.020	±0.013	−1.517[d]	+3.225	±0.026	1.263[c]
FT	−0.003	±0.018	−0.132[c]	−0.005	±0.013	−0.379[c]	+0.325	±0.026	0.127[c]
PC×CN[a]	−0.038	±0.018	−1.977[d]	−0.015	±0.013	−1.137[c]	+0.025	±0.026	0.009[c]
PC×FT[a]	+0.013	±0.018	0.659[c]	+0.030	±0.013	+2.275[e]	+3.225	±0.026	1.263[c]
CN×FT[a]	+0.053	±0.018	2.767[b]	0.030	±0.013	+2.275[e]	+0.725	±0.026	0.284[c]

PC PVA concentration (main effect), *CN* number of freezing-thawing cycles (main effect), *FT* freezing temperature (main effect)

[a] PC×CN, PC×FT, CN×FT=two-factor interactions

[b] Significance≥95%

[c] Significance<80%

[d] 80≤Significance<90%

[e] 90≤Significance<95%

At this PVA level, although $Y_{P/S}$ was not significantly influenced by CN and FT, the interaction between these two independent variables was statistically significant at 95% confidence level, and the maximum $Y_{P/S}$ value (0.63 g g^{-1}) was achieved at CN=5 and FT=−20 °C (Table 1, test n. 5). In contrast, xylitol volumetric productivity was not influenced by any of these variables at 95% confidence level. Although a slight negative effect of PVA concentration on productivity could be identified from the data of Table 1, it was not statistically significant. The same applies to the highest xylitol productivity obtained in test n. 6 (Q_P=0.50 g L^{-1} h^{-1}), which was performed with pellet prepared using PC=80 g L^{-1} and the highest levels of the other two variables (CN=5 and FT=−10 °C). Resuming, although the results obtained according to the selected factorial design did not

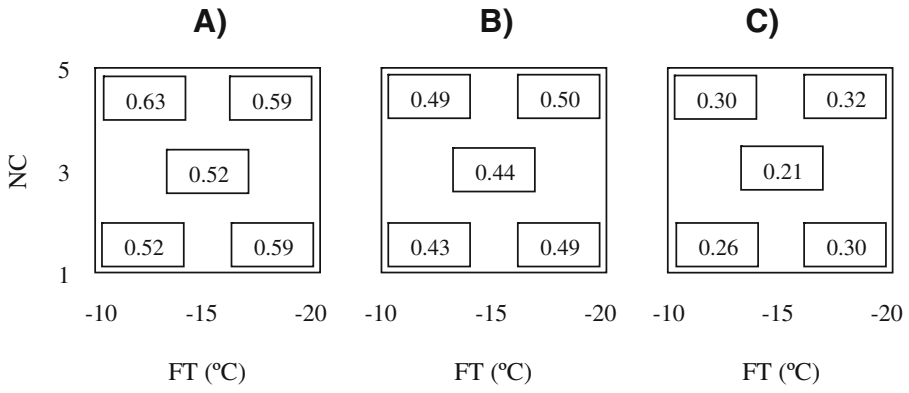

Fig. 1 Interaction plots of the A) yield of xylitol on consumed xylose ($Y_{P/S}$, g g^{-1}), B) xylitol volumetric productivity (Q_P, g L^{-1} h^{-1}) and C) yield of cell retention by the pellet (Y_R, –) as functions of the freezing temperature (*FT*) and number of freezing–thawing cycles (*NC*)

provide a univocal optimization of the system (Fig. 1), they suggested the conditions for future optimization of the immobilization protocol.

Fermentations with Immobilized-Cell Pellet in STR

Figure 2 shows the consumption of sugars (glucose, xylose, and arabinose) and acetic acid as well as xylitol production during fermentations performed in STR on a medium based on sugarcane bagasse hemicellulosic hydrolysate and with pellets prepared using PVA concentration of 80 g L^{-1}, five freezing–thawing cycles and a freezing temperature of −20 °C. As can be seen, glucose was quickly consumed at the beginning (within 12 h) because it is the preferential carbon source for the yeasts and was present in the medium in such a low concentration (about 3 g L^{-1}) that did not affect xylose-to-xylitol bioconversion. The presence of hexoses, such as glucose, in the fermentation medium is in fact a well-known critical factor that regulates xylitol formation by pentose-fermenting yeasts. High glucose levels have been reported to repress the synthesis of xylose reductase, the key enzyme involved in xylose conversion to xylitol, resulting in low yields of this product [31]. Other authors related similar profile of glucose consumption by yeast in hydrolysate-based media using either immobilized cells system [30] or free cells system [32].

As can be observed in Fig. 2 and Table 3, *C. guilliermondii* cells immobilized in PVA pellets were able to convert xylose to xylitol during the fermentation in stirred tank reactor, consuming around 91% xylose and accumulating 28.7 g L^{-1} xylitol after 120 h. The values of xylitol yield (0.49 g g^{-1}) and volumetric productivity (0.24 g L^{-1} h^{-1}) at the end of fermentation were comparable to those reported for hemicellulose hydrolysate fermentation by *C. guilliermondii* cells adsorbed onto porous glass spheres or zeolite, either in flasks or in fluidized bed reactor [13, 24, 25, 30], but lower than those obtained in flasks in this work. The worst xylitol production in the STR system with respect to the flasks could have been the result of an increase in the actual k_La with respect to that selected in the absence of

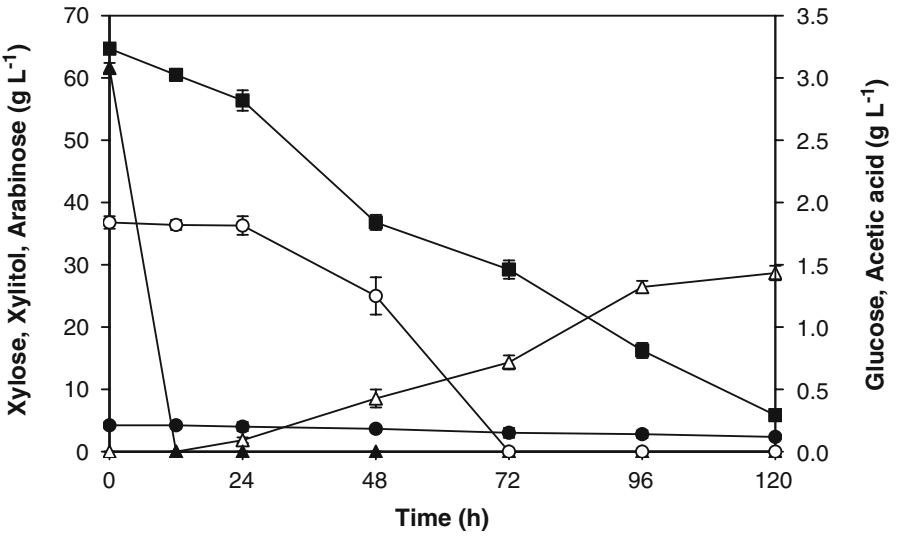

Fig. 2 Consumption of xylose (■), arabinose (●), glucose (▲) and acetic acid (○) as well as xylitol production (△) during fermentation in stirred tank reactor using *C. guilliermondii* cells immobilized in PVA-hydrogel

Table 3 Results of xylose-to-xylitol bioconversion by *C. guilliermondii* cells immobilized in PVA beads after 120 h of cultivation in medium based on sugarcane bagasse hemicellulosic hydrolysate.

Percentage of xylose consumption (%)	91
Final xylitol concentration (g L^{-1})	28.7
Yield of xylitol on consumed xylose (–)	0.49
Yield of biomass on consumed xylose (g g^{-1})	0.18
Xylitol productivity (g L^{-1} h^{-1})	0.24

carrier to avoid excess oxygen dissolution in the medium (k_La=10 h^{-1}) [33]. Paz et al. [34] demonstrated that, under these conditions, the air bubbles in the medium burst and more oxygen is dissolved, thus causing the biomass to grow in the detriment of xylitol formation [25]. This results, which is confirmed by the large biomass growth in STR ($Y_{X/S}$=0.18 g g^{-1}), suggests that optimization of the oxygenation conditions in STR is a fundamental requisite to obtain satisfactory xylitol formation.

Arabinose was poorly and slowly consumed, being about 55% of its starting amount detected in the medium at the end of process. Other authors reported similar behavior in studies performed with both free and immobilized cells [5, 14]. Ethanol was produced at low levels during the fermentations, reaching a final concentration of only 5.2 g L^{-1} (Fig. 3), confirming the results of previous works performed with this yeast either immobilized onto porous glass [13, 24] and zeolite [13] or in suspended-cell system [22]. On the contrary, glycerol concentration in the broth was always negligible.

With regard to acetic acid, it has been proposed that high acetic acid concentrations affect cell metabolism as the result of transfer of this acid in its undissociated form to the inside of the cell and consequent acidification [35]. For example, Felipe et al. [36, 37], in fermentations of *Eucaliptus* wood and sugarcane bagasse hemicellulosic hydrolysates performed with the same yeast, found that 40–50% of the starting acetic acid was consumed

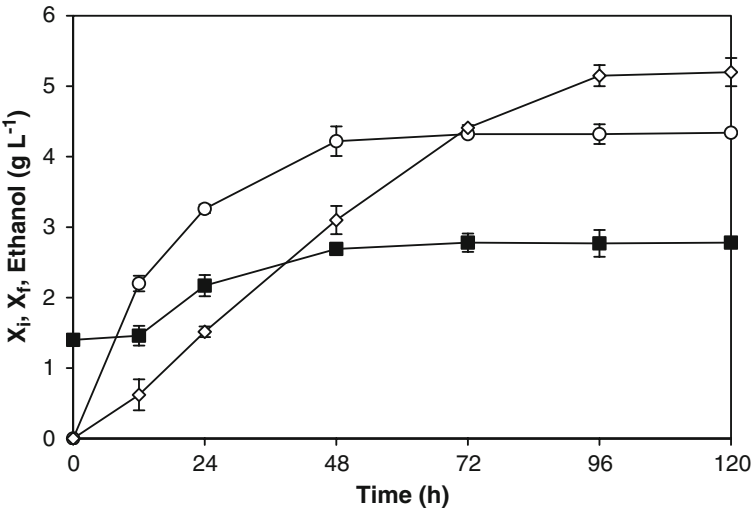

Fig. 3 Concentration profile of ethanol (◇), free cells (X_f, ○) and cells immobilized in the PVA-hydrogel (X_i, ■) during fermentation of sugarcane bagasse hemicellulosic hydrolysate. All concentrations refer to the total reactor volume

simultaneously with sugars after a prolonged lag phase without production of xylitol. Nevertheless, it has been demonstrated that low concentrations of this acid can favor xylose bioconversion by yeast [35, 38], likely because its respiration can favor the bioenergetics of the system [39]. Because the acetic acid level at the beginning of this fermentation (1.8 g L^{-1}) was lower than the inhibitory threshold (2 g L^{-1}) reported by Roberto et al. [40] and it was completely consumed within only 72 h, this suggests that xylose-to-xylitol conversion could have been stimulated by the acetic acid presence in the medium.

Cell growth was considered as the increase of the free-cells concentration (X_f) in the fermentation medium and of immobilized cells in the gel beads (X_i). Figure 3 shows that the immobilized cell concentration increased by 93% up to 48 h and then kept almost constant until the end of the fermentation. This result suggests that the pores of the gel matrix were saturated by immobilized cells after this short time. A portion of these cells was released from the beads and proliferated in the medium, reaching a maximum concentration of 4.34 g L^{-1}. According to Martynenko et al. [41], this cell release, which was already observed by other authors also from alginate [12, 28], PVA [14] and pectin [42] pellets, would take place when the amount of biomass accumulated in the carrier achieves a critical value.

Actually, the explored system was a mixed one, since a fraction of cells was freely suspended in the medium and another one was entrapped in the beads. Oxygen availability is notoriously higher for free cells, for which there is no physical space limitation for growth, thus their higher growth rate with respect to immobilized ones. Nevertheless, in the case of xylose-to-xylitol bioconversion, oxygen limitation is a fundamental requisite for xylitol accumulation, because under these conditions the NADH produced by xylitol dehydrogenation cannot be completely re-oxidized to NAD^+ by the respiratory chain, hence xylitol is excreted to the outside of the cell. Thus, the immobilized cells may have produced higher amount of xylitol than the free ones, mainly at the beginning of the process, when the low concentration of the suspended cells and the relatively high oxygen availability in the medium could have favored their growth in the detriment of xylitol accumulation. After 72 h of fermentation, free cells concentration maintained almost constant, probably due to oxygen limitation conditions created either by high biomass level or oxygen depletion in the broth. After this period, xylitol accumulated in the medium and reached its maximum concentration (28.7 g L^{-1}) at the end of the run. Meanwhile, no less than 40% of total biomass resulted to be entrapped within the pellets, which preserved their shape and size without appreciable wearing even after five successive operations of cell recycling and fermentation (results not shown).

Conclusion

C. guilliermondii cells were successfully immobilized in PVA-hydrogel using the freezing–thawing method using a 2^3 full factorial design to plan the experiments. To this purpose, the PVA concentration, the freezing temperature and the number of freezing–thawing cycles were selected as the independent variables. The pellets were then tested as biocatalyst for the xylose-to-xylitol bioconversion in sugarcane bagasse hemicellulosic hydrolysate. The results obtained in terms of the yield of xylitol on consumed xylose, xylitol volumetric productivity, and cell retention yield suggested a PVA concentration of 80 g L^{-1}, 5 freezing–thawing cycles and a freezing temperature of −20 °C as the most convenient conditions for pellet preparation. When used in a stirred tank reactor, the beads maintained their shape and size without visible wearing. These results demonstrate the feasibility of the

proposed immobilization system to be used in future continuous xylose-to-xylitol bioconversion in hemicellulose hydrolysates.

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