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ORIGINAL PAPER

Inhibitory action of toxic compounds present in lignocellulosic hydrolysates on xylose to xylitol bioconversion by *Candida guilliermondii*

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Abstract The inhibitory action of acetic acid, ferulic acid, and syringaldehyde on metabolism of *Candida guilliermondii* yeast during xylose to xylitol bioconversion was evaluated. Assays were performed in buffered and non-buffered semidefined medium containing xylose as main sugar (80.0 g/l), supplemented or not with acetic acid (0.8–2.6 g/l), ferulic acid (0.2–0.6 g/l), and/or syringaldehyde (0.3–0.8 g/l), according to a 2^3 full factorial design. Since only individual effects of the variables were observed, assays were performed in a next step in semi-defined medium containing different concentrations of each toxic compound individually, for better understanding of their maximum concentration that can be present in the fermentation medium without affecting yeast metabolism. It was concluded that acetic acid, ferulic acid, and syringaldehyde are compounds that may affect *Candida guilliermondii* metabolism (mainly cell growth) during bioconversion of xylose to xylitol. Such results are of interest and reveal that complete removal of toxic compounds from the fermentation medium is not necessary to obtain efficient conversion of xylose to xylitol by *Candida guilliermondii*. Fermentation in buffered medium was also considered as an alternative to overcome the inhibition caused by these toxic compounds, mainly by acetic acid.

Keywords Xylose · Xylitol · *Candida guilliermondii* · Toxic compounds · Fermentation

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Introduction

Xylitol is a sweetener that has drawn the attention of food and drink manufacturers due to its low caloric value, the possibility of its use to reduce or control weight, and its similarity to sugar concerning taste. In addition, xylitol has much potential for application in the odontological and medical areas, being successfully used to combat dental caries, treat illnesses such as diabetes, disorders in lipid metabolism, and parenteral and renal lesions, and to prevent lung infection, otitis, and osteoporosis [10]. Traditionally, xylitol is produced by chemical conversion of xylose, a high-cost method with low product yield (50–60%), which has thus hindered utilization of xylitol in food and medical industries. Microbiological conversion of xylose to xylitol is particularly attractive, since the process is easier than the chemical route and does not need a toxic catalyst, having, as a consequence, lower environmental impact. Moreover, use of enzymes or specific microorganisms that only act on xylose to xylitol conversion may result in higher product yield [4].

Lignocellulosic materials represent an abundant and inexpensive source of sugars which can be microbiologically converted to industrial products. Nevertheless, biotechnological conversion of xylose into xylitol using hydrolysates obtained from the hemicellulosic fraction of lignocellulosic materials is hindered by the presence of several types of compounds released from these materials

or formed during the hydrolysis process, some of which are toxic to the microorganism. The major toxic compounds include hydroxymethylfurfural, furfural, acetic acid, and several aromatic and phenolic compounds (ferulic acid, *p*-coumaric acid, syringaldehyde, vanillin, among others), the latter being considered as having pronounced toxicity towards microbial metabolism [11, 14].

Acetic acid is the main aliphatic acid present in hemicellulosic hydrolysates, released from the hemicellulose structure when lignocellulosic materials are submitted to a hydrolysis process. Ferulic acid and syringaldehyde are aromatic compounds released during the hydrolysis process, due to partial lignin degradation. Such compounds have been identified and quantified in different hydrolysates, their concentration being dependent on the biomass and hydrolysis conditions used [8, 9, 11]. The effects of these compounds on xylose to xylitol bioconversion have not been deeply investigated, and it is known that levels of tolerance of microorganisms to toxic compounds vary according to the strain and cultivation conditions [5]. Therefore, it is of great importance to establish, for a particular microorganism, the maximum concentration of each toxic compound that can be present in the hemicellulosic hydrolysate without affecting the efficiency of the fermentative process.

Experimental designs have been extensively used for optimization of fermentative processes due to the possibility of simultaneously evaluating several factors, as well as verifying the individual and combined effects of each variable [15]. In the present study, an experimental design was thus used to identify and quantify the inhibitory effects of the variables acetic acid, ferulic acid, and syringaldehyde on xylitol production by *Candida guilliermondii* yeast. The maximum concentration of each compound was chosen based on levels present in rice straw hemicellulosic hydrolysate obtained by diluted-acid hydrolysis. The assays were performed in semidefined media, buffered or not with phosphate salts to control possible synergistic effects of this parameter on the responses (cell growth, xylose consumption, xylitol production, and the fermentative parameters values: xylitol yield factor, cell yield factor, and xylitol volumetric productivity).

Materials and methods

Microorganism and inoculum preparation

Candida guilliermondii ATCC 201935 was the microorganism employed in the experiments. Cells of this yeast were maintained on malt extract agar slants at 4°C. The inoculum was prepared by cultivation of the yeast in 125-ml Erlenmeyer flasks containing 50 ml medium composed of (g/l): xylose (30.0), glucose (5.0), $(NH_4)_2SO_4$ (3.0), $CaCl_2{\cdot}2H_2O$ (0.1), and 20% (v/v) rice bran extract. Concentrated solutions of all the nutrients were prepared separately and sterilized at 121°C for 20 min, except the xylose and glucose solutions, which were autoclaved at 112°C for 15 min. To use rice bran as a nutrient, 10% rice bran suspension was autoclaved at 121°C for 20 min and cooled to room temperature. This suspension was then aseptically centrifuged at 1,100*g* for 20 min, and the liquid fraction (rice bran extract, a source of vitamins and amino acids) was used for preparation of the fermentation medium.

Cells were cultivated at 200 rpm, 30°C for 30 h, being subsequently recovered by centrifugation (1,100*g*, 20 min), washed, and resuspended in the fermentation medium.

Media and fermentation conditions

Semidefined media were formulated with the following composition (g/l): xylose (80.0), glucose (15.0), $(NH_4)_2SO_4$ (3.0), $CaCl_2{\cdot}2H_2O$ (0.1), and 20% (v/v) rice bran extract, and their pH adjusted to 5.5. All nutrients were prepared separately as described in the inoculum preparation. After addition of the nutrients, the fermentation media were supplemented with the inhibitor compounds: acetic acid, ferulic acid, and syringaldehyde, at the concentrations given in Table 1. Assays were performed in buffered and nonbuffered fermentation media. Buffered media were formulated by addition of phosphate salts (KH_2PO_4, 68.9 g/l, and K_2HPO_4, 22.1 g/l), as previously described [1].

Fermentations were performed in 250-ml Erlenmeyer flasks containing 100 ml medium inoculated with initial cell concentration of 3.0 g/l. Flasks were agitated in a rotary shaker at 200 rpm, 30°C for 96 h. Fermentation runs were monitored through periodic sampling to determine cell growth, glucose and xylose consumption, xylitol production, and pH variation.

Experimental design

A 2^3 full factorial design with three coded levels leading to 11 sets of experiments was used to establish the effects of three variables (acetic acid, ferulic acid, and syringaldehyde) on xylose fermentation to xylitol. For statistical analysis, variables were coded according to Eq. 1:

$$x_i = (X_i - X_0)/\Delta X_i, \quad (1)$$

where each independent variable is represented by x_i (coded value), X_i (real value), X_0 (real value at the center point), and ΔX_i (step change value). The range and the levels of the variables investigated in this study are given in Table 1. Three assays at the center point were carried out to estimate the random error needed for analysis of

Table 1 Experimental design used to evaluate the inhibitory effect of acetic acid (AA), ferulic acid (FA), and syringaldehyde (SY) on xylose to xylitol bioconversion by *Candida guilliermondii* in buffered and nonbuffered semidefined media

Assay	Variable[a]			Response[b]				
	AA	FA	SY	pH_{final}	C_{Xi} (%)	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	Q_P (g/lh)
1	0	0	0	5.7	98	0.09	0.81	0.87
1*	0	0	0	2.7	74	0.04	0.73	0.62
2	2.6	0	0	5.2	86	0.06	0.77	0.73
2*	2.6	0	0	4.4	62	0.04	0.31	0.21
3	0	0.6	0	5.2	87	0.06	0.72	0.83
3*	0	0.6	0	3.1	87	0.04	0.67	0.64
4	2.6	0.6	0	5.2	64	0.06	0.76	0.59
4*	2.6	0.6	0	4.4	62	0.04	0.33	0.23
5	0	0	0.8	5.5	86	0.07	0.83	0.91
5*	0	0	0.8	2.9	99	0.03	0.84	0.92
6	2.6	0	0.8	5.5	79	0.02	0.71	0.72
6*	2.6	0	0.8	3.8	89	0.04	0.77	0.76
7	0	0.6	0.8	5.2	79	0.06	0.85	0.86
7*	0	0.6	0.8	3.6	77	0.04	0.77	0.65
8	2.6	0.6	0.8	5.2	59	0.07	0.86	0.61
8*	2.6	0.6	0.8	4.8	66	0.05	0.63	0.46
9	1.3	0.3	0.4	5.1	81	0.04	0.75	0.78
9*	1.3	0.3	0.4	4.1	59	0.03	0.49	0.32
10	1.3	0.3	0.4	5.3	79	0.04	0.79	0.82
10*	1.3	0.3	0.4	4.1	58	0.01	0.48	0.31
11	1.3	0.3	0.4	5.3	81	0.04	0.67	0.70
11*	1.3	0.3	0.4	3.9	61	0.04	0.51	0.34

* Assays in nonbuffered media. Values obtained after 72 h fermentation

[a] Values in g/l; [b] C_{Xi}, xylose consumption; $Y_{P/S}$, xylitol yield factor; $Y_{X/S}$, cell yield factor; Q_P, xylitol volumetric productivity

variance. The xylitol yield factor ($Y_{P/S}$), cell yield factor ($Y_{X/S}$), and volumetric productivity (Q_P) calculated at the end of the fermentation runs were taken as the dependent variables or responses of the design experiments. Statistica version 5.0 (Statsoft, USA) software was used for regression and graphical analyses of the obtained data. Statistical significance of the regression coefficients was determined by Student's *t* test.

Analytical methods

Sugars and toxic compounds concentration

Glucose, xylose, xylitol, and acetic acid concentrations were determined by high-performance liquid chromatography (HPLC) using a Waters device with a Bio-Rad Aminex HPX-87H (300 × 7.8 mm) column and a refractive index detector. The samples were diluted, filtered through a Sep Pak C_{18} filter, and thus injected into the chromatograph under the following conditions: column temperature 45°C, 0.01 N sulfuric acid as mobile phase used at flow rate of 0.6 ml/min, and injection volume of 20 µl. Ferulic acid and syringaldehyde concentrations were also determined by HPLC using a Waters device, but with an ultraviolet (UV) detector (at 276 nm) and a Waters Resolve C_{18} 5 µm (3.9 × 300 mm) column at room temperature, using acetonitrile/water (1/8 with 10 g/l acetic acid and 1.6 g/l phosphoric acid) as eluent, flow rate of 0.8 ml/min, and sample volume of 20 µl.

Biomass determination

Cell concentration was estimated from an optical density (OD, 600 nm) versus dry cell weight calibration curve, which was established using spectrophotometric measurements of cells grown in semidefined medium at 200 rpm, 30°C for 30 h. Samples were diluted to a reading band of 0.05–0.5 OD units.

Calculation of fermentative parameters

Xylitol volumetric productivity (Q_P, g/lh) was calculated as the ratio between the concentrations of xylitol (P, g/l) and xylose consumed (g/l). Xylitol yield factor ($Y_{P/S}$, g/g) was defined as the ratio of P to xylose consumed (g/l). Cell yield factor ($Y_{X/S}$, g/g) was defined as the ratio of formed cells to total substrate (xylose and glucose) consumed (g/l).

Results and discussion

Table 1 presents the fermentation results obtained in each experiment. It can be noted that the final pH varied for the nonbuffered media, decreasing from 5.5 to approximately 3.0 in media without acetic acid (AA), and to 4.0 in media supplemented with this acid. The reduction in the initial pH in nonbuffered media is associated with release of free acids due to ammonium consumption by the yeast [17]. On the other hand, the pH decrease in the presence of acetic acid was not so significant, demonstrating that this acid has buffer capacity in a pH range varying between 5.0 and 5.5. Such an effect was also observed during *Saccharomyces cerevisiae* cultivation in defined medium, where a reduction of 2.8 pH units was verified in media without acetic acid, while in the presence of this acid, a variation of less than 1.0 units was observed [13].

Figure 1 shows cell growth and xylitol production obtained according to the experimental design. In general, biomass production was favored in buffered media

(Fig. 1a). The lower cell growth in nonbuffered media may have been a consequence of the pH decrease. When the pH of the medium is low, acetic acid ($pK_a = 4.75$) appears in undissociated form, is liposoluble, and diffuses across the plasma membrane. Once in the cell interior, where pH is 7.4, this acid dissociates and accumulates in the cytoplasm, discharging protons. As a consequence, the internal pH drops, inhibiting cell activity and even causing death [7].

Xylitol production was also higher in buffered media than in nonbuffered media (Fig. 1b). The similar and elevated xylitol concentrations obtained in assays 1 (control) and 5 (supplemented with 0.8 g/l syringaldehyde) (61.5 and 65.5 g/l, respectively) suggest that, under the evaluated experimental conditions, syringaldehyde did not present inhibitory action on xylitol production by *C. guilliermondii*. Another interesting point observed in Fig. 1b is the low xylitol production (<20 g/l) in nonbuffered media containing acetic acid alone (assay 2) or in combination with ferulic acid (assay 4). These results suggest that ferulic acid had no effect on xylitol formation. In fact, in nonbuffered medium containing only ferulic acid (assay 3), xylitol production was similar to that obtained in assay 1 (control), i.e., 45 g/l. It is worth mentioning that xylitol production in medium containing only acetic acid as inhibitor (assay 2) was increased from 15.5 to 46.2 g/l when the fermentation medium was buffered. Such results confirm that the toxic effect of acetic acid was increased when the fermentation pH was decreased.

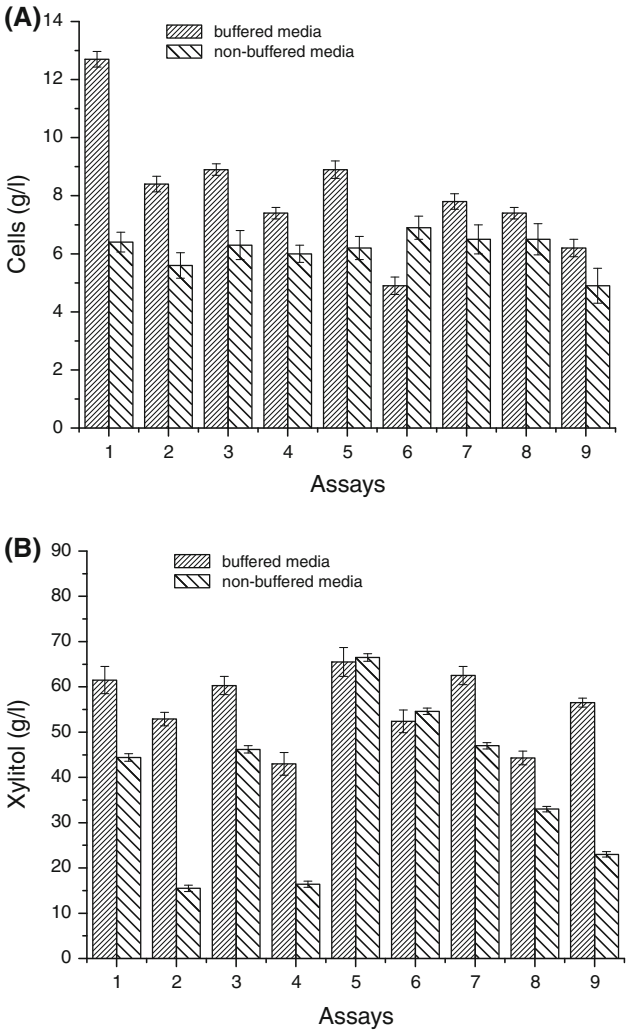

Fig. 1 Cell growth (**a**) and xylitol production (**b**) by *Candida guilliermondii* in semidefined medium under the conditions described in the 2^3 experimental design

As a consequence of the elevated xylitol production, the highest xylitol productivities (Q_P) were obtained in buffered assay 1 (control, 0.87 g/lh) and assay 5 whether buffered or not (supplemented with 0.8 g/l syringaldehyde, 0.91 and 0.92 g/lh, respectively); whereas the lowest Q_P values (0.23 and 0.21 g/lh) were obtained in nonbuffered media supplemented with acetic acid in combination or not with ferulic acid (assays 4 and 2, respectively) (Table 1). The lowest values of xylitol yield factor ($Y_{P/S}$, around 0.30 g/g) were also obtained in assays 2 and 4, due to the presence of acetic acid or its combination with ferulic acid in nonbuffered media. The highest values of this parameter (>0.82 g/g) were obtained in assays 5, 7, and 8 in buffered medium, and in assay 5 in nonbuffered medium.

Xylose consumption varied for each experimental condition (Table 1). In assay 5, for example, which contained only syringaldehyde as toxic compound and gave the highest $Y_{P/S}$ values, xylose consumption was almost total for both buffered and nonbuffered media, suggesting that inhibition of the biosynthesis by this compound did not occur. On the other hand, xylose consumption was reduced to 78% on average in medium containing ferulic acid and syringaldehyde (assay 7), and decreased to approximately 63% when the fermentation medium was supplemented with all three toxic compounds (acetic acid, ferulic acid, and syringaldehyde).

Statistical analysis of the individual and combined effects of the variables on the fermentative parameters of conversion of xylose to xylitol revealed no interaction effects among the variables for any of the evaluated responses (Table 2), in buffered or nonbuffered medium. For the fermentation assays performed in buffered media, only Q_P was influenced by the presence of acetic acid and ferulic acid in the fermentation medium. The main effects of these variables were negative, suggesting that xylitol production was inhibited in the presence of these compounds. The magnitude of these effects also reveals that the inhibition caused by acetic acid was greater than that caused by ferulic acid.

For the nonbuffered media, $Y_{X/S}$ was the only response not influenced by the studied variables. For $Y_{P/S}$ and Q_P

Table 2 Effect estimates (EE), standard errors (SE), and *t* test results for cell yield factor ($Y_{X/S}$), xylitol yield factor ($Y_{P/S}$), and xylitol volumetric productivity (Q_P) obtained during xylose bioconversion by *Candida guilliermondii* in buffered and nonbuffered semidefined media

Effects	$Y_{X/S}$			$Y_{P/S}$			Q_P		
	EE	SE	*t*	EE	SE	*t*	EE	SE	*t*
Buffered media									
Average	0.055	±0.005	11.102	0.774	±0.018	43.898	0.765	±0.013	57.965
Acetic acid (AA)	−0.017	±0.012	−1.493	−0.027	±0.041	−0.664	−0.205	±0.031	−6.619*
Ferulic acid (FA)	0.002	±0.012	0.213	0.017	±0.041	0.423	−0.085	±0.031	−2.744*
Syringaldehyde (SY)	−0.012	±0.012	−1.067	0.047	±0.041	1.148	0.020	±0.031	0.645
AA × FA	0.022	±0.012	1.920	0.052	±0.041	1.268	−0.040	±0.031	−1.291
AA × SY	−0.002	±0.012	−0.213	−0.027	±0.041	−0.664	−0.015	±0.031	−0.484
FA × SY	0.017	±0.012	1.493	0.067	±0.041	1.631	0.005	±0.031	0.161
Nonbuffered media									
Average	0.038	±0.001	25.065	0.593	±0.031	18.600	0.496	±0.053	9.347
Acetic acid (AA)	0.005	±0.003	1.383	−0.242	±0.074	−3.241*	−0.292	±0.124	−2.348*
Ferulic acid (FA)	0.005	±0.003	1.383	−0.062	±0.074	−0.835	−0.132	±0.124	−1.063
Syringaldehyde (SY)	0.000	±0.003	0.000	0.242	±0.074	3.241*	0.272	±0.124	2.188*
AA × FA	0.000	±0.003	0.000	0.002	±0.074	0.033	−0.007	±0.124	−0.060
AA × SY	0.005	±0.003	1.383	0.137	±0.074	1.837	0.117	±0.124	0.943
FA × SY	0.005	±0.003	1.383	−0.042	±0.074	−0.568	−0.152	±0.124	−1.224

* Significant at 95% confidence level

responses, the variables acetic acid and syringaldehyde presented significant main effects (Table 2). The effect of acetic acid on $Y_{P/S}$ was negative (−0.242), while the effect of syringaldehyde was of the same magnitude but positive (+0.242). Such results reveal that the xylitol yield factor was increased by 0.242 g/g in the presence of 0.8 g/l syringaldehyde, while in the presence of 2.6 g/l acetic acid the value of this parameter was reduced by 0.242 g/g, i.e., the negative effect of acetic acid was canceled by the presence of syringaldehyde in the fermentation medium. Similar behavior was observed for Q_P, which was increased by an average of 0.272 g/lh when the medium was supplemented with 0.8 g/l syringaldehyde, but was decreased by 0.292 g/lh when the medium was supplemented with 2.6 g/l acetic acid. The positive effect of syringaldehyde on xylitol production can be easily visualized in Table 1, when comparing the results in nonbuffered media from assays 2 and 4 with those achieved in assays 6 and 8, respectively. When present in the fermentation medium, syringaldehyde canceled the negative effect of acetic acid. Therefore, assays 6 and 8 gave better xylitol production than did assays 2 and 4.

Since only individual effects of the variables were observed, assays were performed in buffered semidefined medium containing different concentrations of acetic acid, ferulic acid or syringaldehyde individually, for better understanding of the maximum concentration of each toxic compound that can be present in the fermentation medium without affecting the microorganism metabolism.

Figure 2 shows that, for all the evaluated inhibitor compounds, yeast cell growth was lower the higher the toxic compound concentration used. For acetic acid concentration, values up to 0.8 g/l did not affect cell growth, but concentration values ≥1.1 g/l caused inhibition of cell growth. Similarly, xylitol production was affected by acetic acid concentrations ≥1.1 g/l, whereas the xylose consumption was only significantly affected by acetic acid concentration of 2.6 g/l (Fig. 2a, b). Under this concentration value, cell growth, xylose consumption, and xylitol production were reduced by 30%, 13%, and 18%, respectively, compared with the control (medium without addition of toxic compounds). The fermentation parameters values (Table 3) reveal that the effect of acetic acid was more evident on cell yield factor ($Y_{X/S}$), which decreased by 33% when the medium was supplemented with 2.6 g/l of this acid, while Q_P and $Y_{P/S}$ were reduced by 16% and 5%, respectively. Similar behavior was reported by Silva et al. [16] when evaluating the effect of acetic acid on xylose to xylitol bioconversion by *C. guilliermondii* from sugarcane bagasse hemicellulosic hydrolysate. According to those authors, a reduction of 17% in cell growth was observed when the fermentation medium contained 2 g/l acetic acid. This inhibitory action was attributed to interference caused by this acid in xylose transportation through the cell membrane, or by deviation of adenosine triphosphate (ATP) required for cell growth to prevent acidification of intracellular pH.

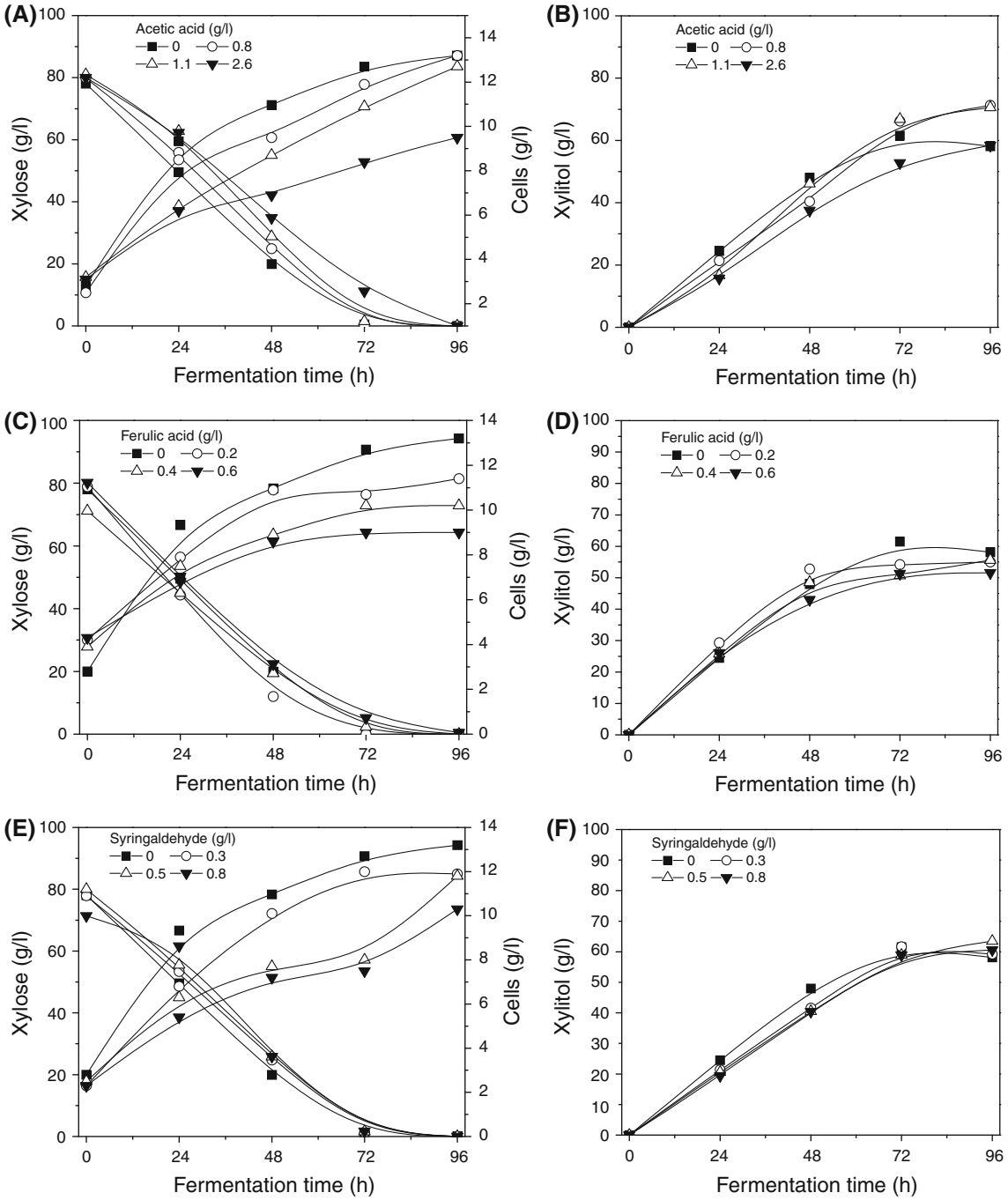

Fig. 2 Kinetic behavior of cell growth, xylose consumption, and xylitol production by *Candida guilliermondii* in semidefined medium supplemented with different concentrations of acetic acid (**a**, **b**), ferulic acid (**c**, **d**) or syringaldehyde (**e**, **f**). Standard deviation less than 5%

Presence of ferulic acid in the fermentation medium affected yeast cell growth at all evaluated concentrations (Fig. 2c), with a 30% reduction in growth being observed in the presence of 0.6 g/l of this acid. On the other hand, xylose consumption was not influenced by the presence of this acid at any of the evaluated concentrations, and xylitol production was slightly reduced (by around 15%) compared with the control when the fermentation medium contained 0.6 g/l ferulic acid (Fig. 2d). Regarding fermentative parameters, addition of 0.2 and 0.4 g/l ferulic acid to the cultivation medium promoted decreases between 10% and 22% in the fermentative parameter values (Table 3). However, increase of the acid concentration to 0.6 g/l caused a 44% reduction in $Y_{X/S}$ value compared with the control, while Q_P and $Y_{P/S}$ were reduced by 18% and 16%, respectively.

Table 3 Effect of different concentrations of acetic acid, ferulic acid or syringaldehyde on fermentative parameters of xylitol production by *Candida guilliermondii* in semidefined medium

	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	Q_P (g/lh)
Acetic acid (g/l)			
0.0	0.09	0.81	0.87
0.8	0.10	0.83	0.92
1.1	0.08	0.84	0.93
2.6	0.06	0.77	0.73
Ferulic acid (g/l)			
0.0	0.09	0.81	0.87
0.2	0.07	0.69	0.75
0.4	0.08	0.73	0.70
0.6	0.05	0.68	0.71
Syringaldehyde (g/l)			
0.0	0.09	0.81	0.87
0.3	0.10	0.80	0.85
0.5	0.06	0.75	0.81
0.8	0.06	0.84	0.81

Larsson et al. [6] evaluated the influence of different ferulic acid concentrations (0.02, 0.2, and 1.0 g/l) on fermentation parameters of ethanol production by *S. cerevisiae*. According to those authors, 0.02 g/l did not influence fermentative parameter values, but increase of this concentration to 0.2 and 1.0 g/l reduced Q_P by 54% and 80%, respectively, and $Y_{X/S}$ by 17% and 90%, respectively, compared with the control. Nevertheless, $Y_{P/S}$ was not affected by any of the ferulic acid concentrations used. They concluded that increase in ferulic acid concentration affected mainly biomass yield, which negatively affected volumetric productivity as a consequence. Such observations are in agreement with the results of the present work, where ferulic acid exerted a more significant inhibitory effect on cell growth than on xylose to xylitol bioconversion.

Similarly as observed for ferulic acid, the presence of any syringaldehyde concentration in the fermentation medium affected cell growth (Fig. 2e), with a reduction of approximately 36% in biomass formation being observed when the medium was supplemented with 0.8 g/l of this acid. Similar behavior was observed by Duarte et al. [3] during cultivation of *Debaryomyces hansenii* in defined medium containing 15 g/l xylose as carbon source. According to those authors, cell growth was decreased by 42% in the presence of 0.75 g/l syringaldehyde. The effect of syringaldehyde (0.8 g/l) on *Candida guilliermondii* cell growth was reflected in a reduction of 33% in $Y_{X/S}$ (Table 3).

In contrast to cell growth, xylose consumption and xylitol production were not influenced by any syringaldehyde concentration in the fermentation medium (Fig. 2e, f). Maybe higher concentrations values could have inhibited the yeast metabolism, as has been observed for other microorganisms. Growth of *Escherichia coli* LY01 on xylose, for example, was only affected by syringaldehyde concentrations higher than 2.5 g/l [18], while growth of *Candida shehatae* and *Pichia stipitis* was affected only by concentrations of this compound higher than 1.5 g/l [2]. The concentration thresholds used in the present work were chosen based on concentration values found in rice straw hemicellulosic hydrolysate obtained by diluted-acid hydrolysis (1% w/v H_2SO_4, 120°C, 30 min), which has been reported as a fermentation medium with great potential for use in xylitol production [12]. Understanding the inhibitory action of the toxic compounds present in this hydrolysate may be of great importance to improve bioconversion results achieved to date.

Conclusions

Acetic acid, ferulic acid, and syringaldehyde are compounds that may affect metabolism of *Candida guilliermondii* yeast (mainly cell growth) during bioconversion of xylose to xylitol. Their toxic effect was dependent on their concentration in the fermentation medium, with inhibition being more pronounced at higher concentrations. However, it was concluded that complete removal of these compounds from the fermentation medium is not necessary to obtain efficient conversion of xylose to xylitol by *Candida guilliermondii*. In addition, fermentation in buffered medium favored biomass production, xylitol yield factor, and productivity, and could be considered as an alternative to overcome the inhibition caused by these toxic compounds, mainly by acetic acid.

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