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ORIGINAL PAPER

Biological detoxification of different hemicellulosic hydrolysates using *Issatchenkia occidentalis* CCTCC M 206097 yeast

Bruno Guedes Fonseca · Rondinele de Oliveira Moutta · Flavio de Oliveira Ferraz · Emílio Rosa Vieira · Andrei Santini Nogueira · Bruno Fernandes Baratella · Luiz Carlos Rodrigues · Zhang Hou-Rui · Sílvio Silvério da Silva

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Abstract This work had as its main objective to contribute to the development of a biological detoxification of hemicellulose hydrolysates obtained from different biomass plants using *Issatchenkia occidentalis* CCTCC M 206097 yeast. Tests with hemicellulosic hydrolysate of sugarcane bagasse in different concentrations were carried out to evaluate the influence of the hydrolysate concentration on the inhibitory compounds removal from the sugarcane bagasse hydrolysate, without reduction of sugar concentration. The highest reduction values of inhibitors concentration and less sugar losses were observed when the fivefold concentrated hydrolysate was treated by the evaluated yeast. In these experiments it was found that the high sugar concentrations favored lower sugar consumption by the yeast. The highest concentration reduction of syringaldehyde (66.67%), ferulic acid (73.33%), furfural (62%), and 5-HMF (85%) was observed when the concentrated hydrolysate was detoxified by using this yeast strain after 24 h of experimentation. The results obtained in this work showed the potential of the yeast *Issatchenkia occidentalis* CCTCC M 206097 as detoxification agent of hemicellulosic hydrolysate of different biomass plants.

Keywords Hydrolysate · Biodetoxification · *Issatchenkia occidentalis* · Furans · Phenolic compounds

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B. G. Fonseca · R. O. Moutta · F. O. Ferraz · E. R. Vieira · A. S. Nogueira · B. F. Baratella · S. S. da Silva (✉)
Deptamento de Biotecnologia Lorena,
Universidade de São Paulo/Escola de Engenharia de Lorena,
Lorena, São Paulo 12.602-810, Brazil
e-mail: silvio@debiq.eel.usp.br

L. C. Rodrigues
Universidade Federal Fluminense-UFF,
Volta Redonda, Rio de Janeiro, Brazil

Z. Hou-Rui
Phytochemical Department, Guangxi Institute of Botany,
The Chinese Academy of Sciences, 541006 Guangxi,
People's Republic of China

Introduction

Lignocellulosic biomass is one of the earth's major carbon and renewable energy sources. These include various wastes and/or agro-industrial by-products such as straw, bagasse, bark, and chips, which are accumulated every year from agricultural production. The accumulation of such wastes leads to environmental and economical problems, as the degradation represents the loss of potential energy resources [1].

Biotechnological processes using lignocellulosic materials in most cases requires preliminary steps of preparation, such as chemical [2, 3] or enzymatic hydrolysis [4, 5], in order to release the components of cellulose and hemicellulose fractions either in the form of mono or oligosaccharides.

High content of pentoses, particularly D-xylose in the hemicellulose fraction of these materials, combined with its easy extraction from the lignocellulosic complex, have been attracting the attention of researchers for the effective use of this fraction in bioconversion processes [6–9].

Culture media using hemicellulosic hydrolysates for D-xylose bioconversion are strongly affected by the presence of some compounds originated from acid hydrolysis of lignocellulosic biomass that are toxic to the microbial metabolism [9]. Due to the high temperatures

and acidic conditions employed in this procedure, furfural, 5-hydroxymethylfurfural (5-HMF), aliphatic acids, and phenolic compounds are produced, which have a negative effect on fermentability of the hemicelluloses hydrolysates and affect cell growth, thereby reducing the enzymatic activity and microbial cell functions [10, 11]. It is important to identify the potential inhibitors present in the hemicellulose hydrolysates before proposing a method or a sequence of methods for detoxification. This knowledge not only helps to choose an efficient and low-cost treatment but also to establish conditions for hydrolysis that minimize its formation. Detoxification treatment with ion-exchange resin [12], activated charcoal [13], organic solvents [14], and overliming [9] are physical and chemical methods that are commonly used for reduction of all or part of these compounds formed after acid hydrolysis. Therefore many disadvantages still exist in these procedures for their use in technical applications.

Hemicellulosic hydrolysate detoxification employing live microorganisms and/or enzymes that assimilate and/or degrade aliphatic acids and aromatic compounds, among them the furans, acids, and phenolic aldehydes, are procedures that deserve mention because they do not generate toxic waste in the environment and especially that they do not cause a decrease in sugar concentrations or volume loss [15].

This study aimed to contribute to the development of a biological detoxification procedure for the removal of the toxic compounds present in different hemicellulose hydrolysates and to establish suitable conditions for the bioconversion of these hydrolysates into various bioproducts.

Materials and methods

Microorganisms and cultivation media

The yeast *Issatchenkia occidentalis* CCTCC M 206097, isolated from industrial wastes containing phenolic compounds [16], was used as a detoxification agent for different hydrolysates. The culture was kept in agar malt extract at 4°C. The inoculum was prepared by the yeast cultivation in 500-ml Erlenmeyer flasks filled with 250 ml of semi-synthetic media constituted of glucose (30 g/l), yeast extract (6.0 g/l), $(NH_4)_2SO_4$ (2.0 g/l), $MgSO_4.7H_2O$ (0.2 g/l), K_2HPO_4 (4.0 g/l), KH_2PO_4 (6.0 g/l), and urea (2.0 g/l). Concentrated solutions of each compound were prepared separately and sterilized in an autoclave or filtered in a 0.22-μm Millipore membrane and then used for the inoculum preparation. The cells were cultivated in a shaker at 200 rpm and 30°C for 24 h. Afterwards, the cells were recuperated by centrifugation at 2,000 rpm for 20 min, washed, and resuspended in sterile distilled water.

Hemicellulosic hydrolysate

First, fiber corn was milled to 20 mesh and then hydrolyzed with acid solution at 110°C for 10 min in an electric autoclave (SOC. FABB Ltd., Model: 103) with 200 mg of H_2SO_4 (98% w/w) per 1.0 g dry weight in a solid/liquid ratio of 1:10. The corn fiber hydrolysate was then concentrated in a vacuum at 70°C to reduce its volume to 1/2.5 of the original volume. The sugarcane straw was subjected to the same conditions of milling and particle size as those used for the corn fiber. Afterwards, the chemical hydrolysis of the sugarcane straw with diluted H_2SO_4 (2.9% w/v) in an electric autoclave at 130°C for 30 min was performed until a solid/liquid ratio of 1:4 (g/ml) was obtained. It was not necessary to concentrate the hydrolysate.

Acid hydrolysis of sugarcane bagasse and coffee husk were made in a stainless-steel reactor with a volume of 30 l, indirectly heated by electrical resistance through an oil jacket. The hydrolysis was operated at 121°C for 10 min, 100 mg of sulfuric acid (98% w/w) per 1.0 g of dry matter in a ratio of dry residue and volume of acidic solution of 1:10. Afterwards, the hydrolysate was submitted to the vacuum concentration process at 70°C to reduce its volume to 1/5 of the original.

Finally, the pH of all hemicellulosic hydrolysates used in this study were adjusted to 5.50 using NaOH and the solids formed were removed by centrifugation. During the process of biological detoxification, the pH was not controlled.

Hydrolysate detoxification

The evaluation of biological treatment was done by detoxification tests with sugarcane straw, coffee husk, and corn fiber hemicellulosic hydrolysates, using 0.5 g/l of concentrated cell suspension of the yeast *I. occidentalis*. The experiments were performed in triplicate in a shaker at 30°C and 200 rpm for 36 h using 125-ml Erlenmeyer flasks filled with 50 ml of media, composed approximately by 46.0 ml of hydrolysate and 3.1 ml of the following nutrients: yeast extract (6.0 g/l), urea (2.0 g/l), $(NH_4)_2SO_4$ (5.0 g/l), $MgSO_4{\cdot}7H_2O$ (0.2 g/l), $CaCl_2$ (0.1 g/l), NaCl (0.1 g/l), and KH_2PO_4 (1.0 g/l). The biodetoxification process was monitored by taking samples periodically each 12 h to analyze the concentrations of D-glucose, D-xylose, acetic acid, furfural, and 5-HMF (5-hydroxymethylfurfural).

For the biodetoxification of sugarcane bagasse hemicellulosic hydrolysates, two different hydrolysate concentrations were used, one of which was concentrated until 1/5 of its original volume (FCH) and the other was not concentrated (NCH), using a 0.5 g/l of concentrated cell suspension of the yeast *I. occidentalis*. The experiments were performed in triplicate in a shaker at 30°C and 200 rpm for

72 h using 125-ml Erlenmeyer flasks filled with 50 ml of the same media composition as described above. For monitoring the biodetoxification process, samples were taken each 24 h and then analyzed to estimate the decrease in the concentrations of D-glucose, D-xylose, acetic acid, furfural, 5-HMF, ferulic acid, and syringaldehyde. Cell growth was also analyzed.

Analytical methods

The concentration of sugars and inhibitors was determined by HPLC. For D-xylose, D-glucose and acetic acid, Waters equipment was used with a refraction index detector and a Bio-Rad Aminex HPX-87H column, and for furans and phenolic compounds (syringaldehyde and ferulic acid), Waters equipment with UV detector and RP18 column was used.

The number of cells was determined by optical density and by optical microscopy in a Neubauer chamber.

Results and discussion

According to Table 1, the hydrolysis conditions employed over the biomass allowed the obtention of a hydrolysate rich in D-xylose and low D-glucose content, showing a selective extraction of the hemicellulose fraction in all cases with formation of fermentative inhibitory compounds. These results are in agreement with literature data, since those indicate that the conditions of acid hydrolysis employed are effective to obtain D-xylose from the hemicellulose fraction, which can be used as a carbon source in fermentation processes [8, 12].

Fermentation inhibitor compounds and their concentrations in hemicellulose hydrolysates depend on raw material and the operating conditions used for hydrolysis. Thus, the variables of the fermentation process—such as physiological condition of cells, dissolved oxygen concentration, and pH of the medium—are also associated with toxicity of these compounds, generally accentuating its toxic effects [17]. By concentrating the hemicellulosic hydrolysate aiming to increase the sugar content, it also increases the concentrations of non-volatile compounds considered as growth inhibitors. The concentration of the toxic compounds can be seen in Table 1 for each of the hydrolysates. It can be seen values of acetic acid concentration around 2.85 g/l, with emphasis on corn fiber with high content of acetic acid (3.79 g/l), which is justified by the composition of the material, that has higher amounts of acetyl groups, and by the more drastic hydrolysis conditions employed.

Furfural and 5-HMF are inhibitory compounds derived from the degradation of D-xylose and D-glucose, respectively, and have been cited as having the greatest inhibitory effect on fermentation. Their concentration in hemicellulose hydrolysates is proportional to the concentration of their relatives sugars and the severity of the hydrolysis reaction. One hypothesis that explains the toxic effect of furan compounds is the fact that by being aldehydes that are chemically reactive, these compounds can react with biological molecules or cause damage to cell membranes [18]. Among the negative effects produced by furfural and 5-HMF on microorganisms in general and on yeast fermentation is the degradation of furfural into acetaldehyde, which the intracellular accumulation causes an increase in the *lag* phase of microbial growth [14].

According to Table 1, coffee husk hydrolysate, obtained employing the same conditions of sugarcane bagasse hydrolysis, generated a high concentration of D-glucose (27.99 g/l) and 5-HMF (0.250 g/l). This suggests that the coffee husk is more susceptible to the hydrolysis conditions employed than other lignocellulosic biomass used in this study, considering that the hydrolysis was more severe for this biomass with higher degradation of cellulose and formation of 5-HMF. The sugarcane straw, which was used in the apparently more drastic hydrolysis conditions, presented the best results on the formation of inhibitors with a content of total furans equal to 0.014 g/l.

The results of hydrolysate characterization after 36 h of detoxification tests with *I. occidentalis* CCTCC M 206097 yeast are shown in Table 2. During the biodetoxification process, D-xylose consumption was not observed in any of the hydrolysates in the first stage of detoxification. The biological detoxification treatment employed had less effect over the coffee husk hydrolysate, which showed a reduction of 63% of total furans. Although this treatment had allowed the removal of 76.6% of furfural from this hydrolysate, 5-HMF removal was very low, leading to a final total furan concentration of 0.25 g/l. It is suggested that the high concentration of 5-HMF had promoted an

Table 1 Concentration (g/l) of the non-detoxified hemicellulose hydrolysate main components of coffee husk, sugarcane straw, and corn fiber (not detoxified) obtained by acid hydrolysis and after the vacuum concentration process

Components	Non-detoxified hemicellulosic hydrolysates (g/l)		
	Coffee husk	Sugarcane straw[a]	Corn fiber
D-glucose	27.99	9.18	2.41
D-xylose	46.43	46.80	35.23
Acetic acid	2.92	2.77	3.79
Furfural	0.018	0.007	0.045
5-HMF	0.250	0.007	0.017
Total furans[b]	0.267	0.014	0.062

[a] The vacuum concentration was not necessary

[b] Total furans are the sum of furfural and 5-HMF

Table 2 Final concentration (g/l) and concentration reduction (%) of the main components of the coffee husk, sugarcane straw, and corn fiber hemicellulosic hydrolysates detoxified by the yeast *I. occidentalis* CCTCC M 206097

Components	Detoxified hemicellulosic hydrolysates (g/l)					
	Coffee husk		Sugarcane straw		Corn fiber	
	Final concentration (g/l)	Reduction (%)	Final concentration (g/l)	Reduction (%)	Final concentration (g/l)	Reduction (%)
D-glucose	27.99	0.0	7.80	15.0	2.38	1.2
Acetic acid	2.92	0.0	1.99	28.2	3.81	0.0
Furfural	0.004	76.6	0.003	54.3	0.005	88.9
5-HMF	0.246	1.4	0.003	54.1	0.016	2.4
Total furans	0.250	6.3	0.007	54.2	0.021	65.5

Table 3 Partial composition (g/l) and percentage reduction in the concentration of the compounds present in the non-concentrated (NCH) and the fivefold concentrated (FCH) sugarcane bagasse hemicellulosic hydrolysate

Compounds	NCH		FCH	
	Initial concentration ($g\ l^{-1}$)	Reduction (%)	Initial concentration ($g\ l^{-1}$)	Reduction (%)
D-xylose	11.30	49.6	51.50	2.5
D-glucose	0.37	100.0	1.83	1.1
Acetic acid	1.13	100.0	3.30	6.1
Syringaldehyde	0.27	92.6	0.60	70.0
Ferulic acid	8.0×10^{-2}	100.0	0.30	76.7
Furfural	1.8×10^{-3}	100.0	1.6×10^{-2}	100.0
5-HMF	4.0×10^{-3}	100.0	2.0×10^{-2}	100.0

inhibitory effect on yeast during fermentation. The best results of the detoxification process occurred with the corn fiber hydrolysate, where the furfural removal also had a higher proportion (88.9%), with a final concentration of 0.021 g/l of total furans. The removal of furfural and 5-HMF in the sugarcane straw hydrolysate occurred in the same proportion, removing 54.2%, leading to a final total furans concentration of 0.007 g/l at the end of the detoxification process.

For the biological detoxification of sugarcane bagasse hydrolysate, different concentrations of this hydrolysate were used in order to evaluate its influence on the reduction efficiency of inhibitory compounds concentration, without sugars losses. For this purpose, the sugarcane bagasse hydrolysate from dilute acid hydrolysis was obtained and part of this hydrolysate was fivefold concentrated, followed by biological detoxification with the yeast *I. occidentalis* CCTCC M 206097. For these tests, samples were taken after preparation and sterilization of the fermentation media. The average composition of these hydrolysates is shown in Table 3.

Table 3 presents the percentage of concentration reduction of sugars, acetic acid, furfural, 5-HMF, syringaldehyde, and ferulic acid in the original (NCH) and the concentrated (FCH) sugarcane bagasse hydrolysate after 72 h of detoxification tests. The smallest decrease in D-xylose concentration (2.5%) was observed when FCH was detoxified with the yeast *I. occidentalis* after 72 h of experimentation. However, the higher concentration reduction of that sugar (49.6%) was obtained in NCH detoxified by the same yeast. When comparing the consumption of D-xylose and the concentration of the hydrolysate, it can be observed that the consumption of that pentose decreases as the concentration of the hydrolysate increases. A removal of 23.9% of D-xylose was observed when the sugarcane bagasse hemicellulosic hydrolysate was treated by pH variation methods followed by treatment with activated charcoal. In some conditions employed in this work, the sugar loss was about 1.0 g/l [19]. As the concentration of D-xylose is one of the major factors that influence the productivity of xylitol and/or ethanol in different fermentative processes, significant losses of this sugar compromise the efficiency of the bioprocess.

Regarding D-glucose consumption, a total concentration reduction of this sugar in both hydrolysate conditions was observed when detoxified the NCH with this yeast strain. The same reduction was not observed in FCH. In those conditions, only 1.1% of that sugar was consumed by the microorganism, i.e., 0.02 g/l. Total consumption of acetic acid was observed when detoxifying NCH with *I.*

occidentalis. However, the removal of the aliphatic acid in FCH was just 6.1% when using this yeast. After 72 h, the concentration of acetic acid in the concentrated hydrolysate did not exceed 3.3 g/l. When using *Pichia stipitis* for production of ethanol from sugarcane bagasse hemicellulosic hydrolysate, Van Zyl et al. [20] verified that the inhibition caused by acetic acid depended not only on the concentration of that acid, but either on the pH value and oxygen availability. The authors demonstrated a decrease of 50% on the volumetric rate of ethanol production when the concentrations of acetic acid were 0.8 and 13.8 g/l at pH 5.1 and 6.5, respectively, under anaerobic conditions.

After 24 and 48 h of bioprocess, 5-HMF was totally removed from the media when the original and concentrated hydrolysates were detoxified, respectively. Figure 1 shows that during the entire biodetoxification test of the concentrate hydrolysate, the removal of 5-HMF was 85%, reducing its concentration in the medium to 0.003 g/l after 24 h. In the original hydrolysate, the total reduction in furfural concentration was observed after 24 h of bioprocess. However, when analyzing the furan concentration when detoxifying the concentrated hydrolysates, the reduction of this inhibitor was approximately 62% after 24 h of process, reaching its total transformation at 48 h of the experiment (Fig. 1). According to Palmqvist and Hahn-Hägerdal [14], the effect of 5-HMF is considered less toxic

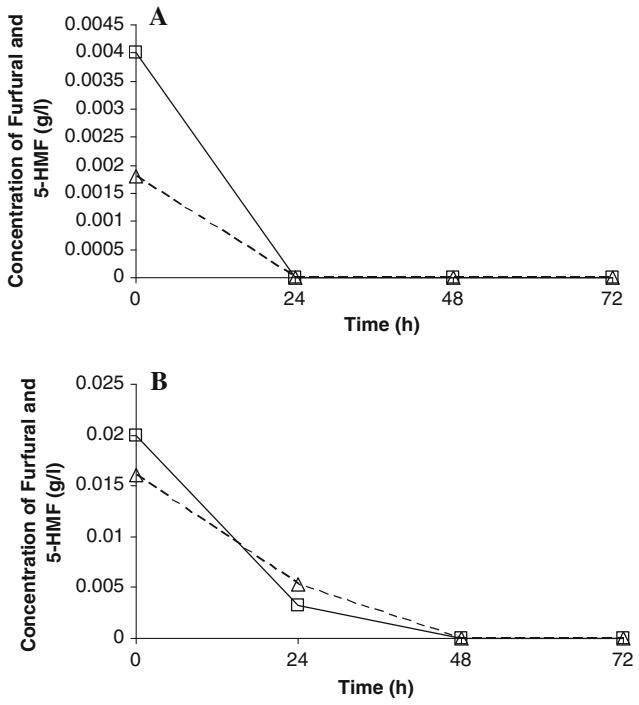

Fig. 1 Reduction in the concentration of furfural (*triangle*) and 5-HMF (*square*) when grown in yeast *I. occidentalis* in hydrolysate of sugarcane bagasse original (**a**) and concentrate (**b**)

than the furfural due to its low concentration in hemicellulosic hydrolysates, since small amounts of hexoses are present in these hydrolysates. As demonstrated by Okuda et al. [15] when they compared the effect of furfural and furoic acid on ethanol production by *Saccharomyces cerevisiae* TJ1 using house wood hydrolysate biologically detoxified by *Ureibacillus thermosphaericus* NCIMB 13819, the oxidized furan did not affect the specific cell growth rate until a concentration of 1.0 g/l, while with 0.9 and 2.7 g/l of furfural they observed a decrease of 20 and 80% on growth, respectively. Nigam [21] showed that concentrations of 1.5 g/l of furfural in wheat straw hemicellulosic hydrolysate interfered with the respiration and growth of *Pichia stipitis* NRRL Y-7124, as well as on ethanol's yield and productivity. Mussato and Roberto [22], utilizing rice straw hemicellulosic hydrolysate for bioconversion of D-xylose to xylitol by *Candida guilliermondii*, used different types of activated charcoal as a detoxifying agent aiming to remove furfural, 5-HMF, and lignin-derived compounds. After treatment, a reduction of 96, 93, and 31% on the concentrations of furfural, 5-HMF, and lignin-derived compounds, respectively, was observed, and a removal of less than 5% of the concentration of D-xylose.

Some physical–chemical methods of detoxification used in hemicellulosic hydrolysate treatment showed less favorable results in the removal of these compounds when compared to the method used in this work. According to Carvalho et al. [23], after detoxification of hemicellulosic hydrolysate of sugarcane bagasse with four different types of resins (anion A-103 S, anion S-860, Applexion cationic and anionic Applexion), the authors observed a furfural removal of 82.1 and 66.5% of 5-HMF. In a similar study using anionic resins at pH 10 to detoxify the hemicellulosic hydrolysates of wood, Larsson et al. [24] observed a drop of 73% in furfural amount and 70% of 5-HMF concentration. Those results are lower than the ones obtained in this work, which were 0.27 and 1.77 g/l of furfural and 5-HMF, respectively. An example of hydrolysate detoxification treatment using organic solvent extraction was evaluated by Frazer and McCasey [25]. These authors, during the detoxification of wood hydrolysate using only ethyl acetate as extracting agent or hexane in combination with calcium hydroxide, observed that these treatments reduced the level of furfural in 83% when started with 0.4 and 0.2 mg/ml of ethyl acetate and hexane, respectively. The same conditions resulted in a decline of approximately 25% of the initial concentration of sugars in both cases. When this method of solvent extraction was compared to detoxification using *I. occidentalis* presented in this work, the later proved to be more efficient to remove these furans.

The phenolic compounds with low molecular weight are those with higher toxic effects on fermentation. The mode

of action of these compounds is established considering: (a) reaction with the cell membrane causing increased permeability and loss of cellular components; (b) inactivation of essential enzymes or enzyme systems, including those involved in energy production and synthesis of structural components; or (c) destruction or inactivation of functional genetic material [26, 27]. The toxic effect of aromatic aldehydes may be related to hydrophobic interaction with certain areas of the cells, causing loss of membrane integrity, thus affecting their ability to act as a selective barrier. For aromatic alcohols, such toxicity is attributed to the damage caused by these compounds in the plasma membrane, while for the aromatic acids is based on mechanisms similar to other aliphatic acids [27].

In this paper, the discussions on the phenolic compounds were based on the removal of ferulic acid and syringaldehyde, some of the compounds found at higher concentrations in hemicellulose hydrolysates [10, 28]. According to Fig. 2, it is observed that the reduction in ferulic acid concentration in the original hydrolysate hits its totality after 72 h of testing. However, after 24 h of bioprocess using yeast as a detoxifying agent, there was a decrease of 62.5% in the concentration of phenol. The reduction in the syringaldehyde concentration presented a different profile under the same hydrolysis conditions. After 24 h of process, there was a 40.74% of reduction in the concentration of syringaldehyde (Fig. 2a). Figure 2b shows the reduction in the concentration of these compounds when the concentrated hydrolysate was treated by the yeast *I. occidentalis*. There was a reduction of 76.7 and 70% in the concentrations of ferulic acid and syringaldehyde, respectively, at the end of 72 h of process. However, a reduction of 73.33 and 66.67% in the concentration of ferulic acid and syringaldehyde, respectively, was achieved in the first 24 h of biodetoxification process. One can notice that the concentration values of syringaldehyde are less than 2.0 g/l, a value which would affect the consumption of D-xylose by *Candida guilliermondii* [29].

Analyzing the detoxified corn hemicellulosic hydrolysate, Nichols et al. [30] observed a reduction of 97 and 84% of the concentrations of ferulic acid and syringaldehyde, respectively. However, the initial concentrations of these compounds were lower than those found in the sugarcane bagasse hemicellulosic hydrolysate used in this work. The initial concentration of these compounds in the Nichols et al. work were 15.0 and 3.0 ppm of ferulic acid syringaldehyde, respectively. In contrast, the FCH detoxified in this work, presented an initial concentration of 0.3 and 0.6 g/l of ferulic acid and syringaldehyde, respectively.

Figure 3 shows the percentage of reduction in the concentrations of sugars and inhibitors present in sugarcane bagasse hemicellulosic hydrolysates, original and concentrated, by employing the yeast *I. occidentalis* as detoxifying agents after 24 h of bioconversion process. It is also observed in this figure that the reduction in sugar concentration was increased when detoxified the original hydrolysate. Similarly, a difference in the reduction of furans and phenolic compounds was observed depending on the concentration of the hydrolysate. It was also observed that in the FCH biodetoxification, the concentration reduction of

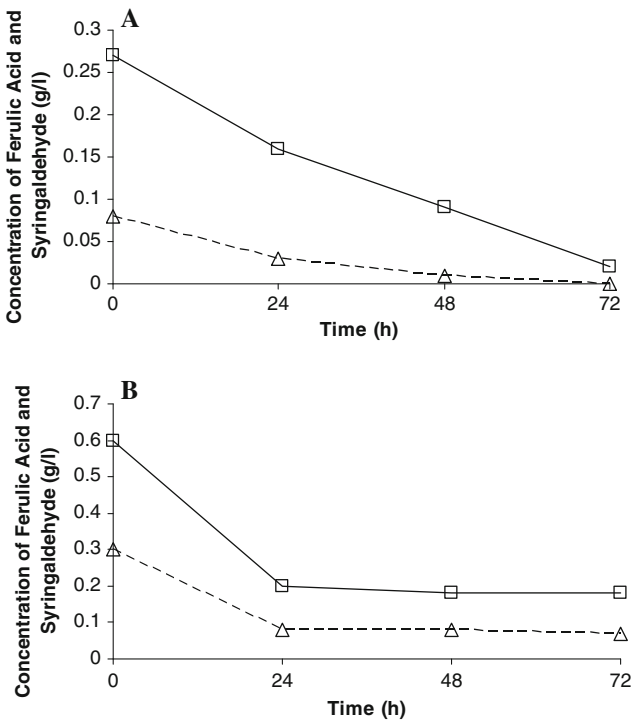

Fig. 2 Reduction in the concentration of ferulic acid (*triangle*) and syringaldehyde (*square*) when grown in yeast *I. occidentalis* in the original (**a**) and concentrate (**b**) sugarcane bagasse hydrolysate

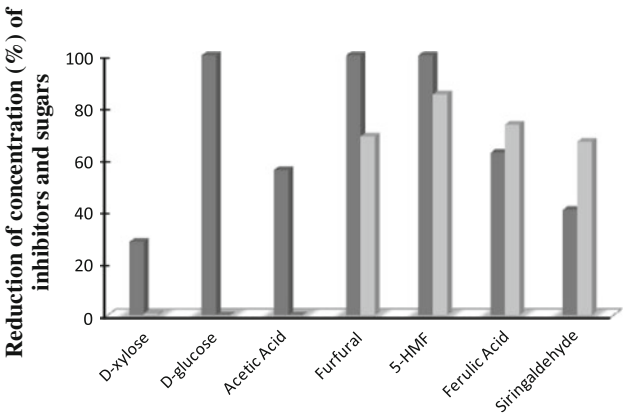

Fig. 3 Consumption of inhibitors and sugars in original (*filled black square*) and concentrate (*filled grey square*) sugarcane bagasse hemicellulosic hydrolysate by *I. occidentalis* CCTCC M 206197

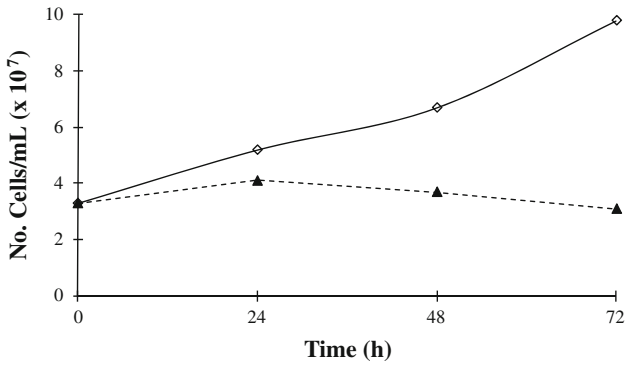

Fig. 4 Cell growth (number of cells/ml) of the yeast *I. occidentalis* in NHC (*diamond*) and in FCH (*triangle*)

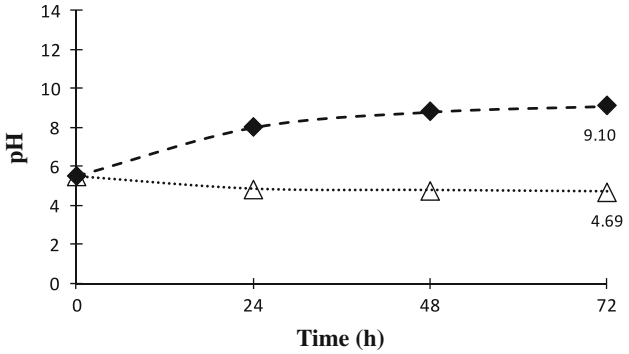

Fig. 5 Variation of pH during the biological detoxification process when the yeast *I. occidentalis* was grown in original (*filled diamond*) and concentrate (*triangle*) sugarcane bagasse hemicellulosic hydrolysate

the phenolic compounds evaluated was higher than in the NHC detoxification, and the reduction of 5-HMF and furfural was lower in the biological treatment of FCH, with reduction greater than 70%.

Figure 4 shows that this strain of *I. occidentalis* presented lower cell growth in the hydrolysate with higher concentration of sugars and toxic compounds than those that were cultivated in the less concentrated hydrolysate. It is important to note that the total concentration of sugars is 4.6 times higher in the more concentrated hydrolysate. This difference in sugar concentration, associated with the presence of other compounds, may have influenced the yeast growth, since high concentrations of substrate can inhibit growth, due to effects over the transport mechanism or even to the increase of the osmotic pressure of the media. An increase of 68.37% was observed in the number of cells at 72 h of cultivation in NHC in comparison to FCH. It was not observed an increase in the number of cells in the concentrated hydrolysate.

Changes in pH during the biodetoxification processes of sugarcane bagasse hydrolysates with *I. occidentalis* yeast had a different behavior in the presence of higher concentrations of sugars, aliphatic acid, furans, and phenolic compounds. It appears that the NHC, when detoxified with the studied yeast, had an increase of 2.50 units in pH value in the first 24 h of treatment (Fig. 5). This fact could be related to the formation of ammonium in the media and/or the excretion of some compounds with basic character resulting from the metabolism of phenolic compounds [31]. In the same condition, higher cell growth and sugar consumption was observed.

On the other hand, the concentrated hydrolysate detoxified with this strain showed a decrease of approximately 0.65 units of pH in the first 24 h of test. By the end of the process, this difference remained constant, decreasing only 0.80 units. This behavior can be justified by the new compounds formed in the biotransformation of furan into their respective acids [32], and/or the release of compounds with acidic properties that resulted from the phenolic compound metabolism [31]. In the treatments where a decrease in pH was observed, cell growth was constant during the detoxification tests. Under the same conditions, the results showed the lowest pentoses and hexoses consumption rates present in the detoxified media. This fact can probably be related to high concentrations of H^+ ions in the media to be detoxified, thus inhibiting yeast growth.

The employment of microorganisms as detoxifying agents of hemicellulosic hydrolysates is a methodology without significant loss of volume and is economically more viable than other processes [16]. In this work, there were no losses greater than 5% of volume when treating NCH and FCH with this yeast strain.

These facts suggest that the yeast *Issatchenkia occidentalis* CCTCC M 206097 presented a metabolic capacity to degrade known inhibitors of fermentative processes, improving the fermentability of the sugars present in the media.

Conclusions

Biodetoxification processes using *Issatchenkia occidentalis* CCTCC M 206097 yeast were applied for the removal of major toxic compounds present in hemicellulosic hydrolysate of sugarcane bagasse, coffee husk, sugarcane straw, and corn fiber. The yeast used in this work is a good biodetoxification agent due to its potential in metabolizing furans and phenolic compounds present in hemicellulosic hydrolysates of biomass. This biological process offers some important advantages compared to other treatments, including the removal of furfural and HMF and the possibility to execute the process in only one stage. In addition, during the biodetoxification process, volume losses greater than 5% were not observed.

Issatchenkia occidentalis CCTCC M 206097 yeast showed lower cell growth when grown on sugarcane bagasse hydrolysate with higher concentrations of sugars and toxic compounds. The pH during the detoxification process in concentrate sugarcane bagasse hydrolysate decreased by 0.80 units. On the other hand, in the original sugarcane bagasse hydrolysate, the pH was increased to 3.5 units. Further studies must be performed to investigate the effects of biological detoxification over the fermentability of hemicellulosic hydrolysates by other microorganisms of industrial interest.

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