J Chem Technol Biotechnol 79:590–596 (online: 2004)
DOI: 10.1002/jctb.1026

Effect of pH and activated charcoal adsorption on hemicellulosic hydrolysate detoxification for xylitol production

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Abstract: Biotechnological conversion of xylose into xylitol using hydrolysates obtained from the hemicellulosic fraction of lignocellulosic materials is compromised by the presence of compounds released or formed during the hydrolysis process, some of them being toxic to microorganisms. In order to improve the bioconversion of these hydrolysates it is necessary to find methods to reduce their toxicity. In the present work, rice straw hemicellulosic hydrolysate was treated by six different procedures (all of them involving pH adjustment, with or without activated charcoal adsorption), before being used as a fermentation medium for xylitol production. The most effective method of treatment was to increase the initial pH (0.4) to 2.0 using solid NaOH, followed by the addition of activated charcoal (25 g kg^{-1}) and increase in the pH to 6.5 using solid NaOH. Lignin degradation products were the most inhibitory compounds present in the hydrolysate; their removal was selective and strongly dependent on the pH employed in the treatment. The highest yield of xylitol was 0.72 g g^{-1} xylose, with a productivity of 0.55 g dm^{-3} h^{-1}.

Keywords: activated charcoal; detoxification; fermentative process; hemicellulosic hydrolysate; pH adjustment; xylitol

1 INTRODUCTION

Xylitol stands out from other sweeteners, not only because it has much potential for application in the odontological and medical areas, but also because it can be produced by the fermentation of xylose derived from hemicellulosic raw materials.[1] During acid hydrolysis of these materials, the liberation of monosaccharides, mainly xylose, is accompanied by the formation or release of compounds such as acetic acid, furfural, 5-hydroxymethylfurfural and lignin degradation products, whose quantities vary according to the raw material and hydrolysis conditions employed. As these compounds are toxic to many microorganisms the fermentative utilisation of sugars in hemicellulosic hydrolysates is limited and product formation is adversely affected.

When compared with the fermentation of commercial sugars or detoxified hydrolysates, the fermentation of untreated hemicellulosic hydrolysates is characterised by slow kinetics, with limited yield and productivity.[2] The levels of dissolved oxygen concentration and pH affect the toxicity of these compounds, and may accentuate the toxic effects.[3] So, the lignocellulosic substrates must be treated to make them more suitable for use as fermentation media.[4–8]

To reduce the concentrations of these compounds, eliminating or decreasing their inhibitory effects, various biological, physical and chemical detoxification methods have been proposed. Among the chemical methods, two stand out as being inexpensive and producing good results: pH adjustment to induce precipitation and/or instability of the toxic compounds,[4,9] and the adsorption of these compounds on activated charcoal.[5,8,10] Combinations of these treatments have been used widely for the detoxification of lignocellulosic hydrolysates. Alves *et al*,[6] for example, employed different combinations of bases and acids to change the initial pH of sugar cane bagasse hemicellulosic hydrolysates with or without treatment with activated charcoal. The best results for xylose-to-xylitol bioconversion ($Y_{P/S} = 0.79$ g g^{-1} and $Q_P = 0.47$ g dm^{-3} h^{-1}) were obtained when the pH was first increased from 0.5 to 7.0 with CaO and then decreased to 5.5 with H_3PO_4, before the addition of activated charcoal (24 g kg^{-1}) to the hydrolysate. However, the effectiveness of activated charcoal treatment depends

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Contract/grant sponsor: FAPESP
(*Received 27 September 2003; accepted 18 January 2004*)

on the levels of the variables used in the adsorption process. The main variables are pH, temperature, contact time and activated charcoal concentration.

In a previous study on the detoxification of rice straw hemicellulosic hydrolysate, using activated charcoal Mussatto and Roberto[5] achieved maximal results using activated charcoal at the rate of 25 g kg^{-1}. In the present study, six different detoxification procedures consisting of pH adjustment, with or without the addition of activated charcoal, were used to remove toxic compounds from rice straw hemicellulosic hydrolysate, with a view to improving the xylose-to-xylitol bioconversion efficiency.

2 MATERIALS AND METHODS

2.1 Preparation of hemicellulosic hydrolysate

Locally produced rice straw containing (g kg^{-1}) 435 cellulose, 220 hemicellulose, 172 lignin and 114 ash, was dried in the sun, milled to obtain particles about 1 cm long and 1 mm thick, (Máquinas Benedetti, Espírito Sto Do Pinhal, SP, Brazil.) hydrolysed in a 350 dm^3 stainless steel pressure bioreactor with 0.1 M H_2SO_4, at a liquid–solid ratio of 10 g g^{-1}, at 121 °C, for 20 min, and filtered under vacuum. The filtrate (hemicellulosic hydrolysate) was stored at 4 °C until it was concentrated under vacuum in a 4 dm^3 evaporator at 70 ± 5 °C to a xylose content of approximately 120 g dm^{-3} (density = 1.160 g cm^{-3}). The concentrated hydrolysate (pH 0.4) was also stored at 4 °C.

2.2 Detoxification methods

The concentrated hydrolysate was divided into six parts, each submitted to a different treatment procedure as follows:

(I) pH increased to 6.5;
(II) pH increased to 8.0 and subsequently decreased to 6.5;
(III) pH increased to 2.0, followed by addition of activated charcoal and pH increased to 6.5;
(IV) pH increased to 2.0, addition of activated charcoal, pH increased to 8.0 and, subsequently, decreased to 6.5;
(V) pH increased to 8.0, pH decreased to 2.0, addition of activated charcoal and pH increased to 6.5;
(VI) pH increased to 8.0, addition of activated charcoal, pH decreased to 2.0, then increased to 6.5.

Solid NaOH (PA-ACS, Labsynh, Brazil) was added to the hydrolysates to increase the pH value, and H_2SO_4 (13 M) was utilised to decrease it. Each time the pH was adjusted, the hydrolysates were centrifuged (1100 × *g* for 15 min) for removal of the precipitate. Activated charcoal was added to the hydrolysates at the rate of 25g kg^{-1}. The charcoal suspensions were agitated at 150 rpm, 45 °C for 60 min, and the activated charcoal was removed by centrifugation (1100 × *g* for 15 min). The detoxified hydrolysates were analysed chromatographically and spectrophotometrically for sugars (glucose, xylose and arabinose) and toxic compounds (acetic acid, furfural, hydroxymethylfurfural and lignin degradation products).

2.3 Microorganism and inoculum preparation

Candida guilliermondii FTI 20 037, selected by Barbosa *et al*,[11] was maintained at 4 °C on malt extract agar slants. Inocula were prepared by growing cells in 125 cm^3 Erlenmeyer flasks containing 50 cm^3 of medium composed of (g dm^{-3}): xylose (20); $(NH_4)_2SO_4$ (3); $CaCl_2.2H_2O$ (0.1) and rice bran extract (20). A 100 g kg^{-1} suspension of rice bran was autoclaved at 121 °C for 20 min, cooled to ambient temperature and centrifuged aseptically at 2000 × *g* for 20 min. The liquid fraction (rice bran extract) was stored at 4 °C for less than 1 day before used. The solutions were sterilised by autoclaving at 121 °C for 20 min, except xylose solution, which was sterilised by autoclaving at 112 °C for 15 min. The inocula were incubated at 30 °C on a rotatory shaker (Tecnal Piracicaba, SP, Brazil TE-420) at 200 rpm for 24 h. The cells were then separated by centrifugation (1100 × *g* for 20 min) and resuspended directly in the fermentation medium.

2.4 Media and fermentation conditions

Erlenmeyer flasks (125 cm^3) were employed for fermentation of the detoxified hydrolysate media and also of a semi-synthetic medium. The hydrolysates without nutrient supplementation were diluted in sterile distilled water to obtain (g dm^{-3}): xylose (90), glucose (20) and L-arabinose (15). The semi-synthetic medium, supplemented with the nutrients used for inoculum cultivation, contained the same concentrations of sugars as the hydrolysates. Each fermentation medium (50 cm^3) was inoculated at an initial cell concentration of 3 g dm^{-3}, and the flasks were agitated at 30 °C on an orbital shaker at 200 rpm. The fermentation runs lasted 116 h and were monitored by periodic sampling to determine cell growth, glucose and xylose uptake, and xylitol production.

2.5 Analytical procedures

Glucose, xylose, arabinose, xylitol and acetic acid concentrations were determined by high-performance liquid chromatography (HPLC) in a chromatograph with a refractive index (RI) detector and a Bio-Rad Hercules, CA, USA HPX-87H (300 × 7.8 mm) column, at 45 °C, using 0.005 M sulfuric acid as the eluent, a flow rate of 0.6 cm^3 min^{-1} and sample volume of 20 mm^3. Hydroxymethylfurfural and furfural were also determined by HPLC, using a UV detector (at 276 nm) and a Waters MilFord, MA, USA Resolve C_{18} 5 μm (3.9 × 300 mm) column at ambient temperature, with acetonitrile/water (1/8 with 10 g dm^{-3} acetic acid) as the eluent, a flow rate of 0.8 cm^3 min^{-1} and sample volume of 20 mm^3.

For the determination of lignin degradation products the pH of the samples was adjusted to 12.0 before they were diluted 1:1000 with distilled water and analysed by a Beckman Fullerton, CA, USA DU 640B spectrophotometer at 280 nm. Cell concentration was also determined spectrophotometrically, at 600 nm, by means of a calibration curve (dry weight vs optical density [OD]) obtained from cells grown on hydrolysate or synthetic medium and agitated on a rotary shaker at 200 rpm, 30 °C, for 72 h. Samples were diluted to a reading band of 0.05 to 0.5 OD units.

3 RESULTS AND DISCUSSION

3.1 Composition of hemicellulosic hydrolysate

The main components of the raw and concentrated hydrolysates are shown in Table 1. It was necessary to concentrate the hydrolysates to increase the xylose content, because the xylitol formation is affected negatively by initial xylose concentrations below 50 g dm^{-3}.[12,13]

Table 1. Composition of raw and concentrated rice straw hemicellulosic hydrolysate

	Hydrolysate composition (g dm^{-3})	
Compounds	Raw	Concentrated
Glucose	3.29	19.80
Xylose	18.33	119.90
Arabinose	3.40	22.90
Acetic acid	1.05	2.28
Furfural	0.10	0.07
Hydroxymethylfurfural	0.17	0.32
Lignin degradation products[a]	0.16	0.52

[a] Absorbance at 280 nm.

Besides increasing the sugar contents (glucose, xylose and arabinose) of the hydrolysate, the concentration process promoted a reduction in the content of furfural by evaporations thereby reducing the toxicity to the yeast. On the other hand, there were moderate increases in the concentrations of acetic acid, hydroxymethylfurfural and lignin degradation products, and consequently in the toxicity of the hydrolysate. To make the hydrolysates more suitable for fermentation, it was necessary to remove these toxic substances.[14]

3.2 Effect of hydrolysate detoxification on removal of sugars and toxic compounds

Several studies have been conducted to identify toxic compounds in hemicellulosic hydrolysates and to minimise their negative effects on the fermentation process.[4–10,14] The present study evaluated six procedures, for two of them (I and II) consisting only of pH adjustment, and the other four being combination' of pH adjustment and activated charcoal adsorption. The percentage removal of sugars and toxic compounds observed for each treatment was determined (Figs 1 and 2).

The extent of sugar removal (Fig 1) was different for each procedure. In all the cases arabinose removal was insignificant, the maximum reduction (8.5%) being observed for treatment IV. Glucose removal was only significant for treatment V (12%) and VI, (14.3%). Xylose, the major sugar in the hydrolysates, was reduced by 18% with treatment IV and by 15% with treatment VI; treatments I, II, III and V provided low xylose removal rates, the highest being 11.5% (treatment II). The removal of xylose should be as low as possible, since this sugar is essential for xylitol production.

The removal of toxic compounds also varied according to the detoxification procedure employed

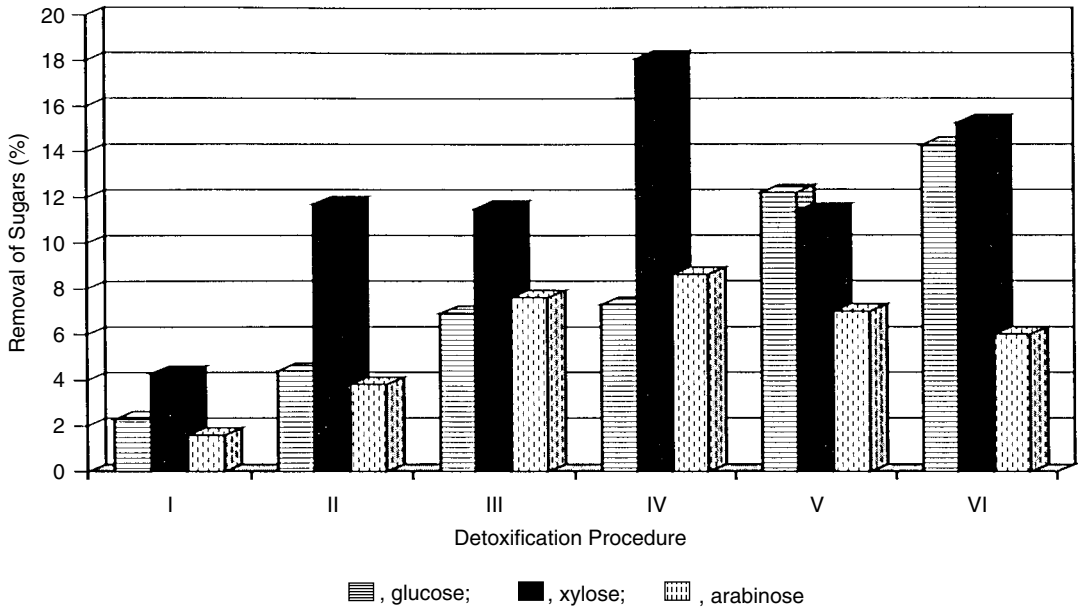

Figure 1. Removal of glucose, xylose and arabinose from rice straw hemicellulosic hydrolysate provided by six detoxification procedures. (For key to detoxification procedures, see Section 2.2).

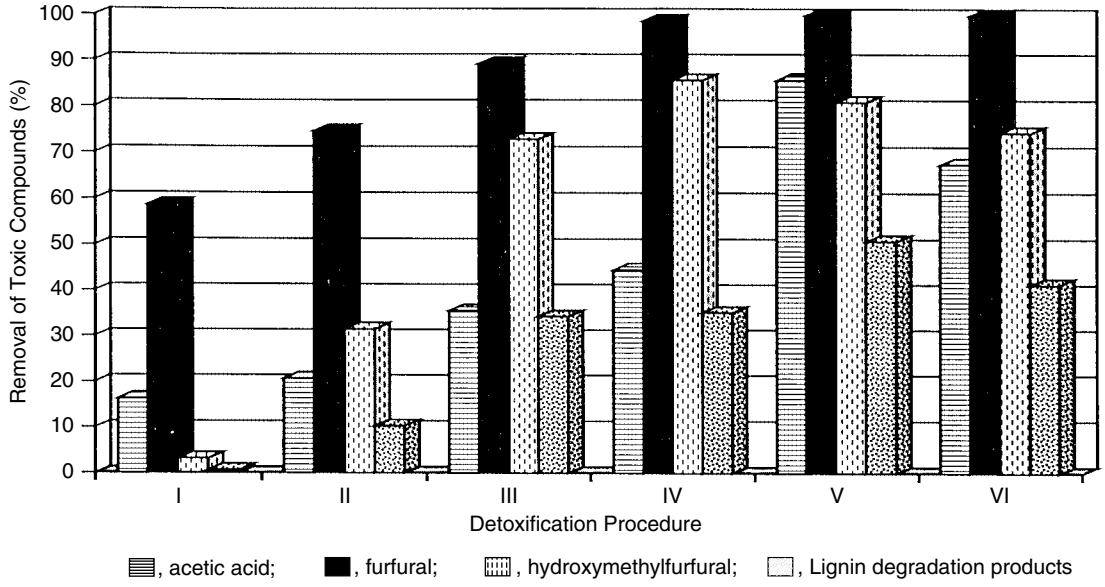

Figure 2. Removal of acetic acid, furfural, hydroxymethylfurfural and lignin degradation products, from rice straw hemicellulosic hydrolysate provided by six detoxification procedures. (For key to detoxification procedures, see Section 2.2).

(Fig 2). The removal of furfural ranged from almost 60% for treatment I to 100% for treatments V and VI. The removal of hydroxymethylfurfural was significant only when pH adjustment was combined with activated charcoal adsorption (treatments III, IV, V and VI). Treatment I caused negligible removal of hydroxymethylfurfural, treatment II gave a removal of 31% and the other four treatments give removals higher than 73%, the maximum (86%) being attained with treatment IV. The removal of acetic acid and lignin degradation products was also higher when pH adjustment and adsorption on activated charcoal were used in combination (treatments, III, IV, V and VI).

Although adjusting the pH to 6.5 (treatment I) caused the lowest levels of sugar removal, this treatment also gave the lowest removal of toxic compounds, thus proving inadequate for hydrolysate detoxification. Increasing the pH to 8.0 and then decreasing it to 6.5 (treatment II), removed more sugar and toxic compounds, but the latter effect was not as high as those attained with procedures III, IV, V and VI. When these four procedures were employed, the removal of toxic compounds varied according to the pH during activated charcoal adsorption.

In treatments III and IV, activated charcoal was added to the hydrolysate after the initial pH had been increased to 2.0. Raising the pH to 8.0 before decreasing it to 6.5 (treatment IV) caused a small increase in the removal of all the toxic compounds compared with treatment III where the pH was adjusted directly to 6.5, but with xylose removal being 1.6 times that in treatment III.

In treatments V and VI, the initial pH of the hydrolysate was first increased to 8.0, then decreased to 2.0 (treatment V) before addition of activated charcoal, or treated with activated charcoal at pH 8.0 (treatment VI). In both cases the pH was finally adjusted to 6.5, treatment VI led to a lower removal of toxic compounds and a greater loss of xylose them treatment V.

By comparing treatments III, IV, V and VI it can be concluded that it was better to add activated charcoal to the hydrolysate at pH 2.0 than at pH 8.0, because it promoted lower sugars removal and higher toxic compounds removal.

After being detoxified, the hydrolysates were diluted to attain xylose concentrations of about 90 g dm^{-3} for use as fermentation media (Table 2). At the

Table 2. Composition of the detoxified hydrolysates at the beginning of fermentation

	Composition of detoxified hydrolysates (g dm^{-3})					
Compounds	I	II	III	IV	V	VI
Glucose	15.26	15.20	15.09	14.75	14.88	14.92
Xylose	91.15	91.92	91.01	89.26	92.00	91.09
Arabinose	18.34	18.10	18.80	18.22	17.68	17.98
Acetic acid	1.501	1.570	1.209	1.149	0.280	0.665
Furfural	0.025	0.016	0.007	0.001	0.000	0.000
Hydroxymethylfurfural	0.240	0.191	0.075	0.042	0.053	0.073
Lignin degradation products[a]	0.410	0.404	0.292	0.305	0.221	0.273

[a] Absorbance at 280 nm. Initial fermentation pH = 6.5 for all treatments. See Section 2.2 for description of treatments I–VI.

beginning of the fermentations the concentrations of acetic acid, hydroxymethylfurfural and furfural were all below the thresholds of inhibition. Low levels of toxic compounds favour the bioconversion processes but, due to synergistic effects, such compounds in combination may inhibit fermentation even when their individual levels are low.[15] Lignin degradation products, which were also present in the detoxified hydrolysates, are more inhibitory than furfural and hydroxymethylfurfural, even at low concentrations.[16]

3.3 Evaluation of xylose-to-xylitol bioconversion from detoxified hydrolysates

The results of the fermentations using the detoxified hydrolysates are shown in Fig 3. Xylose was almost totally consumed when the hydrolysate was treated by pH adjustment combined with activated charcoal adsorption (treatments III, IV, V and VI), but when only pH adjustment was used (treatments I and II) the residual amounts of xylose were above 10 g dm^{-3} (Fig 3(A)). Although xylose was not totally consumed after treatment I, the result obtained with this procedure was better than that reported by Roberto *et al*[9] when the pH of rice straw hemicellulosic hydrolysate was raised to 6.5 using $Ca(OH)_2$. In this case, only 20% of the xylose was consumed after 130 h of fermentation, which suggests that NaOH is more favourable than $Ca(OH)_2$ for xylose consumption.

Xylitol production was also improved by the combination of pH adjustment and adsorption on activated charcoal, especially when the charcoal was added to the hydrolysate at pH 2.0, without previous pH increase to 8.0 (treatments III, IV—Fig 3(B)).

With detoxification procedures V and VI (pH increased initially to 8.0) xylitol production was lower than with procedures III and IV (pH increased initially to 2.0). As the concentrations of acetic acid, furfural and hydroxymethylfurfural in the hydrolysates detoxified by these four treatments were low, the differences observed could be attributed to the lignin degradation products, which include a high proportion of phenolic compounds. According to Fox[17] when the pH of the medium is low, phenolic compounds are readily adsorbed on activated charcoal because they are in the neutral or non-ionised form, whereas at high pH they become anions (phenolate ions), which are poorly adsorbed. This is why in this work, although removal ratios of 51 and 41% were attained for lignin degradation products with treatments V and VI respectively, these two procedures were not able to remove large quantities of those lignin degradation products that appear in the medium when the hydrolysate pH is

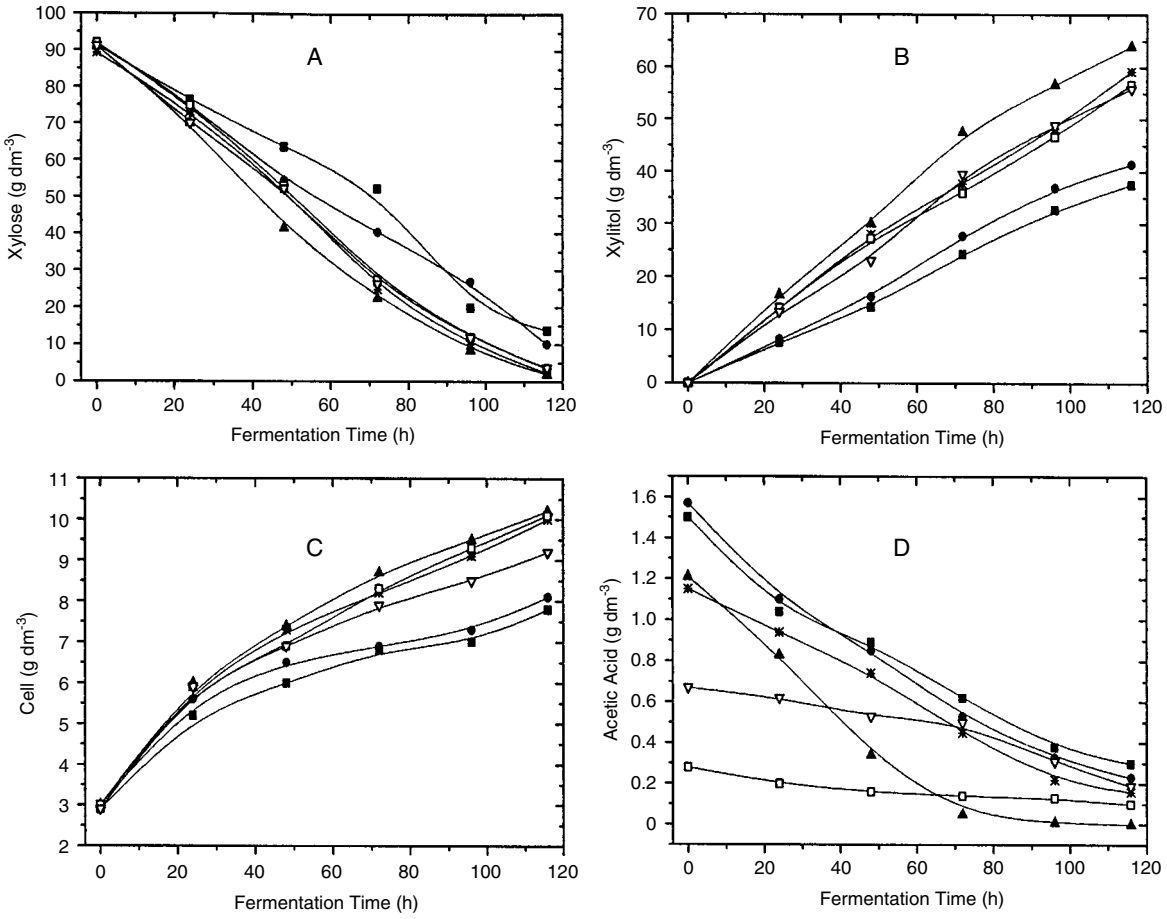

Figure 3. Xylose uptake (A), xylitol production (B), cell growth (C) and acetic acid consumption (D), during the fermentation of rice straw hemicellulosic hydrolysate, detoxified by procedures I (■), II (●), III (▲), IV (✱), V (□) and VI (▽). (For key to detoxification procedures, see Section 2.2).

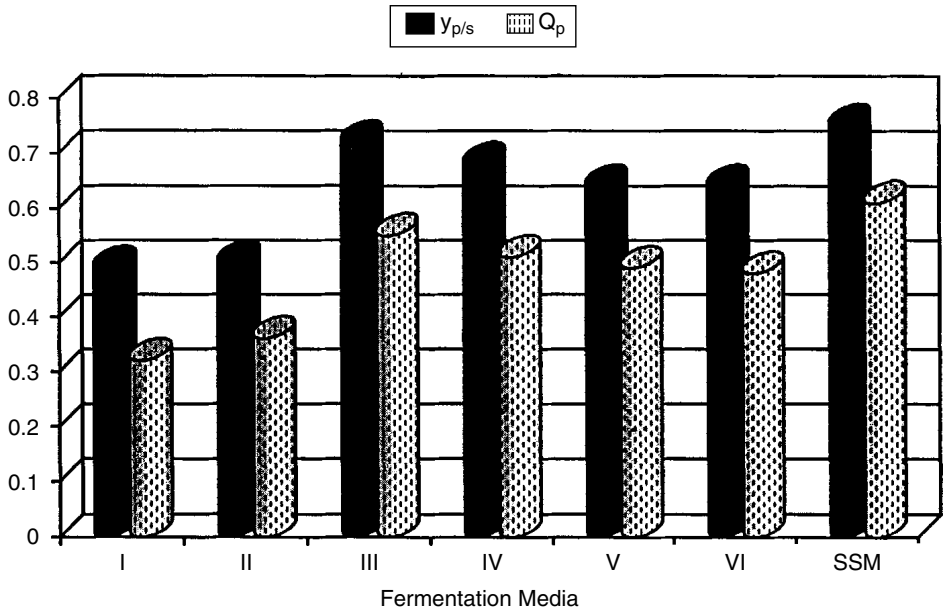

Figure 4. Xylitol yield coefficients ($Y_{P/S}$ as g xylitol g^{-1} xylose consumed) and volumetric productivity (Q_P as g xylitol dm^{-3} h^{-1}) for the fermentation processes carried out with detoxified hydrolysate media (methods I–VI) and semi-synthetic medium (SSM). (For key to detoxification procedures, see Section 2.2).

increased to 8.0, and that are considered as the most potent inhibitors of microbial metabolism.[18] As a consequence, the bioconversion process was ineffective.

The results of the fermentation processes led us to conclude that the pH employed during the detoxification of the hydrolysate by activated charcoal strongly influenced the removal of toxic compounds, mainly lignin degradation products, so that the addition of activated charcoal at pH 2.0 enhanced cell growth (Fig 3(c)) and xylitol production.

Acetic acid in the media was almost totally consumed by the yeast during the fermentation of all the detoxified hydrolysates (Fig 3(D)), showing that the six detoxification procedures were able to reduce the acetic acid concentration to levels that were not inhibitory to the microorganism. As the concentrations of hydroxymethylfurfural and furfural in detoxified rice straw hemicellulosic hydrolysates are always low, lignin degradation products can be considered the most inhibitory compounds present. Likewise, the toxicity of wood hydrolysate media is principally due to the presence of lignin degradation products.[14,19]

Figure 4 shows the xylitol yield coefficients ($Y_{P/S}$) and volumetric productivity (Q_P) for the six detoxified hydrolysates (I, II, III, IV, V and VI) and for the semi-synthetic medium (SSM). The lowest values of xylitol yield and volumetric productivity ($0.49\,g\,g^{-1}$ and $0.32\,g\,dm^{-3}h^{-1}$ respectively) were associated with medium I, and the highest values ($0.72\,g\,g^{-1}$ and $0.55\,g\,dm^{-3}h^{-1}$ respectively) with medium III, giving increases of 47 and 72% respectively. Detoxification procedure III proved to be the best, giving results very similar to those for the semi-synthetic medium.

4 CONCLUSIONS

Rice straw hemicellulosic hydrolysate obtained by acid hydrolysis contains, besides a mixture of sugars, several toxic compounds (acetic acid, hydroxymethylfurfural, furfural and lignin degradation products). As the concentrations of these compounds (except furfural) increase during the concentration of the hydrolysate it is necessary to detoxify the hydrolysates before they can be used as fermentation media. Although pH adjustment alone was not adequate to produce a suitable medium for xylose-to-xylitol bioconversion, the combination of pH adjustment and activated charcoal adsorption resulted in higher rates of removal, of toxic compounds and consequently in significantly improved fermentation processes. The effectiveness of adsorption on activated charcoal varied according to the pH of the hydrolysate. Selective removal of lignin degradation products (the most potent inhibitors present in rice straw hemicellulosic hydrolysate) was observed when the pH value during the adsorption was 2.0 or 8.0. The best results for the fermentation process ($Y_{P/S} = 0.72\,g\,g^{-1}$ and $Q_P = 0.55\,g\,dm^{-3}\,h^{-1}$) were attained when the hydrolysate was detoxified through the following procedure: increase in the initial pH (0.4) to 2.0 using solid NaOH, addition of activated charcoal to the hydrolysate at the rate of $25\,g\,kg^{-1}$ under agitation (150 rpm, 45 °C, 60 min) and increase in the pH to 6.5 using solid NaOH. With this method the levels of sugar loss were all below 11.5% and the levels of removal of toxic compounds were 35.6% for acetic acid, 72.9% for hydroxymethylfurfural, 89.3% for furfural and 34.3% for lignin degradation products. Also with this treatment, the fermentation efficiency (78.5%) was almost as high as that observed for the semi-synthetic medium (81.8%) formulated with the

same concentrations of sugars as the hydrolysate, but with no toxic compounds.

ACKNOWLEDGEMENTS

The authors acknowledge the financial assistance from FAPESP and thank Maria Eunice M. Coelho for her valuable efforts in the review of this manuscript.

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