Optimal Experimental Condition for Hemicellulosic Hydrolyzate Treatment with Activated Charcoal for Xylitol Production

ARTICLE *in* BIOTECHNOLOGY PROGRESS · JANUARY 2004

Impact Factor: 2.15 · DOI: 10.1021/bp034207i · Source: PubMed

2 AUTHORS:

Solange I Mussatto
Delft University of Technology
187 PUBLICATIONS **3,351** CITATIONS

SEE PROFILE

Ines Roberto
University of São Paulo
144 PUBLICATIONS **3,381** CITATIONS

SEE PROFILE

Optimal Experimental Condition for Hemicellulosic Hydrolyzate Treatment with Activated Charcoal for Xylitol Production

Solange I. Mussatto* and Inês C. Roberto

Department of Biotechnology, Faculty of Chemical Engineering of Lorena, Rodovia Itajubá-Lorena Km 74.5, 12600-970 Lorena, SP, Brazil

Rice straw was hydrolyzed into a mixture of sugars using diluted H_2SO_4. During hydrolysis, a variety of inhibitors was also produced, including acetic acid, furfural, hydroxymethylfurfural, and lignin degradation products (several aromatic and phenolic compounds). To reduce the toxic compounds concentration in the hydrolyzate and to improve the xylitol yield and volumetric productivity, rice straw hemicellulosic hydrolyzate was treated with activated charcoal under different pH values, stirring rates, contact times, and temperatures, employing a 2^4 full-factorial design. Fermentative assays were conducted with treated hydrolyzates containing 90 g/L xylose. The results indicated that temperature, pH, and stirring rate strongly influenced the hydrolyzate treatment, temperature and pH interfering with all of the responses analyzed (removal of color and lignin degradation products, xylitol yield factor, and volumetric productivity). The combination of pH 2.0, 150 rpm, 45 °C, and 60 min was considered an optimal condition, providing significant removal rates of color (48.9%) and lignin degradation products (25.8%), as well as a xylitol production of 66 g/L, a volumetric productivity of 0.57 g/L·h, and a yield factor of 0.72 g/g.

Introduction

Rice straw is an agricultural residue produced in large quantities in many countries, and in Brazil only its production reached 14 million tons in 2002. Accumulations of this residue in the soil interfere with the ecosystem, and its elimination by burning has a damaging effect on the air and consequently on the public health (*1*, *2*). A solution to this problem would be the utilization of rice straw as a raw material to generate useful products such as ethanol, xylitol, or even paper. This is quite possible, because rice straw is a fibrous lignocellulosic material that has a high nutritional value and contains 43.5% cellulose, 22% hemicellulose, 17.2% lignin, and 11.4% ash (*3*). Its high pentosan content (hemicellulosic fraction) provides a hydrolyzate basically composed of D-xylose and L-arabinose, which is suitable for use as a culture medium in fermentative processes aiming at the production of xylitol (*4*, *5*).

Xylitol, a sugar alcohol derived from xylose, is equivalent to sucrose in sweetness, but unlike sucrose it is anticariogenic and can be consumed by diabetics because it is metabolized by an insulin-independent pathway. Disorders in the lipid metabolism, as well as parenteral and renal lesions, can be treated with xylitol, which also prevents otitis, osteoporosis, and lung infections (*6*). Xylitol is currently produced by a chemical process based on the catalytic hydrogenation of D-xylose from hemicellulosic hydrolyzates, a high-cost method that requires extensive xylose purification steps and results in a relatively expensive final product (about $ 7/Kg) (*7*). The biotechnological method has been considered as a more economic alternative for xylitol production, since it requires very little xylose purification and employs enzymes or specific microorganisms that only act on xylose-to-xylitol conversion, providing a higher product yield (*8*). Nevertheless, the biotechnological conversion of xylose into xylitol using hydrolyzates obtained from the hemicellulosic fraction of lignocellulosic materials is damaged 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 and furfural (sugars degradation products), acetic acid (substance released from the hemicellulosic structure), and several aromatic and phenolic compounds (lignin degradation products) that are considered more toxic than furfural or hydroxymethylfurfural, even at low concentrations (*9*). Besides the problem of toxicity, phenolic oxidation reactions occur during the hydrolyzate neutralization, resulting in brown-colored pigments that are detrimental to the quality of processed food (*10*).

To improve the bioconversion of hydrolyzates, several attempts have been made to identify the toxic compounds (*11*−*13*) or to find alternative methods of treating the hydrolyzates to reduce their toxicity (*14*−*18*). Among such methods, adsorption on activated charcoal stands out as an efficient and low-cost technique, but its effectiveness depends on the levels of the variables used in the adsorption process. These variables include pH, temperature, stirring rate, contact time, and activated charcoal concentration (*3*). As each of these operational variables interacts with the others and influences the adsorption of the compounds, it is essential to optimize the treatment conditions through a method taking these interactions into account, so that a set of optimal experimental conditions can be determined. Optimization

* To whom correspondence should be addressed. Tel: (0055) 1231595026. Fax: (0055) 1231533165. E-mail: solange@debiq.faenquil.br.

10.1021/bp034207i CCC: $27.50
Published on Web 10/16/2003

through factorial design and response surface methodology (RSM) particularly fulfils this requirement (*19*).

An earlier study on xylitol production established the most adequate activated charcoal concentration for detoxification of rice straw hemicellulosic hydrolyzate (*18*). In the present work, rice straw hydrolyzate was treated with activated charcoal and the variables pH, stirring rate, contact time, and temperature employed in the treatment were statistically optimized with the help of a 2^4 full-factorial design using response surface methodology. The responses statistically analyzed were the removal of color and lignin degradation products, both obtained after each treatment, and the xylitol yield factor and volumetric productivity attained after fermentation of each treated hydrolyzate.

Materials and Methods

Microorganism and Inoculum. *Candida guilliermondii* FTI 20037, maintained at 4 °C on malt extract agar slant, was grown in 125-mL flasks containing 50 mL of medium composed of (g/L) xylose (20), $(NH_4)_2SO_4$ (3.0), $CaCl_2 \cdot 2H_2O$ (0.1), and 20% (v/v) rice bran extract. Solutions prepared with each component separately were sterilized at 121 °C for 20 min, except the xylose solution, which was autoclaved at 112 °C for 15 min. To use rice bran as a nutrient, a 10% suspension of rice bran acquired from local farmers was autoclaved at 121 °C for 20 min, cooled to room temperature, and aseptically centrifuged at 1100*g* for 20 min (*5*). The culture was incubated at 30 °C on a rotary shaker (Tecnal TE-420) at 200 rpm for 24 h, and the cells were subsequently separated by centrifugation (1100*g* for 20 min) and resuspended on the fermentation medium.

Hemicellulosic Hydrolyzate and Treatment Conditions. Rice straw locally obtained was dried in the sun and milled to attain particles of about 1 cm in length and 1 mm in thickness. Acid hydrolysis was conducted in a 350-L stainless steel pressure reactor under the following conditions: 0.1 M H_2SO_4, liquid:solid ratio 10:1 (v/w), 121 °C, and 27 min (*4*). The resulting solid material was removed by filtration under vacuum, and the filtrate (hemicellulosic hydrolyzate) was concentrated in a 4-L evaporator at 70 ± 5 °C, to obtain a xylose content of approximately 120 g/L (density = 1.160 g/mL). The concentrated hydrolyzate was treated with powder activated charcoal using a charcoal:hydrolyzate ratio of 1:40 (g/g) and different pH values, stirring rates, contact times, and temperatures, according to a 2^4 full-factorial design.

All treatments were carried out in 125-mL Erlenmeyer flasks that contained 50 mL of hydrolyzate and were agitated on a rotatory shaker. After each treatment, the precipitate was removed by centrifugation at 1100*g* for 20 min, and the hydrolyzate was analyzed to determine the removal of sugars (glucose, xylose, and arabinose), color, and toxic compounds (hydroxymethylfurfural, furfural, acetic acid, and lignin degradation products).

Experimental Design and Optimization by Response Surface Methodology. A 2^4 full-factorial design with three coded levels leading to 19 sets of experiments was made to establish the effects of four different activated charcoal treatment variables: pH, stirring rate, contact time, and temperature. For statistical analysis, the variables were coded according to

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

Table 1. Experimental Ranges and Levels of the Independent Process Variables According to the 2^4 Full Factorial Design

		range and levels		
independent variable	symbol	−1	0	+1
pH	X_1	2.0	5.0	8.0
stirring rate (rpm)	X_2	150	200	250
contact time (min)	X_3	10	35	60
temperature (°C)	X_4	25	35	45

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 in the center point were carried out to estimate the random error needed for the analysis of variance, as well as to examine the presence of curvature in the response surface. The removal rates of lignin degradation products (LDPR) and color (CR), plus xylitol yield factor ($Y_{P/S}$) 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.

The model for predicting the optimal point was expressed as

$$\hat{y}_i = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j \quad (2)$$

where $\hat{y}_i$ represents the response variable; b_0 is the interception coefficient; b_i, b_{ii}, and b_{ij} are the regression coefficients; n is the number of variables studied; and X_i and X_j represent the variables. Where possible, the model was simplified by elimination of statistically insignificant terms.

Statistica v.5.0 (Statsoft, USA) software was used for regression and graphical analyses of the data obtained. The statistical significance of the regression coefficients was determined by Student's *t*-test, and the proportion of variance explained by the model was given by the multiple coefficient of determination, R^2. The optimum values of the variables were obtained by graphical and numerical analyses using the Statistica program and the criterion of desirability.

Fermentation Conditions. To be used as a fermentation medium, each treated hydrolyzate was diluted with sterile distilled water to obtain 90 g of xylose per liter and inoculated with an initial cell concentration of 3 g/L. For fermentation, 125-mL Erlenmeyer flasks containing 50 mL of medium were agitated at 30 °C in an orbital shaker at 200 rpm. The fermentation runs lasted 116 h and were monitored through periodic sampling to determine cell growth, glucose and xylose uptake, and xylitol production.

Analytical Procedures. Glucose, xylose, xylitol, acetic acid, hydroxymethylfurfural, and furfural concentrations were determined by high-performance liquid chromatography (HPLC) as described previously (*3*). To determine the lignin degradation products, the pH of the samples was adjusted to 12.0 before they were diluted (1:1000 mL) in distilled water and analyzed at 280 nm by a Beckman DU 640B spectrophotometer. For the color determination, the pH of the samples was adjusted to 5.5 before they were diluted in distilled water (1:50 mL) and analyzed at 440 nm. Cell concentration was determined at 600 nm, by means of a calibration curve (dry weight × optical density [OD]) obtained from cells grown on hydrolyzate medium agitated on a rotary shaker at

Table 2. Characterization of Rice Straw Hemicellulosic Hydrolyzate before and after Concentration

	original hydrolyzate	concentrated hydrolyzate
	Concentration (g/L)	
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
	Absorbance	
lignin degradation products[a]	0.16	0.52
color[b]	0.10	0.47

[a] Absorbance at 280 nm. [b] Absorbance at 440 nm.

200 rpm, 30 °C, for 72 h. Samples were diluted to a reading band of 0.05−0.5 OD units.

Results and Discussion

Toxicity of Rice Straw Hemicellulosic Hydrolyzate. To be employed in the process of xylitol production, rice straw was initially submitted to acid hydrolysis under conditions able to promote a selective separation of its hemicellulosic fraction. The resulting hemicellulose syrup contained, in addition to xylose (the major sugar), several compounds that are toxic to yeasts, namely, acetic acid, furfural, hydroxymethylfurfural, and lignin degradation products.

Because the amount of xylose in the hydrolyzate, 18 g/L (Table 2), was inadequate to produce xylitol by fermentative process, it had to be increased by concentrating the hydrolyzate. In addition to providing high xylitol production rates, increased initial xylose concentrations are interesting from the economic point of view, since the final product concentration also increases, making the separation process more viable. Nevertheless, the increase in sugars concentrations is accompanied by the increase in the contents of hydroxymethylfurfural, acetic acid, and lignin degradation products, resulting in higher degrees of toxicity and inhibition of the microbial metabolism. Table 2 shows the hydrolyzate composition before and after the concentration process. As can be seen, concentrating the hydrolyzate caused the concentrations of all of the compounds to increase except furfural, which is volatile in the conditions employed in this process. Lowering the levels of toxic compounds results in improved fermentative activity of the microorganism and in a more effective bioconversion of xylose into xylitol.

In general, the levels of furfural, hydroxymethylfurfural, and acetic acid present in concentrated hydrolyzates are below the threshold of inhibition. Furfural at concentrations lower than 1 g/L has been described as practically harmless to the microorganism in fermentative processes (*20*−*22*). Similarly, hydroxymethylfurfural has been reported to inhibit the microbial metabolism only when its concentration in the medium is higher than 1 g/L *(23*, *24*), and acetic acid has been found to be toxic to the microorganism in fermentative processes for xylitol production only at concentrations higher than 3 g/L (*25*).

Lignin degradation products, considered to be the most potent toxic compounds present in lignocellulosic hydrolyzates, are more toxic to the microorganism than furfural or hydroxymethylfurfural, even when presents in low concentrations (*9*, *26*, *27*). In view of this fact and considering that in our work the concentration of lignin degradation products increased when the hydrolyzate was concentrated, a detoxification procedure was required before the hydrolyzate could be used as a fermentation medium. Another consequence of concentrating the hydrolyzate was the increase in the intensity of its color, which passed from light brown to dark brown. According to Fengel and Weneger (*28*), the hydrolyzate darkening is due both to the oxidation of the phenolic compounds and to the increase in their concentrations in the medium.

Table 3. Experimental Matrix for Color Removal (CR), Lignin Degradation Products Removal (LDPR), Xylitol Yield Factor ($Y_{P/S}$), and Volumetric Productivity (Q_P), with Coded Levels of Variables According to a 2^4 Full-Factorial Design with Three Replicates at the Center Point

	independent variables[a]				responses			
runs	X_1	X_2	X_3	X_4	CR (%)	LDPR (%)	$Y_{P/S}$ (g/g)	Q_P (g/L·h)
1	−1	−1	−1	−1	37.0	29.8	0.50	0.47
2	+1	−1	−1	−1	4.3	5.4	0.60	0.45
3	−1	+1	−1	−1	21.9	22.9	0.53	0.40
4	+1	+1	−1	−1	0.5	9.4	0.58	0.36
5	−1	−1	+1	−1	46.5	31.5	0.50	0.47
6	+1	−1	+1	−1	3.1	12.3	0.63	0.46
7	−1	+1	+1	−1	38.5	33.0	0.52	0.41
8	+1	+1	+1	−1	0.1	10.0	0.55	0.34
9	−1	−1	−1	+1	48.5	36.2	0.74	0.55
10	+1	−1	−1	+1	3.5	13.5	0.64	0.48
11	−1	+1	−1	+1	47.1	35.1	0.55	0.41
12	+1	+1	−1	+1	2.1	23.3	0.57	0.44
13	−1	−1	+1	+1	48.9	25.8	0.72	0.57
14	+1	−1	+1	+1	0.0	15.8	0.68	0.50
15	−1	+1	+1	+1	48.3	32.0	0.55	0.43
16	+1	+1	+1	+1	2.5	17.7	0.57	0.40
17	0	0	0	0	20.7	21.7	0.62	0.41
18	0	0	0	0	23.3	20.0	0.65	0.43
19	0	0	0	0	22.2	23.7	0.65	0.42

[a] X_1 = coded values of pH; X_2 = coded values of stirring rate; X_3 = coded values of contact time; X_4 = coded values of temperature

The next stage of this work consisted in optimizing the conditions of the hydrolyzate treatment with activated charcoal for removal of the lignin degradation products and color, which are the main indicators of toxicity of rice straw hydrolyzate.

Statistical Analysis of the Adsorption of Compounds on Activated Charcoal and of Xylitol Production from Treated Hydrolyzates. Detoxification with activated charcoal has been previously reported to decrease the toxicity of hemicellulosic hydrolyzates obtained from different lignocellulosic materials (*12*, *14*, *15*, *17*). However, the adsorption of compounds on activated charcoal can vary as a function of the levels of the process variables, which include pH, temperature, stirring rate, and contact time. To optimize the treatment conditions, the first step is identifying the variables that have the greatest influence on the responses. In the present work, experiments were planned according to a 2^4 full-factorial design composed of three replicates at the center point. The design of this experiment is given in Table 3, together with the experimental results, which indicate that the removal of color and lignin degradation products was dependent on the conditions employed in the treatment of the hydrolyzate with activated charcoal. On the other hand, the results of the fermentative process ($Y_{P/S}$ and Q_P) from treated hydrolyzates were dependent on the removal of lignin degradation products obtained to each treatment.

The analysis of the estimated effects (Table 4) shows a positive and main significant effect of temperature on

Table 4. Effect Estimates, Standard Errors (SE), and *t*-Test Results for Color Removal, Lignin Degradation Products Removal (LDPR), Yield Factor, and Volumetric Productivity for Xylose-to-Xylitol Bioconversion by *Candida guilliermondii*, According to the 2^4 Full-Factorial Design

	color removal			LDPR			xylitol yield factor			volumetric productivity		
variables and interactions	estimated effects	SE	*t*	estimated effects	SE	*t*	estimated effects	SE	*t*	estimated effects	SE	*t*
X_1	−40.075	±1.301	30.80[a]	−17.363	±1.547	11.22[a]	0.026	±0.022	1.21	−0.035	±0.013	2.59[b]
X_2	−3.850	±1.301	2.96[b]	1.638	±1.547	1.06	−0.074	±0.022	3.41[a]	−0.095	±0.013	7.05[a]
X_3	2.875	±1.301	2.21[c]	0.313	±1.547	0.20	0.001	±0.022	0.06	0.003	±0.013	0.18
X_4	6.125	±1.301	4.71[a]	5.638	±1.547	3.64[a]	0.076	±0.022	3.53[a]	0.053	±0.013	3.89[a]
X_1X_2	2.425	±1.301	1.86[c]	1.713	±1.547	1.11	0.004	±0.022	0.17	0.008	±0.013	0.55
X_1X_3	−4.050	±1.301	3.11[b]	0.738	±1.547	0.48	0.009	±0.022	0.40	−0.010	±0.013	0.74
X_1X_4	−6.100	±1.301	4.69[a]	2.663	±1.547	1.72	−0.051	±0.022	2.37[b]	0.000	±0.013	0.00
X_2X_3	1.575	±1.301	1.21	0.188	±1.547	0.12	−0.011	±0.022	0.52	−0.010	±0.013	0.74
X_2X_4	3.625	±1.301	2.79[b]	2.563	±1.547	1.66	−0.061	±0.022	2.83[b]	−0.010	±0.013	0.74
X_3X_4	−3.250	±1.301	2.50[b]	−4.513	±1.547	2.92[b]	0.004	±0.022	0.17	0.003	±0.013	0.18

[a] $P < 0.01$. [b] $P < 0.05$. [c] P, 0.10; X_1 = pH; X_2 = stirring rate; X_3 = contact time; X_4 = temperature.

Table 5. Model Equations for Response Surfaces Fitted to Experimental Data Points and the Respective R^2

response	model equations[a]	R^2
color removal (CR in %)	$CR = 22.053 - 20.037X_1 - 1.925X_2 + 1.437X_3 + 3.062X_4 + 1.212X_1X_2 - 2.025X_1X_3 - 3.050X_1X_4 + 1.812X_2X_4 - 1.625X_3X_4$	0.99
lignin degradation products removal (LDPR in %)	$LDPR = 22.058 - 8.681X_1 + 2.819X_4 - 2.256X_3X_4$	0.90
xylitol yield factor ($Y_{P/S}$ in g/g)	$Y_{P/S} = 0.597 + 0.013X_1 - 0.037X_2 + 0.038X_4 - 0.026X_1X_4 - 0.031X_2X_4$	0.82
volumetric productivity (Q_P in g/L.h)	$Q_P = 0.442 - 0.017X_1 - 0.047X_2 + 0.026X_4$	0.88

[a] X_1 = pH; X_2 = stirring rate; X_3 = contact time; X_4 = temperature.

all responses ($P < 0.01$), indicating that all of them increased with the increase in temperature.

In regard to lignin degradation products removal and color removal, the variables temperature and pH were significant at 99% confidence level ($P < 0.01$), and the pH decrease favored both removals. In fact, the hydrolyzate color is directly related to the presence of phenolic compounds (lignin degradation products), and so a substantial loss of color can be obtained by removing these compounds from the hydrolyzate. According to Talcott and Howard (*10*), when submitted to polymerization reactions, phenolic compounds result in brown-colored pigments, the browning rates being pH-dependent. Initially, yellow pigments are formed, but as the pH rises, brown pigments begin to appear. The pH increase also causes the phenolic compounds to be transformed into phenolate ions. As these ions are poorly adsorbed on activated charcoal, they remain in the hydrolyzate, which consequently becomes dark. In the present work, pH 2.0 produced better results than pH 8.0 for removal of both color and lignin degradation products, probably as a result of the low formation of phenolate ions.

The influence of temperature increase on removal of color and lignin degradation products was also observed by other authors (*29*−*31*). According to Ravi et al. (*30*), the rise in temperature promotes an increase in the density of the packing of phenolic molecules in the activated charcoal pores. When the phenolic compounds are removed, the color intensity of the hydrolyzate also decreases.

Table 4 also shows that the xylitol yield ($Y_{P/S}$) was strongly influenced by the temperatures and stirring rates employed in the treatment of the hydrolyzate with activated charcoal ($P < 0.01$ for both variables). The pH interfered with $Y_{P/S}$ when in interaction with temperature ($P < 0.05$). For Q_P, the interactions between the variables were not significant, but the linear terms including temperature and agitation ($P < 0.01$) and pH ($P < 0.05$) were significant.

The statistical significance of main and interaction effects of the variables was determined by analysis of variance, and a multiple regression analysis was performed to fit the polynomial equations to the experimental data points (Table 5). All responses were described as a function of a first-order polynomial, and the high values of R^2 (> 0,80) indicate that the selected models are suitable for the process, showing a close agreement between the experimental results and the theoretical values predicted by the models.

Tridimensional response surfaces described by the above-mentioned first-order polynomials were fitted to the experimental data points concerning the removal of color and lignin degradation products, xylitol yield factor, and volumetric productivity (Figures 1 and 2). In all the cases, the response was fitted to a flat surface.

The response surfaces described by the model equations for color removal and lignin degradation products removal (Figure 1) clearly show that the increase in both removals occurs linearly with the increase in temperature and decrease in pH. Maximum values were obtained from the hydrolyzate treated at 45 °C and pH 2.0.

Temperature and stirring rate had main significant effects on xylitol yield factor (Figure 2). The response surface shows that the maximum $Y_{P/S}$ was attained from the medium provided by hydrolyzate treated at 45 °C and 150 rpm. For this response, the pH was only significant when in interaction with temperature, and to minimize the error determination, the main effect of the pH was kept in the model equation, as can be seen in Table 5.

The last response evaluated was volumetric productivity, and the response surface was plotted as a function of temperature and agitation, which had main significant effects on Q_p (Figure 2). As in the case of $Y_{P/S}$, the fitted surface also shows that the maximum Q_P value was attained from medium provided by hydrolyzate treated at 45 °C and 150 rpm.

These results on the whole demonstrate that the best conditions for removal of color and lignin degradation products, as well as for xylose-to-xylitol bioconversion from concentrated rice straw hemicellulosic hydrolyzate, are pH 2.0, 45 °C, 60 min, and 150 rpm.

The results of the fermentative processes were very similar for the hydrolyzates treated under pH 2.0, 150 rpm, and 45 °C, irrespective of the contact time (10 or

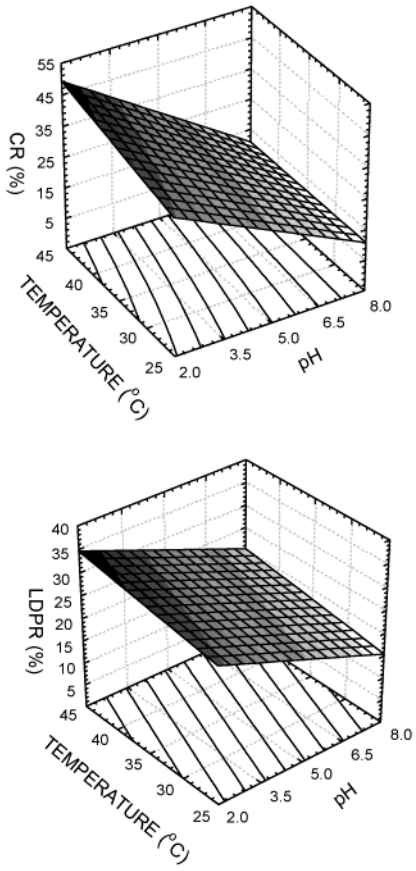

Figure 1. Response surface fitted to the experimental data points corresponding to the color removal (CR in %) and the lignin degradation products removal (LDPR in %) from hydrolyzate detoxified with activated charcoal.

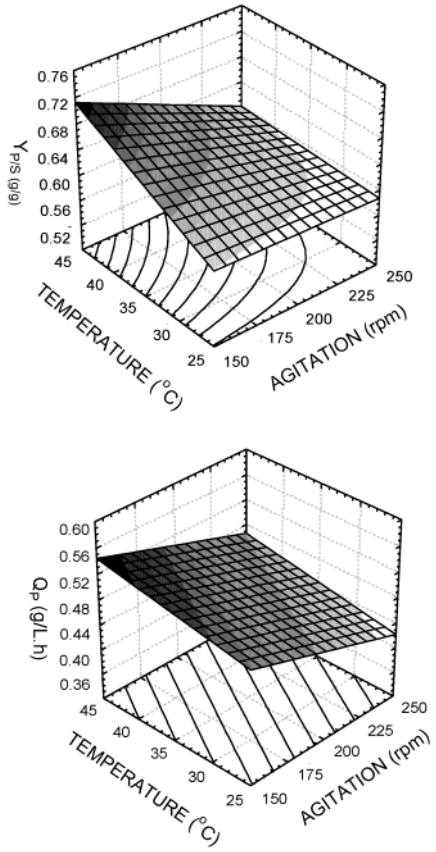

Figure 2. Response surface fitted to the experimental data points corresponding to the xylitol yield factor ($Y_{P/S}$ in g/g) and the volumetric productivity (Q_P in g/L·h) attained in the bioconversion from hydrolyzate detoxified with activated charcoal.

60 min). In hydrolyzate treated for 60 min the xylitol production was 66 g/L, and the volumetric productivity was 0.57 g/L·h. In hydrolyzate treated for 10 min, the results were 64 g/L and 0.55 g/L·h, respectively. Nevertheless, the contact time increase had a positive effect in color removal. For this reason, 60 min was considered a more adequate contact time to treat rice straw hemicellulosic hydrolyzate with activated charcoal.

It should be pointed out that the charcoal treatment employing pH 2.0, 150 rpm, 45 °C, and 60 min removed 25.8% of lignin degradation products from the hydrolyzate. Although the treatment under other conditions removed up to 36% of these compounds, the bioconversion process was not so effective, which suggests that, depending on the conditions employed in the treatment, the removal of the lignin degradation products is selective. As lignin degradation products include a large variety of compounds such as catechol, hydroquinone, coniferyl, 4-methylcatechol, guaiacol, vanillyl alcohol, syringic, vanillic and palmitic acids, and others (*32*), a study on the effect of each of these compounds on the xylose-to-xylitol bioconversion could be useful to determine which one is the most potent inhibitor of the microbial metabolism. Identifying this inhibitor would enable the establishment of suitable hydrolysis conditions to minimize its formation and to produce a less toxic hydrolyzate.

Conclusions

A broad range of compounds was liberated from the rice straw into the hydrolyzate or formed during the dilute acid hydrolysis. Some of these compounds were toxic to the microorganism. The most toxic ones were lignin degradation products, since the concentrations of acetic acid, furfural, and hydroxymethylfurfural were below the threshold of inhibition. The lignin degradation products were partially removed from the hydrolyzate by the treatment with activated charcoal, which improved the hydrolyzate fermentability and decreased the intensity of its color. Temperature and pH were the treatment variables that had the strongest effect on the removal of these compounds.

Candida guilliermondii yeast was able to grow and produce xylitol in all treated hydrolyzates. Nevertheless, the xylose-to-xylitol bioconversion was dependent on the conditions of treatment employed. The use of pH 2.0, 150 rpm, 45 °C, and 60 min proved to be the best condition, providing a removal of 25.8% of lignin degradation products, a xylitol production of 66 g/L, and a yield and volumetric productivity of 0.72 g/g and 0.57 g/L·h, respectively. These results show that a relatively simple method of treatment can be used to detoxify hemicellulosic hydrolyzates, improving their fermentability.

Acknowledgment

The authors gratefully acknowledge financial support of FAPESP (Brazil) and also thank Maria Eunice Machado Coelho for revising this manuscript.

References and Notes

(1) Torigoe, K.; Hasegawa, S.; Numata, O.; Yazaki, S.; Matsunaga, M.; Boku, N.; Hiura, M.; Ino, H. Influence of Emission from Rice Straw Burning on Bronchial Asthma in Children. *Pediatr. Int.* **2000**, *42*, 143−150.

(2) Weber, S.; Stubner, S.; Conrad, R. Bacterial Populations Colonizing and Degrading Rice Straw in Anoxic Paddy Soil. *Appl. Environ. Microbiol.* **2001**, *67*, 1318−1327.
(3) Mussatto, S. I. Influência do Tratamento do Hidrolisado Hemicelulósico de Palha de Arroz na Produção de Xilitol por *Candida guilliermondii*. MSc. Dissertation, Faculty of Chemical Engineering of Lorena, Brazil, 2002.
(4) Roberto, I. C.; Mussatto, S. I.; Rodrigues, R. C. L. B. Dilute-Acid Hydrolysis for Optimization of Xylose Recovery from Rice Straw in a Semi-Pilot Reactor. *Ind. Crops Prod.* **2003**, *17*, 171−176.
(5) Silva, C. J. S. M.; Roberto, I. C. Statistical Screening Method for Selection of Importante Variables on Xylitol Byosynthesis from Rice Straw Hydrolysate by *Candida guilliermondii* FTI 20037. *Biotechnol. Tech.* **1999**, *13*, 743−747.
(6) Mussatto, S. I.; Roberto, I. C. Xylitol: A Sweetner with Benefits for Human Health. *Braz. J. Pharm. Sci.* **2002**, *38*, 401−413.
(7) Leathers, T. D. Bioconversion of Maize Residues to Value-Added Coproducts Using Yeast-Like Fungi. *FEMS Yeast Res.* **2003**, *3*, 133−140.
(8) Faria, L. F. F.; Gimenes, M. A. P.; Nobrega, R.; Pereira, N., Jr. Influence of Oxygen Availability on Cell Growth and Xylitol Production by *Candida guilliermondii*. *Appl. Biochem. Biotechnol.* **2002**, *98*−*100*, 449−458.
(9) Parajó, J. C.; Domínguez, H.; Domínguez, J. M. Biotechnological Production of Xylitol. Part 3: Operation in Culture Media Made from Lignocellulose Hydrolysates. *Bioresour. Technol.* **1998**, *66*, 25−40.
(10) Talcott, S. T.; Howard, L. R. Phenolic Autoxidation is Responsible for Color Degradation in Processed Carrot Puree. *J. Agric. Food Chem.* **1999**, *47*, 2109−2115.
(11) Ranatunga, T. D.; Jervis, J.; Helm, R. F.; McMillan, J. D.; Hatzis, C. Identification of Inhibitory Components Toxic Toward *Zymomonas mobilis* CP4 (pZB5) on Xylose Fermentation. *Appl. Biochem. Biotechnol.* **1997**, *67*, 185−198.
(12) Larsson, S.; Reimann, A.; Nilvebrant, N.; Jönsson, L. J. Comparison of Different Methods for the Detoxification of Lignocellulose Hydrolyzates of Spruce. *Appl. Biochem. Biotechnol.* **1999**, *77*−*79*, 91−103.
(13) Palmqvist, E.; Hahn-Hägerdal, B. Fermentation of Lignocellulosic Hydrolysates I: Inhibition and Detoxification. *Bioresour. Technol.* **2000**, *74*, 17−24.
(14) Alves, L. A.; Felipe, M. G. A.; Silva, J. B.; Silva, S. S.; Prata, A. M. R. Pretreatment of Sugar Cane Bagasse Hemicellulose Hydrolysate for Xylitol Production by *Candida guilliermondii*. *Appl. Biochem. Biotechnol.* **1998**, *70*−*72*, 89−98.
(15) Jönsson, L. J.; Palmqvist, E.; Nilvebrant, N. O.; Hahn-Hägerdal, B. Detoxification of Wood Hydrolysates with Laccase and Peroxidase from the White-Rot Fungus *Trametes versicolor*. *Appl. Microbiol. Biotechnol.* **1998**, *49*, 691−697.
(16) Martinez, A.; Rodriguez, M. E.; Wells, M. L.; York, S. W.; Preston, J. F.; Ingram, L. O. Detoxification of Dilute Acid Hydrolysates of Lignocellulose with Lime. *Biotechnol. Prog.* **2001**, *17*, 287−293.
(17) Rodrigues, R. C. L. B.; Felipe, M. G. A.; Almeida e Silva, J. B.; Vitolo, M.; Gómez, P. V. The Influence of pH, Temperature and Hydrolysate Concentration on the Removal of Volatile and Nonvolatile Compounds from Sugarcane Bagasse Hemicellulosic Hydrolysate Treated with Activated Charcoal Before or After Vacuum Evaporation. *Braz. J. Chem. Eng.* **2001**, *18*, 299−311.
(18) Mussatto, S. I.; Roberto, I. C. Hydrolysate Detoxification with Activated Charcoal for Xylitol Production by *Candida guilliermondii*. *Biotechnol. Lett.* **2001**, *23*, 1681−1684.
(19) Barros Neto, B.; Scarmino, I. S.; Bruns, R. E. Planejamento e Otimização de Experimentos. Editora da Universidade Estadual de Campinas. Campinas, **1995**, 299 p.
(20) Parajó, J. C.; Dominguez, H.; Dominguez, J. M. Improved Xylitol Production with *Debaryomyces hansenii* Y-7426 from Raw or Detoxified Wood Hydrolysates. *Enzyme Microb. Tech.* **1997**, *21*, 18−24.
(21) Delgenes, J.; Moletta, R.; Navarro, J. M. Effects of Lignocellulose Degradation Products on Ethanol Fermentations of Glucose and Xylose by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipitis*, and *Candida shehatae*. *Enzyme Microb. Tech.* **1996**, *19*, 220−225.
(22) Roberto, I. C.; Lacis, L. C.; Barbosa, M. F. S.; Mancilha, I. M. Utilization of Sugarcane Bagasse Hemicellulosic Hydrolysate by *Pichia stipitis*, for the Production of Ethanol. *Process Biochem.* **1991**, *26*, 15−21.
(23) Martinez, A.; Rodriguez, M. E.; York, S. W.; Preston, J. F.; Ingram, L. O. Effects of $Ca(OH)_2$ Treatments ("Overliming") on the Composition and Toxicity of Bagasse Hemicellulose Hydrolysates. *Biotechnol. Bioeng.* **2000**, *69*, 526−536.
(24) Felipe, M. G. A.; Mancilha, I. M.; Vitolo, M.; Roberto, I. C.; Silva, S. S.; Rosa, S. A. M. Preparation of Xylitol by Fermentation of a Hydrolysate of Hemicellulose Obtained from Sugarcane Bagasse. *Braz. Arch. Biol. Technol.* **1993**, *36*, 103−114.
(25) Felipe, M. G. A.; Veira, M. V.; Vitolo, M.; Mancilha, I. M.; Roberto, I. C.; Silva, S. S. Effect of Acetic Acid on Xylose Fermentation to Xylitol by *Candida guilliermondii*. *J. Basic Microbiol.* **1995**, *35*, 171−177.
(26) Lee, W. G.; Lee, J. S.; Shin, C. S.; Park, S. C.; Chang, H. N.; Chang, Y. K. Ethanol Production Using Concentrated Oak Wood Hydrolysates and Methods to Detoxify. *Appl. Biochem. Biotechnol.* **1999**, *77*−*79*, 547−559.
(27) Converti, A.; Perego, P.; Domínguez, J. M. Xylitol Production From Hardwood Hemicellulose Hydrolysates by *Pachysolen tannophilus*, *Debaryomyces hansenii* e *Candida guilliermondii*. *Appl. Biochem. Biotechnol.* **1999**, *82*, 141−151.
(28) Fengel, D.; Weneger, G. Utilization of Wood and Wood Components for Chemicals and Energy. In *Wood Chemistry, Ultrastructure, Reactions*; Walter de Gruyter: Berlin, 1989; pp 526−562.
(29) Ahmedna, M.; Marshall, W. E.; Rao, R. M. Production of Granular Activated Carbons from Select Agricultural byproducts and Evaluation of their Physical, Chemical and Adsorption Properties. *Bioresour. Technol.* **2000**, *71*, 113−123.
(30) Ravi, V. P.; Jasra, R. V.; Bhat, T. S. G. Adsorption of Phenol, Cresol Isomers and Benzyl Alcohol from Aqueous Solution on Activated Carbon at 278, 298 and 323 K. *J. Chem. Technol. Biotechnol.* **1998**, *71*, 173−179.
(31) Gurgel, P. V.; Mancillha, I. M.; Peçanha, R. P.; Siqueira, J. F. M. Xylitol Recovery from Fermented Sugarcane Bagasse Hydrolyzate. *Bioresour. Technol.* **1995**, *52*, 219−223.
(32) Zaldivar, J.; Martinez, A, Ingram, L. O. Effect of Alcohol Compounds Found in Hemicellulose Hydrolysate on Growth and Fermentation of Ethanologenic *Escherichia coli*. *Biotechnol. Bioeng.* **2000**, *68*, 524−530.

Accepted for publication September 16, 2003.

BP034207I