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Optimization of Acid Hydrolysis of Sugarcane Bagasse and Investigations on its Fermentability for the Production of Xylitol by *Candida guilliermondii*

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Abstract

The dilute-acid hydrolysis of sugarcane bagasse was optimized using a statistical experimental design resulting in hydrolysates containing 57.25 g/L of xylose, which were fermented with a high inoculum concentration (10 g/L of the yeast *Candida guilliermondii* IM/UFRJ 50088). The addition of urea reduced the time of conversion (t_C) to 75 h (without nitrogen source addition t_C > 127 h), and, consequently, improving the rates of xylitol bioproduction. Fermentator experiments, using the optimized conditions, resulted in enhanced conversion rates, reducing t_C to 30 h. The stability of the yeast in the hydrolysate was also verified in a 480-h cultivation.

Index Entries: Sugarcane bagasse; dilute-acid hydrolysis optimization; sugarcane bagasse hydrolysate; xylitol; bioproduction.

Introduction

Sugarcane bagasse is a lignocellulosic material that constitutes the fibrous residue of sugarcane after undergoing conventional milling. About 50% of this residue are needed to generate heat and power to run the sugar milling process, the remainder is stockpiled. The stockpiled bagasse is of low economic value and constitutes an environmental problem to the sugar mill area and its surroundings, because it can spontaneously burn *(1)*. Lignocellulosic materials, such as bagasse, are an abundant source for fermentation feedstock, because more than 70% of its dry mass consists of carbohydrates. Hemicelluloses are het-

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eropolymers of pentoses and hexoses and can represent 10–40% of the lignocellulosic materials *(2).* From all the dry mass of sugarcane bagasse, 23.3% is xylose, which can be converted to xylitol, a natural carbohydrate with sweetner power similar to sucrose and bearing a lower caloric content than this sugar. This polyalcohol has anticariogenic properties and is insulin-independent when metabolized by humans *(3–6).* Dilute-acid hydrolysis of surplus lignocellulosic materials into fermentation sugar offers enormous opportunity worldwide to add incremental value to farming operations *(7).* Currently, xylitol is produced by catalytic hydrogenation from lignocellulosic hydrolysate, an expensive process mainly because of the number of purification stages required. The bioconversion of xylose to xylitol by yeasts is an attractive alternative, because microbial production of this polyalcohol is a simple process, with great specificity and low energy requirements *(8).* Despite being a cheap raw material for xylitol production, lignocellulosic hydrolysate can possess a considerable amount of toxic components, such as furfural, hydroxymethylfurfural, acetic acid, aromatics, and polyaromatic compounds, which may inhibit the bioconversion process *(9).* In an attempt to circumvent the inhibitory effect of the toxic compounds, several efforts have been made, including the use of high initial biomass into the bioconversion process and the acclimatization of the process biocatalyst. In this work, the dilute-acid hydrolysis of sugarcane bagasse was optimized through the utilization of statistical experimental design. The optimized hydrolysis was used to produce a hydrolysate that was fortified with different nitrogen sources, separately, and submitted to bioconversion with a high initial biomass, with the objective of minimizing the inhibitory effects of the hydrolysates' toxic compounds. Complementary, the ability of the yeast *Candida guilliermondii* IM/UFRJ 50088 to acclimatize to the hydrolysate was investigated, as well as the effect of a gradual decreasing aeration rate, allowing minimum xylitol consumption.

Materials and Methods

Sugarcane Hydrolysis Optimization

The sugarcane bagasse was dried at 60°C and stored at 4°C to avoid contamination. Hydrolysis optimization was assayed in a three-stage statistical experimental design, generated and evaluated with a statistical computational software (Statistica for Windows, version 5.1, Statsoft, Inc., 1995) as shown in Table 1. The first two stages consisted of a 2^4-factorial design with five replications of the center point. The third stage was a 3^2-full factorial design with three replication of the two best results. All hydrolysis experiments were carried out in an autoclave and the liquid fraction was extracted through manual compression of the material.

Table 1
Stages of the Statistical Experimental Design and its Values

		Factor			
Stage	Level	Solid:liquid ratio (g: mL)	H_2SO_4(%)	Pressure (atm)	Time (min)
1st	−1	1:9	3.0	0.5	20
	0	1:8	1.75	1.0	30
	+1	1:7	1.0	1.5	40
2nd	−1	1:8	7.0	0.5	20
	0	1:7	5.0	1.0	30
	+1	1:6	3.0	1.5	40
3rd	−1	—	5.0	0.5	—
	0	—	3.0	1.0	—
	+1	—	1.0	1.5	—

Hydrolysate Treatment

The pH of the hydrolysate was adjusted to 7 by the addition of CaO. The material was vacuum filtrated using filter paper (No. 5) and cellulose acetate membranes (0.45 μm pore size) to remove all the solid fraction. The hydrolysate was supplemented with nitrogen sources, when needed, and sterilized (10 min at 110°C) before its utilization for the bioconversion assays.

Microorganism, Preparation of Medium and Inoculum

Three loopfuls of *C. guilliermondii* IM/UFRJ 50088 cells, maintained at 4°C on semisynthetic agar slants *(10)*, were inoculated into a medium containing: 20 g/L of D-xylose, 1.1 g/L of potassium phosphate, 1.25 g/L of urea, 1.5 g/L of yeast extract, 40.0 mL of mineral salt solution, and citric acid *(11)*. The pH of the medium was adjusted to 6 with 2 N $NaOH_o$. The yeast was grown in 500-mL Erlenmeyer flasks containing 200 mL of medium, previously sterilized (15 min at 110°C), for 36 h at 30°C on a rotary shaker (300 rpm). The grown cells were then used as inoculum for the bioconversion assays.

Nitrogen Source Addition Evaluation

Inorganic and organic nitrogen sources were added to a hydrolysate containing 55.05 g/L of xylose, 19.43 g/L of glucose, and 4.77 g/L of arabinose, obtained with the optimized hydrolysis condition. The nitrogenous compounds were added to the hydrolysate at a sufficient amount to reach approximately 0.93 g/L of nitrogen concentration. The nitrogen sources and their concentrations were: sodium nitrate (5.7 g/L), ammonium sulfate (4.4 g/L), soy peptone (9.7 g/L), meat peptone (7 g/L), meat extract (7.8 g/L), yeast extract (8.1 g/L), tryptose (7 g/L), and urea (2 g/L).

After selecting the best nitrogen source for the bioconversion, four different concentrations of it were added to the hydrolysate medium (1 g/L, 2 g/L, 3 g/L, and 4 g/L).

Acclimatization Assay

After growing for 96 h in the hydrolysate, the yeast was harvested through centrifugation (5000 rpm for 5 min) and aseptically re-inoculated into a new hydrolysate medium. The inoculation in the first hydrolysate medium was carried out with cells obtained from cultivation in synthetic medium.

Bioconversion Assay Conditions

The nitrogen source effect and the acclimatization assays were performed in 125-mL Erlenmeyer flasks containing 50 mL of fortified hydrolysate medium (pH 7), with two replicates of each experiment. All the flasks were closed with polyurethane plugs in a attempt to ensure a uniform aeration rate. The initial biomass was 10 g/L of the yeast and the cultivation was done at 30°C for 127 h in a rotary shaker (150 rpm). Samples were aseptically collected at 24-h intervals.

The effect of aeration was studied in a 1.6-L fermentator (Bioflo III; New Brunswick), containing baffles and two flat blade turbines. The fermentator system was equipped with temperature, pH and DO controllers. The air stream was filtered trough 0.2-μm cellulose acetate membranes. The experiment took place with initial biomass of 10 g/L of the yeast in 1.2 L of urea fortifying (1 g/L) hydrolysate medium at 30°C and pH 6, with mechanical stirring and a varying supply of oxygen according to the $K_L a$ required *(12).* At 12-h intervals the $K_L a$ was modified, beginning with 50 h^{-1} and finishing with 25 h^{-1}. Samples were aseptically collected at 3-h intervals.

Analytical Methods

Xylose, glucose, arabinose, and xylitol were analyzed by high-performance liquid cromatography (Waters) using a Shodex Sugar SC1011 column (300 × 8 mm) at 75°C, and degasified Milli-Q water as mobile phase at a flow rate of 0.8 mL/min. Prior to this, the sample was filtered at a 0.45 μm cellulose acetate membrane, followed by another filtration with SEP-PAK C-18 (Millipore). Cell growth was estimated by turbidimetry at 570 nm. The cell concentration was determined using a standard curve, which correlated absorbance and dry cell weight.

Results and Discussion

Table 2 shows the results of the three stages of the statistical experimental design. The experiments were carried out sequentially, beginning

Table 2
Xylose Concentration Obtained with the Statistical Experimental Design Hydrolysis Experiments

	Xylose (g/L)			
Experiment	First stage	Second stage	Experiment	Third stage
1 (−1,−1,−1,−1)	3.06	23.99	18 (−1,−1)	45.85
2 (+1,−1,−1,−1)	2.84	32.94	19 (0,−1)	44.34
3 (−1,+1,−1,−1)	23.12	28.69	20 (+1,−1)	40.41
4 (+1,+1,−1,−1)	24.77	33.47	21 (−1,0)	43.41
5 (−1,−1,+1,−1)	20.01	37.72	22 (0,0)	57.25 (±1.69)[b]
6 (+1,−1,+1,−1)	22.48	38.63	23 (+1,0)	51.71 (±2.06)[b]
7 (−1,+1,+1,−1)	23.80	37.10	24 (−1,+1)	38.31
8 (+1,+1,+1,−1)	33.55	39.78	25 (0,+1)	46.12
9 (−1,−1,−1,+1)	8.81	28.17	26 (+1,+1)	46.51
10 (+1,−1,−1,+1)	8.37	43.74	—	—
11 (−1,+1,−1,+1)	26.31	30.14	—	—
12 (+1,+1,−1,+1)	32.37	43.37	—	—
13 (−1,−1,+1,+1)	20.91	23.15	—	—
14 (+1,−1,+1,+1)	24.52	29.91	—	—
15 (−1,+1,+1,+1)	23.18	20.61	—	—
16 (+1,+1,+1,+1)	28.73	20.77	—	—
17 (0,0,0,0)	28.02 (±0.55)[a]	34.90 (±1.61)[a]	—	—

[a] Five center point mean and standard error.;
[b] Replicates mean and stantard error.
Variables for the frist and second experiments: Sol:Liq ratio (g:mL), H_2SO_4 (%), pressure (atm), time (min). Variables for the third experiment: H_2SO_4 (%), pressure (atm).

with the experiments of the first stage. The analysis of Fig. 1 reveals that the concentration of H_2SO_4 and pressure are the most important factors positively affecting the dilute-acid hydrolysis. In contrast, the interaction between these two factors negatively affects the hydrolysis. Neureiter *et al. (2)* show that the interaction of these two factors strongly collaborate to furfural yield, leading to a xylose loss. Knowing the effects of H_2SO_4 concentration and pressure, the second stage of statistical experimental design was assayed increasing the levels of acid percentage in the hydrolysis, but maintaining the level of pressure in a safe grade due to autoclave limitations. The solid:liquid ratio was also modified, because the increasing of the level from 1 g:9 mL to 1 g:7 mL generates higher xylose concentration.

Figure 2 shows that the solid:liquid ratio turns out to be a major factor affecting positively the hydrolysis, indicating that levels of acid concentrations greater than 3% are unnecessary to the hydrolysis process when a high solid load is applied. The interaction between pressure and time has the most important effect when acid concentration is between 3% and 7% and solid:liquid ratio is higher than 1 g:6 mL. However, this was an negative effect. The solid:liquid ratio interaction with time promotes a

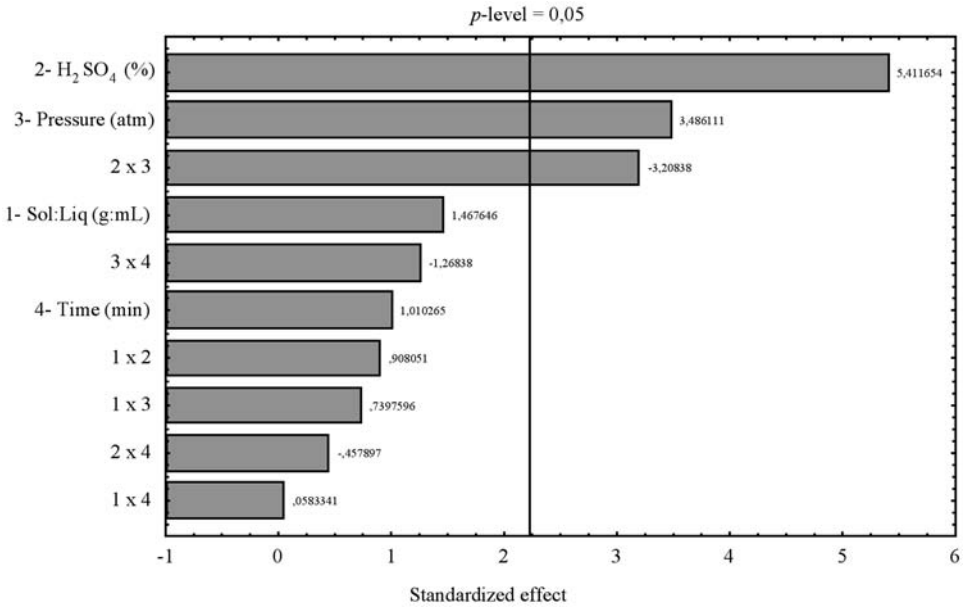

Fig. 1. Standardized Pareto chart for xylose in the first stage of statistical experimental design.

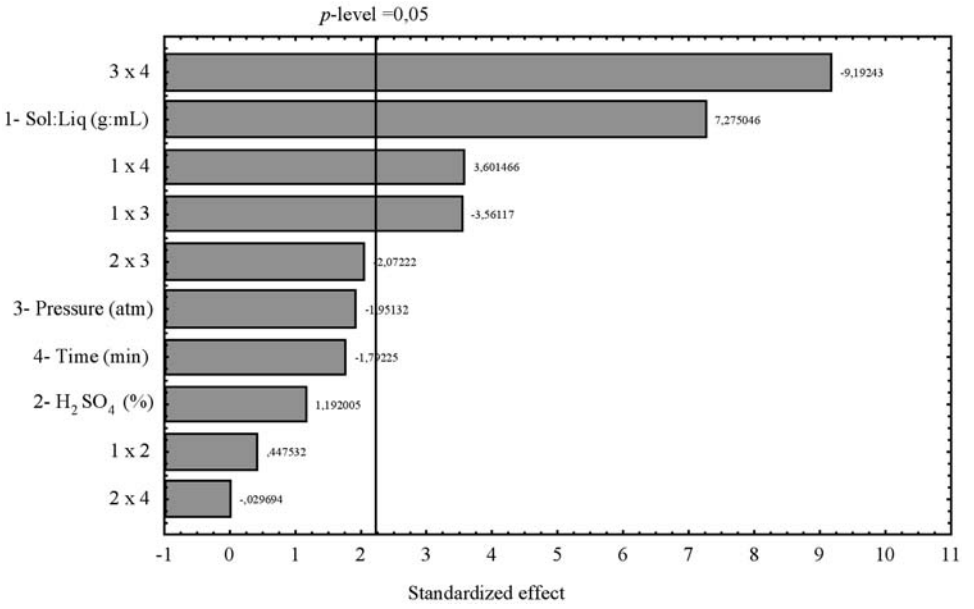

Fig. 2. Standardized Pareto chart for xylose in the second stage of statistical experimental design.

positive effect to the hydrolysis, but its synergism with pressures was negative.

Knowing that acid concentrations higher than 3% should be of no use for the hydrolysis, and that high solid ratio in interaction with time is very

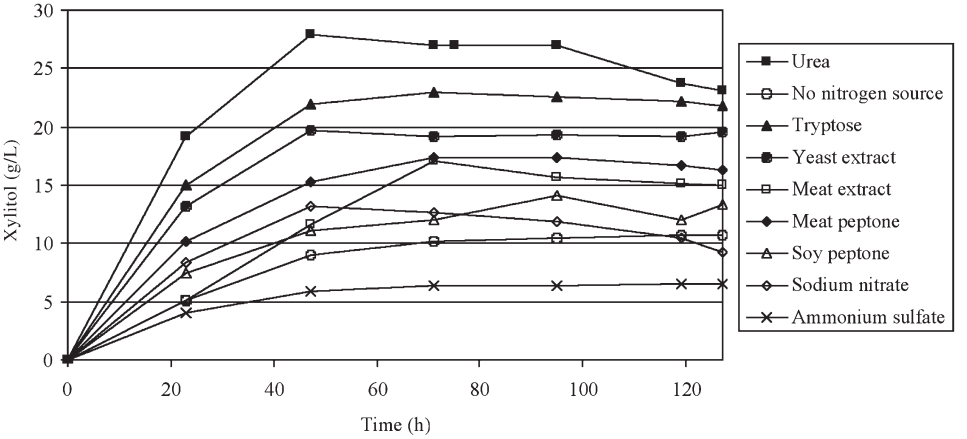

Fig. 3. Xylitol production in nitrogen-enriched hydrolysate.

effective to the xylose extraction, a third stage of experiments was carried out. The solid ratio factor was maintained at the maximum value that can be achieved. Levels higher than 1 g:4 mL are not feasible, because the liquid fraction is not extractable from the hydrolyzed material. Time was set at 40 min, because it shows a positive effect in interaction with high solid: liquid ratios. The acid concentration was maintained in a range of 1–5%, because the best concentration was not met; however, we knew that levels much higher than 3% did not effect the hydrolysis. The pressure was maintained at the initial levels (between 0.5 and 1.5 atm), because this factor showed a positive effect in acid concentrations lower than 3%. The results are show in Table 2. The maximum xylose concentration (57.25 ± 1.69 g/L) was achieved at experiment 5, with 1 g of sugarcane bagasse:4 mL of H_2SO_4 (3%) submitted to 1.0 atm for 40 min.

The bioconversion experiments were carried out using the hydrolysate obtained with the optimized hydrolysis conditions. No sugar concentration was necessary, because the hydrolysate showed to have a relatively high xylose concentration.

Nitrogen sources were added to the hydrolysate medium and bioconversion assays were carried out with high inoculum concentration. Considering that the added nitrogen source was the only supplied, and xylose, glucose, and arabinose the only carbon source in the hydrolysate, the C:N ratio used in the experiments was, approximately, 34.1 g of C:1 g of N. Figure 3 and Table 3 show the results of the addition of nitrogen sources to the hydrolysate media. Urea clearly promoted the best results. With the addition of this nitrogen source, all the xylose was consumed within 75 h. In the other enriched hydrolysates, as in the non-enriched hydrolysate, xylose was detected even after 127 h of cultivation. The maximum xylitol concentration was obtained with the addition of urea, achieving 27.93 g/L after 47 h of cultivation, although, xylitol was consumed thereafter, and its

Table 3
Measured and Calculated Variables from the Xylitol Production in Nitrogen-Enriched Hydrolysate.

NS	X_0 (g/L)	X_f (g/L)	S_0 (g/L)	S_f (g/L)	Xol (g/L)	t_C (h)	$Y_{Xol/S}$ (g/g)	$Y_{Xol/X}$ (g/g)	Q_{Xol} [g/(L⁻h)]	$Y_{X/S}$ (g/g)	Q_X [g/(L⁻h)]
U	10	12.6	55.05	0	27.03	75	0.491	2.145	0.360	0.047	0.035
NE	10	10.4	55.05	15.45	10.76	>127	0.272	1.035	0.085	0.010	0.003
T	10	10.0	55.05	7.42	21.84	>127	0.459	2.184	0.172	0.000	0.000
YE	10	11.1	55.05	12.23	19.52	>127	0.456	1.759	0.154	0.026	0.009
ME	10	11.9	55.05	8.31	15.00	>127	0.321	1.261	0.118	0.041	0.015
MP	10	12.4	55.05	9.27	16.24	>127	0.355	1.310	0.128	0.052	0.019
SP	10	10.5	55.05	34.59	13.24	>127	0.647	1.261	0.104	0.024	0.004
SN	10	12.6	55.05	1.54	9.27	>127	0.173	0.736	0.073	0.049	0.020
AS	10	9.5	55.05	13.32	6.56	>127	0.157	0.691	0.052	0.000	0.000

NS, nitrogen source; U, urea; NE, non-enriched hydrolysate; T, tryptose; YE, yeast extract; ME, meat extract; MP, meat peptone; SP, soy peptone; SN, sodium nitrate; AS, ammonium sulfate.
X_o: Biomass initial concentration, g/L; X_f: Biomass final concentration, g/L; S_0: Xylose initial concentration, g/L; S_f: Xylose final concentration, g/L; Xol: Xylitol concentration, g/L; t_c: time of conversion, h; $Y_{Xol/S}$: Xylitol yield on Xylose consumed, g/g; $Y_{Xol/X}$: Xylitol yield on Biomass, g/g; $Y_{X/S}$: Biomass yield on Xylose consumed, g/g; Q_{Xol}: Xylitol volumetric productivity, g/[L⁻h]; Q_X: Biomass volumetric productivity, g/[L⁻h].

final concentration in the hydrolysate was 27.03 g/L (Table 3). Lu et al. *(13)* obtained similar results in synthetic medium with initial xylose concentration of 114 g/L and 3 g/L of urea, achieving a xylitol yield of 0.88 g/g, xylitol volumetric productivity of 0.46 g/[L⁻h] and time for xylose depletion of 65.5 h.

The effects of urea addition can be explained by the follow considerations. In the urea catabolism, urease cleaves the nitrogen source to allophanate, which was further decomposed to NH^{4+} and CO_2. Allophanate is a natural promoter of most proteins involved in the regulatory complex of nitrogen source consumption. As allophanate is generated, the nitrogen catabolism repression (NCR), an important metabolic phenomenon that regulates the use of nitrogen to the production of several proteins, including those related enzymes and transporters of xylose catabolism, are repressed, allowing the constant consumption of urea. The presence of NH^{4+} in the cell might be repressive to the NCR, affecting the xylitol production. However, urea is a poor nitrogen source, being of slow assimilation, allowing low intracellular levels of ammonia ions. The presence of allophanate and the low NH^{4+} concentration granted the constant consumption of urea, allowing good action of the enzymes involved in xylose catabolism *(14–17).* The negative influence exerted by the addition of ammonium sulfate can be explained through the presence of high intracellular NH^{4+} concentration. Ammonium sulfate is a high-quality nitrogen source, easily and quickly assimilated, resulting in high intracellular levels of ammonia ions, activating the NCR, and, consequently, repressing the xylose metabolism-related proteins. In the non-fortified hydrolysate medium, the natural nitrogen sources (hydrolysate

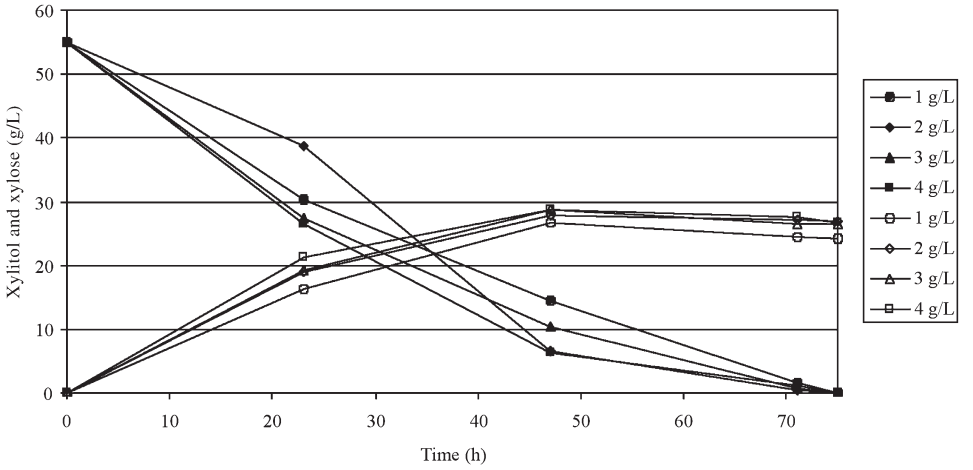

Fig. 4. Xylitol production and xylose consumption in different urea concentrations; solid symbols, xylose; open symbols, xylitol.

Table 4
Measured and Calculated Variables from the Xylitol Production in Different Urea Concentrations

Urea	C:N (gC: 1gN)	X_0 (g/L)	X_f (g/L)	S_0 g/L	S_f (g/L)	Xol (g/L)	$Y_{Xol/S}$ (g/g)	$Y_{Xol/X}$ (g/g)	Q_{Xol} [g/(L h)]	$Y_{X/S}$ (g/g)	Q_X [g/(L h)]
1 g/L	67.4	10	12.9	55.05	0	24.20	0.440	1.876	0.323	0.053	0.039
2 g/L	34.1	10	12.6	55.05	0	27.03	0.491	2.145	0.360	0.047	0.035
3 g/L	22.6	10	12.2	55.05	0	26.40	0.480	2.164	0.352	0.040	0.029
4 g/L	16.7	10	11.8	55.05	0	26.75	0.486	2.267	0.357	0.033	0.024

X_0: Biomass initial concentration, g/L; X_f: Biomass final concentration, g/L; S_0: Xylose initial concentration, g/L; S_f: Xylose final concentration, g/L; Xol: Xylitol concentration, g/L; t_c: time of conversion, h; $Y_{Xol/S}$: Xylitol yield on Xylose consumed, g/g; $Y_{Xol/X}$: Xylitol yield on Biomass, g/g; $Y_{X/S}$: Biomass yield on Xylose consumed, g/g; Q_{Xol}: Xylitol volumetric productivity, g/[L h]; Q_X: Biomass volumetric productivity, g/[L h].

proteins) fulfill the cell demand, without the NCR activation or in a lower level than in the ammonium sulfate containing medium.

The addition of different urea concentrations was assayed and the results are shown in Fig. 4 and Table 4. No significant differences were observed, revealing that only 1 g/L of urea is sufficient to improve the xylitol bioproduction from the sugarcane bagasse hydrolysate. This urea concentration was added to the hydrolysate medium for the fermentator bioconversion assay.

The results of the fermentor experiment are shown in Fig. 5 and Table 5. The maintenance of pH at 6, the optimum value for the xylitol bioproduction by *C. guilliermondii* IM/UFRJ 50088 *(11)*, and a gradually decrease in aeration, in addition to the presence of urea in the hydrolysate, resulted in a great improvement in the xylitol conversion rates. The time of conversion decreased to 30 h, the xylitol volumetric productivity achieved

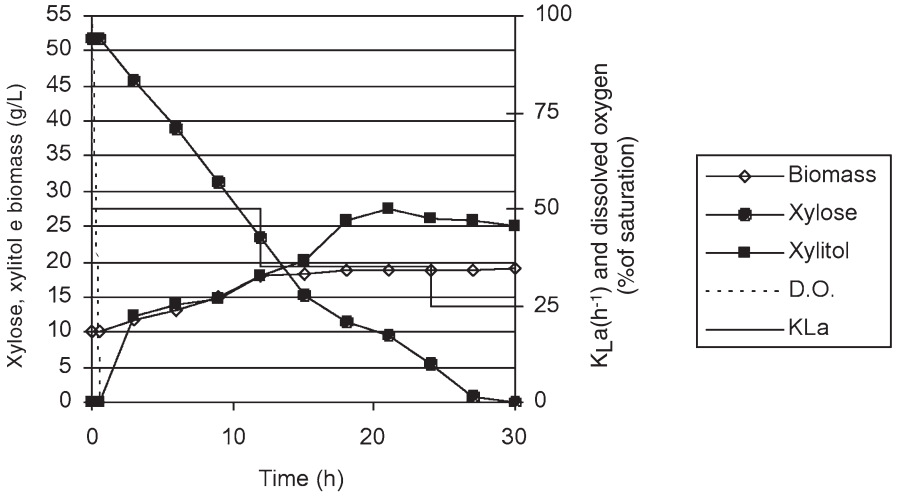

Fig. 5. Fermentator experiment.

Table 5
Measured and Calculated Variables from the Xylitol Production in Fermentator Experiment

Measured variables		Response variables	
X_0	10 g/L	$Y_{Xol/S}$	0.49 g/g
X_f	19 g/L	$Y_{Xol/X}$	1.13 g/g
S_0	51.7 g/L	Q_{Xol}	0.84 g/(L⌉h)
S_f	0 g/L	$Y_{X/S}$	0.30 g/g
t_C	30 h	Q_X	0.30 g/(L⌉h)
Xol	25.1 g/L	Consumed xylose	100%
Maximum xylitol (t_C)	27.5 g/L (21 h)		

X_o: Biomass initial concentration, g/L; X_f: Biomass final concentration, g/L; S_0: Xylose initial concentration, g/L; S_f: Xylose final concentration, g/L; Xol: Xylitol concentration, g/L; t_c: time of conversion, h; $Y_{Xol/S}$: Xylitol yield on Xylose consumed, g/g; $Y_{Xol/X}$: Xylitol yield on Biomass, g/g; $Y_{X/S}$: Biomass yield on Xylose consumed, g/g; Q_{Xol}: Xylitol volumetric productivity, g/[L⌉h]; Q_X: Biomass volumetric productivity, g/[L⌉h].

0.84 g/[L⌉h], and the xylitol yield was 0.49 g/g. The best results were achieved at 21 h of cultivation (xylitol = 27.5 g/L), reaching a volumetric productivity of 1.31 g/[L⌉h] and a xylitol yield of 0.651 g/g. Between 21 and 30 h of cultivation, a small amount of xylitol was consumed. The low oxygen concentration is detrimental to the regeneration of NAD^+ cofactor, which is, on the other hand, essential to the enzyme xylitol dehydrogenase, responsible for the consumption of the polyalcohol. Table 6 shows a comparison between the Erlenmeyer flask experiments and the one carried out in a fermentor.

Table 6
Comparison between Erlenmeyer flasks and Fermentator Experiments

	X_0 (g/L)	X_f (g/L)	S_0 (g/L)	S_f (g/L)	Xol (g/L)	t_C (h)	$Y_{Xol/S}$ (g/g)	$Y_{Xol/X}$ (g/g	Q_{Xol} [g/(L h)]	$Y_{X/S}$ (g/g)	Q_x [g/(L h)]
Erlenmeyer[a]	10	10.4	55.05	15.45	10.76	>127	0.272	1.035	0.085	0.010	0.003
Erlenmeyer[b]	10	12.9	55.05	0	24.20	75	0.440	1.876	0.323	0.053	0.039
Fermentator[b]	10	19.0	51.70	0	25.10	30	0.486	1.130	0.837	0.300	0.300

[a] Non-enriched hydrolysate.
[b] Urea enriched (1 g/L) hydrolysate.
X_o: Biomass initial concentration, g/L; X_f: Biomass final concentration, g/L; S_0: Xylose initial concentration, g/L; S_f: Xylose final concentration, g/L; Xol: Xylitol concentration, g/L; t_c: time of conversion, h; $Y_{Xol/S}$: Xylitol yield on Xylose consumed, g/g; $Y_{Xol/X}$: Xylitol yield on Biomass, g/g; $Y_{X/S}$: Biomass yield on Xylose consumed, g/g; Q_{Xol}: Xylitol volumetric productivity, g/[L h]; Q_X: Biomass volumetric productivity, g/[L h].

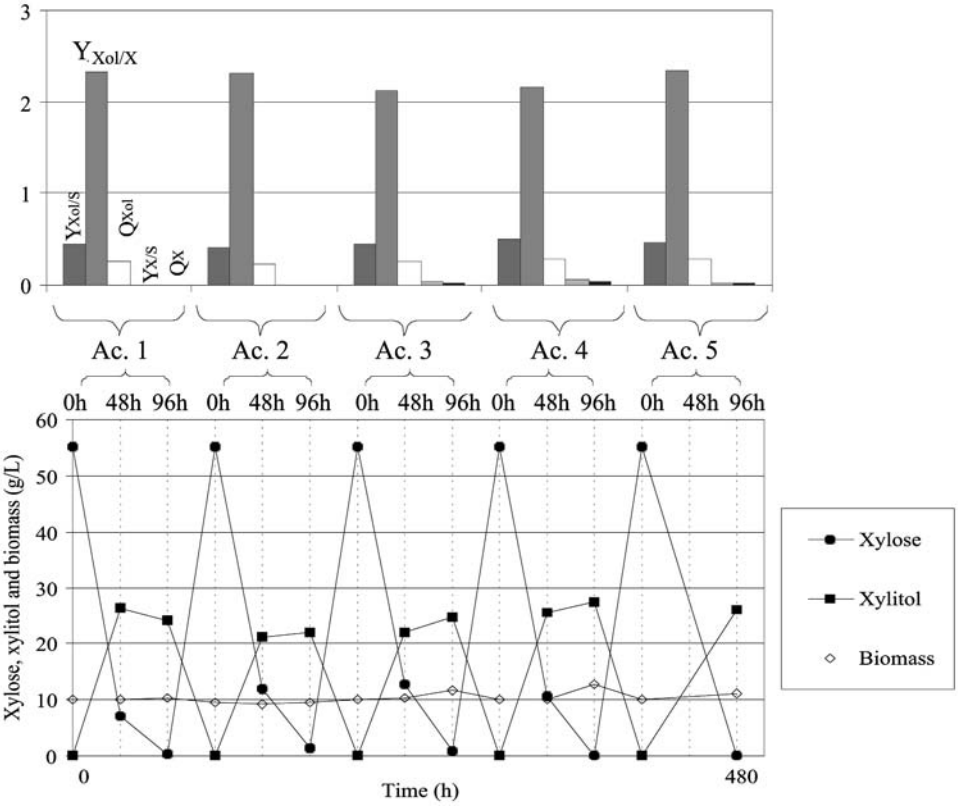

Fig. 6. Stability data in a 480-h cultivation of *C. guilliermondii* IM/UFRJ 50088.

The yeast showed stability when grown in the hydrolysate without addition of nitrogen sources. After 480 h of cultivation, the rates of conversion remained roughly constant (Fig. 6). These data show that only a first inoculum of the yeast *C. guilliermondii* IM/UFRJ 50088, grown in synthetic medium, may be produced, and the cells can be harvested from the fermented hydrolysate medium and re-inoculated in a new hydrolysate medium, at least for 480 h.

Conclusion

The solid:liquid ratio is one of the most important factors to the dilute-acid hydrolysis of sugarcane bagasse. The optimized hydrolysis allows the achievement of hydrolysate-containing media with more than 50 g/L of xylose, suppressing the sugar concentration stage. The xylose concentration of 57.25 (±1.69) g/L are the best result reported at the literature concerning the dilute-acid hydrolysis of sugarcane bagasse, according to our knowledge. The addition of urea to the hydrolysate, combined to a high inoculum concentration strategy, and the maintenance of pH value and aeration control, significantly improved xylitol bioproduction by *C. guilliermondii* IM/UFRJ 50088. The time of conversion lowered from 127 to 30 h, and the rates of the process were enhanced: xylitol yield raised from 0.272 to 0.486 g/g, and xylitol volumetric productivity increased from 0.085 to 0.837 g/[L h]. The addition of urea and the high inoculum concentration made the use of hydrolysate feasible, because the effects of the toxic compounds of the medium were minimized. The yeast *C. guilliermondii* IM/UFRJ 50088 was show to be stable in the presence of toxic compounds at the non-enriched hydrolysate, in 480-h cultivation, allowing cell reutilization for repeated batch processes.

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References

1. Lavarack, B.P., Griffin, G.J., and Rodman, D. (2000), *Catalysis Today* **63,** 257–265.
2. Neureiter, M., Danner, H., Thomasser, C., Saidi, B., and Braun, R. (2002), *Appl. Biochem. Biotechnol.* **98–100,** 49–58.
3. Lee, J. (1997), *J. Biothecnol.* **56,** 1–24.
4. Hÿvonen, L., Koivistoinen, P., and Voirol, F. (1982), *Adv. Food Res.* **28,** 373–403.
5. Scheinin, A., Mäniken, K.K., and Ylitalo, K. (1975), *Acta Odontol. Scand.* **33,** 383–412.
6. Ylikari, R. (1979) *Adv. Food. Res.* **25,** 159–180.
7. Cuzens, J.C. and Miller, J.R. (1997) *Renewable Energy* **10,** 285–290.
8. Barbosa, M.F.S., Medeiros, M.B., Mancilha, I.M., Schneider, H., and Lee, H. (1998), *J. Ind. Microbiol.* **3,** 241–251.
9. Palmqvist, E. and Hahn-Hägerdall, B. (2000), *Bioresour. Technol.* **74,** 17–24.
10. Faria, L.F.F., Gimenez, M.A.P., Nobrega, R., and Pereira N. Jr., (2002) *Appl. Biochem. Biotechnol.* **98–100,** 449–458.
11. Couto, M.A.P.G. (2002), DSc thesis, Post Graduate Program in Chemical and Biochemical Processes Technology, School of Chemistry, UFRJ, Rio de Janeiro, Brazil.
12. Aguiar, W.B., Jr., Faria, L.F.F., Couto, M.A.P.G., Araujo, O.Q.F., and Pereira N. Jr., (2002), *Biochem. Eng. J.* **3608,** 1–11.
13. Lu, J., Tsai, L.B., Gong, C. S., and Tsao, G.T. (1995), *Biothecnol. Lett.* **17,** 167–170.
14. Sosa, E., Aranda, C., Riego, L., et al. (2003), *Biochem. Biophys. Res. Commun.* **310,** 1175–1180.
15. ter Schure, E.G., van Riel, N. A. W., and Verrips, T. (2000), *FEMS Microbiol. Rev.* **24,** 67–83.
16. Messenguy, F. and Dubois, E. (2000), *Food Technol. Biotechnol.* **38,** 277–285.
17. Rousselet, G., Simon, M., Ripoche, P., and Buhler, J. -M. (1995), *FEBS Lett.* **359,** 215–219.