Appl Biochem Biotechnol (2010) 162:1195–1205
DOI 10.1007/s12010-009-8892-5

Xylooligosaccharides Production from Alkali-Pretreated Sugarcane Bagasse Using Xylanases from *Thermoascus aurantiacus*

Michel Brienzo · Walter Carvalho · Adriane M. F. Milagres

Received: 29 August 2009 / Accepted: 14 December 2009 /
Published online: 12 January 2010

Abstract Sugarcane bagasse hemicellulose was isolated in a one-step chemical extraction using hydrogen peroxide in alkaline media. The polysaccharide containing 80.9% xylose and small amounts of L-arabinose, 4-*O*-methyl-D-glucuronic acid and glucose, was hydrolyzed by crude enzymatic extracts from *Thermoascus aurantiacus* at 50 °C. Conditions of enzymatic hydrolysis leading to the best yields of xylose and xylooligosaccharides (DP 2-5) were investigated using substrate concentration in the range 0.5–3.5% (*w*/*v*), enzyme load 40–80 U/g of the substrate, and reaction time from 3 to 96 h, applying a 2^2 factorial design. The maximum conversion to xylooligosaccharides (37.1%) was obtained with 2.6% of substrate and xylanase load of 60 U/g. The predicted maximum yield of xylobiose by a polynomial model was 41.6%. Crude enzymatic extract of *T. aurantiacus* generate from sugarcane bagasse hemicellulose 39% of xylose, 59% of xylobiose, and 2% of other xylooligosaccharides.

Keywords Xylooligosaccharides · *Thermoascus aurantiacus* · Lignocellulose degradation · Biomass · Optimization · Enzyme

Introduction

Xylooligosaccharides (XOS) are sugar oligomers produced during hydrolysis of xylan, the major component of plant hemicellulose. XOS with a low degree of polymerization (DP) have been proven to promote proliferation of bifidobacteria, beneficial microorganisms in human intestine. Demand for this food additive has shown rapid growth over the last two decades [1]. XOS can be prepared by hydrolysis of xylan by endoxylanases (E.C. 3.2.1.8) [2]. The best known endoxylanases have been classified into two glycoside hydrolase families, families10 and 11, based on primary structure comparisons of the catalytic domains. Enzymes belonging to family 10 exhibit greater catalytic versatility and cleave

M. Brienzo · W. Carvalho · A. M. F. Milagres (✉)
Department of Biotechnology, School of Engineering of Lorena, University of São Paulo—USP Estrada Municipal do Campinho, s/n°- CP 116, 12602-810 Lorena, SP, Brazil
e-mail: adriane@debiq.eel.usp.br

glycosidic linkages in the xylan main chain closer to side chain substituents, than endoxylanases of family 11 [3].

The enzymes produced by *Thermoascus aurantiacus* proved to be extremely thermostable [4–6]. Its major endoxylanase belongs to family 10. Catalytic properties of this enzyme proved to be very important for its application [7, 8]. This enzyme requires only two unsubstituted consecutive xylopyranosyl residues to cleave the xylan main chain [9], which is in contrast to xylanases of family 11 requiring three unsubstituted consecutive xylopyranosyl residues [3].

A rich source of xylan decorated with branches of L-arabinose and 4-*O*-methyl-D-glucuronic acid is sugarcane bagasse [10]. Since xylan bound in bagasse is directly not susceptible to enzymatic attack, it has to be extracted before the enzyme hydrolysis. In this work, sugarcane bagasse xylan, obtained by an alkaline hydrogen peroxide extraction, was hydrolyzed to oligosaccharides and xylose by extracellular hemicellulases produced by the thermophylic ascomycete, *T. aurantiacus* ATCC [11–13]. This study focuses on production of low DP XOS from sugarcane hemicellulose by xylanolytic enzymes from *T. aurantiacus*. Specific attention was dedicated to optimization of XOS production, their purification, and preliminary characterization. Furthermore, the efficient use of enzymes depends on process variables such as temperature, pH, reaction time, enzyme concentration, substrate concentration, intensity of agitation, and presence of other chemical species that may inhibit or accelerate their rates of reaction. Consequently, for the enzymatic process be performed with high efficiency, an optimization of the hydrolysis conditions is required. The present work evaluated the effect of enzyme loading and substrate concentration on enzymatic hydrolysis of hemicellulose from sugarcane bagasse. The assays were performed according to a 2^2 full factorial design in an attempt to establish the optimal conditions for maximum xylooligosaccharides yield. The products of hydrolysis of sugarcane hemicellulose were also compared with the action of the enzyme on birchwood xylan and oat spelt xylan. The objective of the present work was to apply statistical methods to optimize the enzymatic hydrolysis for the improvement of XOS production by xylanase of *T. aurantiacus*.

Materials and Methods

Materials

Birchwood xylan, oat spelt xylan, and xylose were obtained from Sigma Chemical-Aldrich, USA. Xylobiose, xylotriose, xylotetraose, and xylopentaose were from Megazyme, Ireland. All other chemicals were analytical grade obtained either from Sigma Company, USA, or Merck, Germany. Sugarcane bagasse was obtained from the São Martinho Company, SP, Brazil. Separation columns Aminex HPX 42A (300×7.8 mm; average particle size: 25 μm) and Aminex HPX 87H (300×7.8 mm; average particle size: 25 μm) were purchased from Bio-Rad Laboratories, CA, USA.

Extraction of Hemicellulose

Hemicellulose was extracted from dewaxed sugarcane bagasse by alkaline hydrogen peroxide and further purified by fractional precipitation with ethanol [10]. A sample of 10 g of dewaxed bagasse was treated with alkaline peroxide (6%) and magnesium sulfate (0.5%) with the pH adjusted to 11.6 with NaOH 2 M in a reaction volume of 200 mL and

incubated in thermostat-controlled water bath (20 °C). After 4 h, the insoluble residue was collected by filtration, washed with distilled water until the pH was neutral, and then dried at 45 °C. The supernatant fluid was adjusted to pH 6 with 6 M HCl and then concentrated to about 1/3 of its volume under air circulation at 45 °C. The concentrated sample was poured into three volumes of 95% ethanol to precipitate hemicellulose that was then washed four times with 70% ethanol. The decanted hemicellulose was dried by air circulation at 45 °C.

Microorganism and Enzyme Production

T. aurantiacus (ATCC 204492) was cultivated in potato–dextrose agar medium for 7 days at 45 °C. Spore suspensions were prepared by adding 10 mL of distilled water to slant cultures whose surfaces were gently scraped with a sterile wire loop. The spores were counted in a Neubauer chamber, and a standardized suspension (10^4 spores/g wheat bran) was inoculated into Erlenmeyer flasks of 300 mL with 15 g of wheat straw with the moisture adjusted to 80% with 2% *v*/*v* Vogel solution. After 4 days of stationary incubation at 45 °C, the content of each flask was washed three times with 100 mL of 50 mM sodium acetate buffer at pH 5.5 under agitation for 1 h at 60 rpm. The combined washings were clarified by filtration. Xylanase activity in the filtrate was determined on 1% (*w*/*v*) birchwood xylan according to the method of Bailey et al. [14]. One unit of activity is defined as the amount of enzyme liberating 1 μmol equivalents of xylose in 1 min. Activity of α-arabinofuranosidase was determined according to Biely et al. [15].

Enzymatic Hydrolysis of Hemicelluloses

Hemicellulose was hydrolyzed by xylanase in 50-mL tubes with the volume made up to 6 mL using acetate buffer (0.05 M, pH 5.0). The effect of time of hydrolysis was determined using hemicellulose (2%) and 60 U/g of xylanase. The tubes were incubated at 150 rpm, and samples were taken at 3, 6, 12, 24, 48, 72, and 96 h of hydrolysis, heated on boiling water bath for 10 min to denature the enzymes, and finally cooled to room temperature. An aliquot was used to determine the amount of arabinose, xylose, and XOS released. To study the effect of enzyme loading, 2% of hemicellulose was hydrolyzed by 10, 20, 40, 80, 120, and 200 U/g of xylanase for 24 h, and the products were analyzed by HPLC.

A 2^2 full factorial design with three replicates at the midpoint was used to evaluate the influence of two different variables: substrate concentration (x_1) and enzyme loading (x_2) in the enzymatic hydrolysis of hemicellulose from sugarcane bagasse. All combinations of the hemicellulose and enzyme concentrations examined (the 2^2 design) are listed in Table 3. The enzymatic reaction was carried out in a shaking water bath maintained at 50 °C for 96 h, and the reaction was stopped by keeping the reaction mixture in a boiling water bath for 10 min. The xylose, xylobiose, and XOS yields were taken as dependent variables or responses of the experimental design. In order to fit an empirical second-order polynomial model, a central composite design was performed. The results were analyzed using the technique of the analysis of variance, and the responses and variables were correlated by the "Response Surface Analysis" of the Statistica 6.0 software.

The feasibility of the regression models was also carried out in 250-mL conical flask containing 50-mL reaction mixture, consisting of 1.3 g hemicellulose and 60 U of xylanase, and the volume was made up to 50 mL using acetate buffer (0.05 M, pH 5.0). The enzymatic reaction was carried out in a shaking water bath maintained at 50 °C for 96 h. At the end of specified incubation time, the reaction was stopped by keeping the reaction mixture in a boiling water bath for 10 min.

Analysis of Products of Hydrolysis of Hemicellulose

The products of hydrolysis were analyzed by HPLC equipped with a refractive index detector using a HPX87H column at 45°C, eluted at the 0.6 mL/min with 0.005 mol/L sulfuric acid for monomeric sugars. The concentration of xylooligomers (XOS) were determined using a HPX42A column at 60°C, eluted at the 0.6 mL/min with deionized water. The chromatographic of the monosaccharides and XOS indicate the percentage distribution sugars in the hydrolysate. The XOS formed was quantified by comparing the peak area of XOS with that of standards (xylobiose, xylotriose, xylotetraose, and xylopentaose) and is expressed as mg/mL of hydrolysate. The XOS yield (*w*/*w*) was determined as xylobiose/hemicellulose weight, since the other XOS were produced in very low amounts.

Hemicellulose (40 mg) was incubated with 4U of pure xylanase of *T. aurantiacus*, endoxylanase family11, endoxylanase family 5, crude xylanase of *T. aurantiacus* incubated for 6, 12, 48, and 96 h. Xylose and XOS were separated by thin-layer chromatography on silicagel in the system ethyl acetate/acetic acid/2-Prop/Formic acid/water (25:10:5:1:15, by vol.). Sugars were localized on dry sheets with the aniline/phthalate reagent.

Uronic Acid Determination

The quantification of uronic acid groups in hemicellulose was performed using the colorimetric uronic acid assay [16] in a test tube on 50–400 nmol of uronic acids or 1 mg of dried pulp sample in 0.4 mL of water. To the sample, 40 μL of 4 mol/L sulfamic acid–potassium sulfamate was added followed by 2.4 mL of concentrate H_2SO_4. When the mixture reached room temperature, 100 μL of the carbazole reagent (0.1% *w*/*v* of carbazole in ethanol) was added. The tube was placed in a boiling water bath for 20 min, followed by cooling in an ice-water bath until room temperature was reached. The light absorbance of the solution at 525 nm was measured.

Results

Effect of Enzyme Loading on the Enzymatic Hydrolysis of Sugarcane Bagasse Hemicellulose

Physical access to xylosic linkage in hemicellulose is restricted by the surrounding lignocellulosic components as well as by the substituents on its backbone. The sugarcane bagasse was pretreated before enzymatic degradation using the modified alkaline extraction method to expose the polysaccharide components to enzymatic hydrolysis [10]. The carbohydrate composition of hemicellulose extracted from sugarcane bagasse was xylose 80.9%, arabinose 3.8%, glucose 4.2%, and uronic acids 3.2%. The content of xylose, arabinose, and uronic acids in isolated bagasse hemicellulose suggests that the ratio Xyl/L-Ara and Xyl/uronic is approximately 20:1. This means that, in average, every tenth xylopyranosyl residue carries a branch which will cause generation of a branched oligosaccharide with most probably three or four xylopyranosyl residues. Following this consideration, one could expect that if *T. aurantiacus* endoxylanase preparation would not contain accessory enzymes, it could produce xylose and unsubstituted xylooligosaccharides, mainly xylobiose, theoretically in 60–70% yield referring to the number of xylopyranosyl residues in the main chain. About 30% of the hemicellulose main chain

would be converted to branched xylooligosaccharides, identity of which could be examined by treatment with pure enzymes, such as α-L-arabinofuranosidase, β-xylosidase, and α-glucuronidase.

This composition suggested that the expected products of enzymatic hydrolysis with an enzyme system, which contains mainly a family 10 endoxylanase, will be predominantly generate xylobiose and xylose. As shown in Table 1, this was actually the case regardless of the enzyme load on the level of enzymatic hemicellulose hydrolysis.

It is well known that the maximum rate of the enzymatic reaction is proportional to the enzyme concentration. This is why determining the optimum concentration of enzyme is very significant for the study of this process. We examined this influence in the concentration interval of 10 to 200 U/g. Through experiments, we established that for the enzyme employed, the optimal concentration was 120 U/g, reaching 31.50% XOS yield. From the results presented in Table 1, it is evident that for enzyme concentration greater than 40 U/g, the rate of hydrolysis decreases. We suppose that this was most likely due to the modification that occurred on substrate structure during reaction with the formation of fragments, which are worse substrates than the polysaccharide. Mode of action of xylanases seems to be influenced by L-arabinofuranosyl and 4-*O*-methyl-D-glucuronic acid substituents linked to hemicellulose [17, 18]. The progress of hydrolysis diminishes if these substituents are not totally removed.

The production of other XOS of higher molecular weight was not significant with any enzyme dose, and the amount of released xylose was also independent of the enzyme load (Table 1). These data proved our assumption for the difficulty for enzyme action during the hydrolysis process on the employed hemicellulose at higher enzyme concentrations (greater than 40 U/g).

Effect of Enzymatic Hydrolysis Time on Sugar Yields

Hydrolysis of sugar cane bagasse hemicellulose was carried out with xylanase (load 60 U/g hemicellulose) for various times. The yields of formed sugars are presented in Table 2. Both xylose and XOS concentration in the hydrolysates were proportional to the time of hydrolysis. Maximum yield was achieved after 96 h. The sum of released xylose and xylobiose reaches the theoretical maximum (60%) only in the case of 96 h long treatment with 60 xylanase U/g of isolated hemicellulose (Table 2). Yang et al. [19] have reported that xylanase of *Thermobifida fusca* produced 3.7 mg/mL of XOS from hemicellulose extracted

Table 1 Influence of the concentration of *T. aurantiacus* xylanase on the level of enzymatic sugarcane bagasse hemicellulose hydrolysis.

Xylanase (U/g)	Monosaccharides and xylooligosaccharide yield (%)					
	Xylose	X2	X3	X4	X5	XOS (mg/mL)
10	17.07	3.77	0.08	–	0.01	0.61
40	9.78	17.53	0.19	–	0.04	2.96
80	14.22	24.82	0.15	–	0.03	3.92
120	17.98	31.50	0.13	–	0.03	4.75
200	16.79	21.34	0.29	–	0.08	3.04

XOS produced after 24-h hydrolysis

X2 xylobiose, *X3* xylotriose, *X4* xylotetraose, *X5* xylopentaose, (–) not detected

Table 2 Time course study of XOS production from sugar cane bagasse hemicellulose using enzymes (60 U xylanase/g of hemicellulose) from *T. aurantiacus*.

Reaction time	Monosaccharides and xylooligosaccharide yield (%)[a]					XOS (mg/mL)
	Xylose	X2	X3	X5	Total XOS	
3	9.34±0.57	13.94±0.94	0.22±0.01	0.05±0	14.21±0.94	2.13
6	9.65±0.71	11.79±1.81	0.20±0	0.05±0	12.04±1.81	1.81
12	10.61±0.73	13.75±0.99	0.19±0.02	0.07±0.01	14.01±1.02	2.10
24	12.97±0.21	21.58±0.67	0.19±0	0.05±0	21.82±0.68	3.27
48	19.42±2.37	28.87±3.01	0.13±0.02	0.05±0.01	29.05±3.04	4.36
72	20.33±1.11	33.01±1.43	0.11±0.01	0.04±0.01	33.17±1.45	4.98
96	22.48±0.77	38.48±0.99	0.08±0	0.02±0	38.58±1.0	5.79

X2 xylobiose, *X3* xylotriose, *X5* xylopentaose

[a] Mean and standard deviations for $n=3$

from bagasse by 48 h of reaction time. The present study has a distinct advantage in terms of the lesser period of reaction for obtaining 3.27 mg/mL in the hydrolysate at 24 h of reaction corresponding to 21.82±0.68% of XOS. The thin-layer chromatography (TLC) confirmed that xylobiose was the major end product yielded. As the reaction time increased, the xylotriose concentration decreased with a simultaneous increase of xylobiose and xylose concentration. These results also confirmed the endo-acting nature of *T. aurantiacus* xylanase (Fig. 2). The percentage of other XOS was higher at the 6 h, indicating the random hydrolysis of xylan during the initial stages of reaction. This primarily results in long chain oligosaccharides, which on further hydrolysis results in the formation of smaller oligosaccharides.

The mixtures were analyzed by TLC to compare the products of hydrolysis. The main difference between the products liberated by endoxylanases of family 10 and 11 concerned the length of the products containing 4-*O*-methyl-D-glucuronic acid. The enzyme of family 10 of *T. aurantiacus* liberated aldouronic acid having chromatographic mobility of aldotetrauronic acid as the shortest acidic xylooligosaccharide (Fig. 1).

The HPLC analysis did not provide sufficient information on branched oligosaccharides (Fig. 2). The oligosaccharide, which elutes as "X5," could be one of such products; however, its content appears much lower that one would be expect in the case of a branched arabinoxylooligosaccharide. The hydrolyzate should contain also some aldouronic acids, presumably the aldotetraouronic acid (MeGlcA)Xyl-Xyl-Xyl, the shortest acidic product of xylanase GH10, which should not be eluted in the volume of neutral sugars [3].

Fitting Model with Response Surface Method

To optimize the XOS production from sugarcane bagasse hemicellulose by enzymatic hydrolysis, a statistical design was used to evaluate the effect of substrate concentration and enzyme loading, as variables. Given the chemical composition of employed hemicellulose, optimal XOS yield averages could be obtained with enzyme dose of 60 U/g and XOS release of 300 μg/g substrate at the central point (38.58%) (Table 3). The xylobiose yield followed the same trend as that of total XOS, reaching the maximum about 38%, which is considerably high in comparison to the literature reports [20]. The yield results are related to XOS concentration; however, similar XOS concentrations gave different yields because

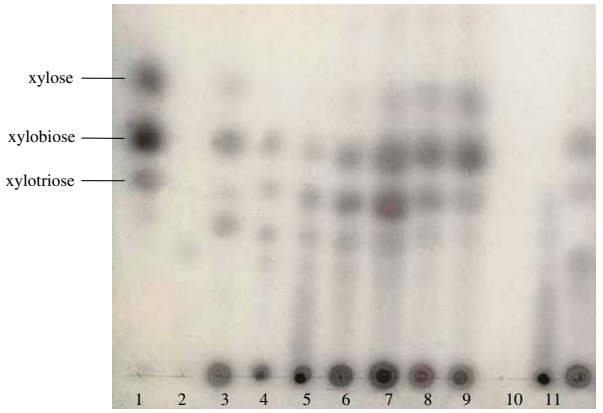

Fig. 1 Time course of hydrolysis of sugarcane bagasse hemicellulose by the enzymatic extract of *T. aurantiacus*. Controls: *1* mixtures containing xylose, xylobiose, and xylotriose, *2* aldohexauronic, *10* sugarcane hemicellulose, *11* pure xylanase of *T. aurantiacus*, *12* mixtures containing xylose, xylobiose, and xylotriose. Hemicellulose (40 mg) was incubated with 4 U of each enzyme (*3* pure xylanase of *T. aurantiacus*, *4* endoxylanase family11, *5* endoxylanase family 5, *6* crude xylanase of *T. aurantiacus* (TA) 6 h, *7* TA 12 h, *8* TA 48 h, *9* TA 96 h), and products were analyzed by TLC

initial hemicellulose content differs among the assays (40–80%). The yield results are useful to compare the enzyme efficiency to hydrolyze hemicellulose, since the production of high content xylobiose depends on substrate is a time consuming and expensive process [21].

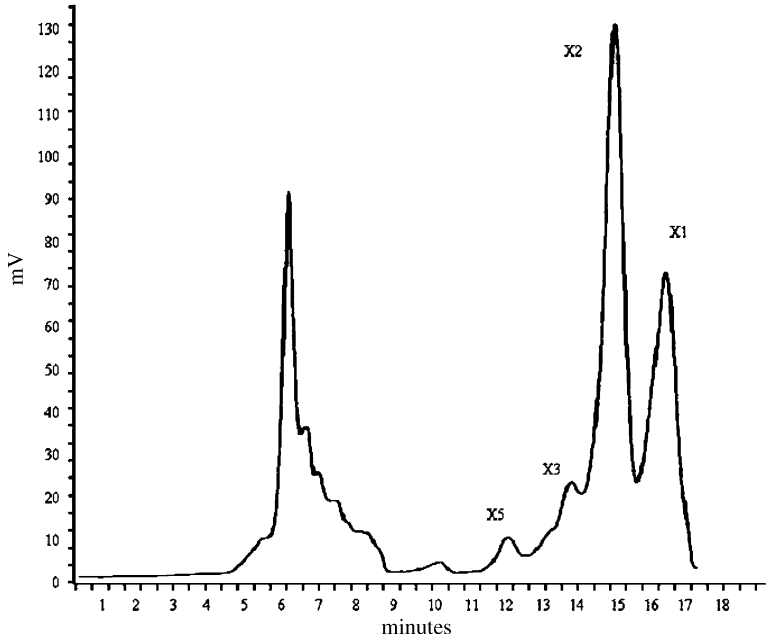

Fig. 2 HPLC analysis of monosaccharides and XOS of sugarcane bagasse hemicellulose by enzymatic extract of *T. aurantiacus*. (Peaks correspond to elution of standards of x_1 xylose, x_2 xylobiose, x_3 xylotriose, and x_5 xylopentaose)

Table 3 Experimental design and the results of the 2^2 full factorial central composite design.

Run	Coded level		Real level		Monosaccharides and xylooligosaccharide yield (%)						XOS (mg/mL)
	x_1	x_2	Substrate (%)	Enzyme (U/g)	Arabinose (%)	Xylose	X2	X3	X5	Total XOS	
1	−1	−1	0.5	40	0	35.98	11.30	0.03	0.01	11.35	0.43
2	−1	1	0.5	80	0	34.91	8.49	0.05	0.01	8.55	0.32
3	1	−1	3.5	40	1.48	13.65	25.65	0.08	0.02	25.76	6.76
4	1	1	3.5	80	2.73	16.83	30.30	0.04	0.01	30.36	7.97
5	0	−1	2	40	2.52	16.61	27.29	0.10	0.03	27.42	4.11
6	0	1	2	80	2.67	28.03	27.04	0.05	0.02	27.11	4.07
7	−1	0	0.5	60	2.89	37.87	11.28	0.12	0.02	11.42	0.43
8	1	0	3.5	60	1.38	17.56	34.97	0.05	0.02	35.04	9.20
9	0	0	2	60	2.25	22.08	38.11	0.08	0.02	38.21	5.73
10	0	0	2	60	1.04	21.98	37.72	0.07	0.02	37.82	5.67
11	0	0	2	60	1.22	23.37	39.60	0.08	0.02	39.70	5.96

Experiments were performed at incubation time of 96 h

x_1 substrate, x_2 enzyme, *X2* xylobiose, *X3* xylotriose, *X5* xylopentaose

Central point experiments from 9 to 11

According to Table 4, only the substrate concentration had a highly significant effect ($p<0.01$) on XOS production, indicating that there is an optimal amount of substrate to be degraded in order to maintain a certain level of XOS production. The statistical significance of main and interaction effects of the variables was determined by analysis of variance. The quadratic model was found to be fitted to describe the relationship between substrate concentration and enzyme dose on XOS yield. The model was expressed by Eq. 1, representing XOS yield (Y_1) with significant variable (x_1 and x_2 coded value).

$$Y_1 = 37 + 9.97x_1 - 11.62{x_1}^2 - 7.59{x_2}^2 \quad (1)$$

The XOS yield values for different concentration of hemicellulose and enzyme activity can be predicted from the response surface and contour plots (Fig. 3).

Table 4 Analysis of variance (ANOVA) parameters to the significant effects on the XOS yield during the enzymatic hydrolysis of sugarcane bagasse hemicellulose.

Factor	SS	DF	MS	*F* value	*p*
Curvature	369.45	1	369.45	652.50	0.0015
x_1	184.43	1	184.43	330.31	0.0030[a]
x_2	0.46	1	0.46	0.82	0.4603
x_1 x_2	7.69	1	7.69	13.80	0.0654
Error	1.12	2	0.56		
Total	563.15	6			

R^2 =0.998

SS sum of squares, *DF* degrees of freedom, *MS* mean square, x_1 substrate, x_2 enzyme

[a] Significant at 95% confidence level

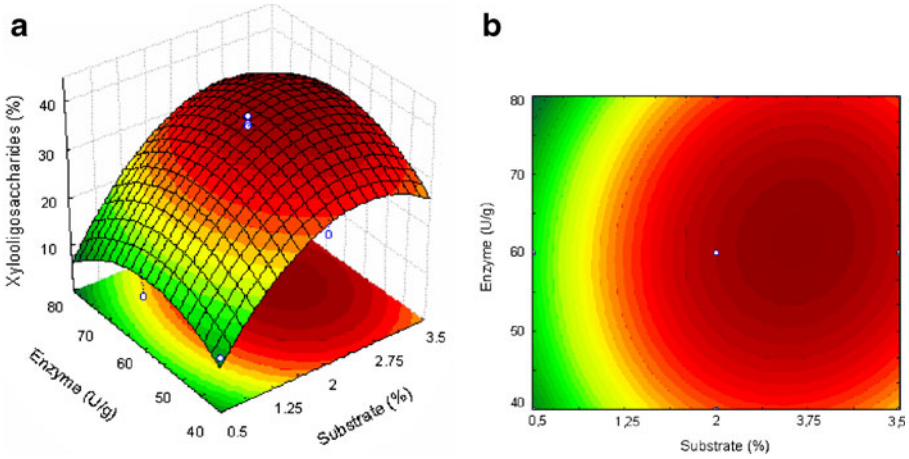

Fig. 3 **a**–**b** Quadratic fit of the effect of enzyme dose and substrate concentration on change in XOs yield

This is a reconfirmation that the fitted surface has a maximum point, which is 2.6% substrate (x_1=0.4) and 60 U/g substrate (x_2=0). The model predicted a maximum response of 41.6±0.45% for this point. To confirm these results, experimental rechecking was performing using a medium representing this maximum point, and a value of 37.1±4.27% was obtained. The good correlation between these two results confirms the validity of the response model and the existence of an optimal point.

The feasibility of the regression models was also carried out in a 250-mL Erlenmeyer under optimized conditions (hemicellulose concentration=2.6% and 60 U/g hemicellulose), which resulted in 24.1±0.2% xylose and 39.4± 0.1% X_2, similar to the results obtained at the previous conditions.

Table 5 Extensive hydrolysis of hemicelluloses (2.6%) at 50°C for 96 h.

	U/g	Monosaccharides and xylooligosaccharide yield (%)					XOS (mg/mL)
		Arabinose	Xylose	X2	X3	X5	
SBH	5	0.60	7.63	14.06	0.20	0.05	2.79
	10	1.01	10.32	20.16	0.20	0.05	3.98
	20	0.68	14.21	27.08	0.17	0.05	5.32
	30	0.31	16.78	32.55	0.13	0.04	6.38
BWX	5	0.07	9.73	15.82	0.11	–	3.11
	10	–	11.89	20.31	0.09	–	3.98
	20	–	14.90	26.71	0.06	–	5.22
	30	–	17.00	35.21	–	–	6.87
OSX	5	–	9.63	9.26	0.14	0.04	1.84
	10	1.51	13.16	14.99	0.15	0.04	2.96
	20	1.05	16.58	21.22	0.14	0.04	4.17
	30	0.49	19.13	25.69	0.12	0.03	5.04

SBH sugarcane bagasse hemicellulose, *BWX* birchwood xylan, *OSX* oat spelt xylan, (–) not detected

Enzymatic Hydrolysis of Different Hemicelluloses

In order to determine the effect of enzyme on substrate, we have done experiments with different hemicelluloses in the range from 5 to 30 U/g. The hydrolysis results of sugarcane bagasse hemicellulose, oat spelts xylans, and birchwood xylan by crude *T. aurantiacus* xylanase are given in Table 5. Sugarcane bagasse hemicellulose and birchwood xylan, which have similar composition, were hydrolyzed to about the same extent, similar as the results reported by Christakopoulos et al. [22]. Xylose and xylobiose were the predominant end products of hydrolysis. Arabinose liberation was also observed in the present experiment, especially from oat spelt xylan, and its level reached maximally 1% of the hemicellulose. This indicates the presence of α-L-rabinofuranosidase in the used xylanolytic system, which was confirmed on 4-nitrophenyl α-L-arabinofuranoside. Under the conditions used for growing *T. aurantiacus*, the quantity of α-arabinofuranosidase obtained was 2.2 U/g after cultivation. Thus, the presence of branch points in the xylan chain seems to prevent the complete hydrolysis of xylans by crude filtrate of *T. aurantiacus.* The effect of enzyme concentration on XOS production was also studied in same range to this work to hydrolysis of alkali pretreated corncob powder [23]. The amount of XOS produced was around 5.9 mg/mL in the lowest enzyme charge, which increased to a value of 10.62 mg/mL at an enzyme concentration of 14 U/mL, and thereafter, the increase was very insignificant with respect to increase in enzyme concentration. At this concentration of endoxylanase activity, the percentage XOS formed was much more superior as that produced by *T. aurantiacus* xylanase (Table 5). Pretreated corncob powder was greatly altered during alkali pretreatment, making it more accessible for enzymatic hydrolysis.

Conclusion

In this study, XOS production was duplicated by optimizing the concentrations substrate and enzyme. Two-level experimental designs and RS methodology were efficient tools in this process. The XOS yield predicted by the model at optimal conditions agreed very well with experimental data, thus confirming the validity of the model. The result obtained in this study is satisfactory, although a superior gain in XOS production will be still necessary to facilitate further work on the purification of the xylobiose. A scale-up of the hydrolysis was carried out in a 250-mL Erlenmeyer flask to reconfirm the maximum xylose and X_2 production of 5.8 mg/mL after 96 h hydrolysis under optimized conditions.

Acknowledgments The authors gratefully acknowledge the financial support of Fapesp and CNPq.

References

1. Vazquez, M. J., Alonso, J. L., Dominguez, H., & Parajo, J. C. (2000). *Trends in Food Science & Technology, 11*, 387–393.
2. Zhu, Y., Kim, T. H., Lee, Y. Y., Chen, R., & Elander, R. T. (2006). *Applied Biochemistry and Biotechnology, 129–132*, 586–598.
3. Biely, P., Vršanská, M., Tenkanen, M., & Kluepfel, D. (1997). *Journal of Biotechnology, 57*, 151–166.
4. Alam, M., Gomes, I., Mohiuddin, G., & Hoq, M. M. (1994). *Enzyme and Microbial Technology, 16*, 298–302.
5. Gomes, I., Gomes, J., Gomes, D. J., & Steiner, W. (2000). *Applied Microbiology and Biotechnology, 53*, 461–468.

6. Yu, E. K. C., Tan, L. U. L., Chan, M. K. H., Deschatelets, L., & Saddler, J. N. (1987). *Enzyme and Microbial Technology, 9*, 16–24.
7. Vardakou, M., Katapodis, P., Samiotaki, M., Kekos, D., Panayotou, G., & Christakopoulos, P. (2003). *International Journal of Biological Macromolecules, 33*, 129–134.
8. Kalogeris, E., Christakopoulos, P., Vrsanska, M., Kekos, D., Biely, P., & Macris, B. J. (2001). *Journal of Molecular Catalysis. B, Enzymatic, 11*, 491–501.
9. Kolenova, K., Vrsanska, M., & Biely, P. (2006). *Journal of Biotechnology, 121*, 338–345.
10. Brienzo, M., Siqueira, A. F., & Milagres, A. M. F. (2009). *Biochemical Engineering Journal, 46*, 199–204.
11. Kalogeris, E., Christakopoulos, P., Katapodis, P., Alexiou, A., Vlachou, S., Kekos, D., et al. (2003). *Process Biochemistry, 38*, 1099–1104.
12. Milagres, A. M. F., Santos, E., Piovan, T., & Roberto, I. C. (2004). *Process Biochemistry, 39*, 1387–1391.
13. Santos, E., Piovan, T., Roberto, I. C., & Milagres, A. M. F. (2003). *Biotechnological Letters, 25*, 13–16.
14. Bailey, M. J., Biely, P., & Poutanen, K. (1992). *Journal of Biotechnology, 23*, 257–270.
15. Biely, P., Mastihubová, M., van Zyl, W. H., & Prior, B. A. (2002). *Analytical Biochemistry, 311*, 68–75.
16. Li, J., Kisara, K., Danielsson, S., Lindstrom, M. E., & Gellerstedt, G. (2007). *Carbohydrate Research, 342*, 1442–1449.
17. Khandke, K. M., Vithayathil, P. J., & Murthy, S. K. (1989). *Archives of Biochemistry and Biophysics, 274*, 511–517.
18. Roche, N., Desgranges, C., & Durand, A. (1994). *Journal of Biotechnology, 38*, 43–50.
19. Yang, C.-H., Yang, S.-F., & Liu, W.-H. (2007). *Journal of Agricultural and Food Chemistry, 55*, 3955–3959.
20. Jiang, Z. Q., Deng, W., Zhu, Y. P., Li, L. T., Sheng, Y. J., & Hayashi, K. (2004). *Journal of Molecular Catalysis B, Enzymatic, 27*, 207–213.
21. Jeong, K. J., Park, I. Y., Kim, M. S., & Kim, S. C. (1998). *Applied Microbiology and Biotechnology, 50*, 113–118.
22. Christakopoulos, P., Katapodis, P., Kalogeris, E., Kekos, D., Macris, B. J., Stamatis, H., et al. (2003). *International Journal of Biological Macromolecules, 31*, 171–175.
23. Aachary, A. A., & Prapulla, S. G. (2009). *Bioresource Technology, 100*, 991–995.