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Xylose production from sugarcane bagasse by surface response methodology

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ABSTRACT

The aim of this study was to optimize the production of xylose from sugarcane bagasse. The hydrolysis process was carried out to evaluate the effect of temperature and sulphuric acid concentration on the xylose production at 18, 30, and 50 min of hydrolysis. A Central Composite Rotatable Design (CCRD) was used to study two process variables, temperature (111.5; 114.0; 120.0; 126.0 and 128.5 °C) and sulphuric acid concentration (0.20; 0.70; 1.90; 3.10 and 3.60% v v^{-1}). Sulphuric acid had a greater influence on the hydrolysis process than temperature. At concentrations of sulphuric acid higher than 3.10% (v v^{-1}), raising temperature had little influence on the yield of xylose production. The best condition to obtain xylose (266.73 mg g^{-1} of dry bagasse) was at 18 min of reaction, at 126 °C and 3.10% (v v^{-1}) of sulphuric acid, representing 96.0% of the theoretical maximum.

Key words: hemicellulose, hydrolysis, HPLC, aldopentose, renewable carbon source

Produção de xilose a partir do bagaço de cana-de-açúcar por metodologia de superfície de resposta

RESUMO

Propôs-se neste trabalho, otimizar a produção de xilose a partir do bagaço de cana-de-açúcar. O processo de hidrólise foi realizado para avaliar o efeito de temperatura e concentração de ácido sulfúrico na produção de xilose, em 18, 30 e 50 min de hidrólise. Um planejamento completo com pontos centrais foi usado para se estudar duas variáveis do processo, temperatura (111,5; 114,0; 120,0; 126,0 e 128,5 °C) e concentração de ácido sulfúrico (0,20; 0,70; 1,90; 3,10 e 3,60% v v^{-1}). O ácido sulfúrico teve maior influência no processo de hidrólise do que a temperatura. Nas concentrações de ácido sulfúrico acima de 3,10% (v v^{-1}), o aumento da temperatura teve pouca influência no rendimento da produção de xilose. A melhor condição para obtenção da xilose (266,73 mg g^{-1} de bagaço seco) foi a 18 min de reação, 126 °C e 3,10% (v v^{-1}) de concentração de ácido sulfúrico, representando 96,0% do máximo teórico.

Palavras-chave: hemicelulose, hidrólise, HPLC, aldopentose, fonte renovável de carbono

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INTRODUCTION

Among several potential sources of biomass, the sugarcane bagasse has been one of the most promising industrial residues obtained from the sugar and alcohol industries (Saska & Ozer, 1994). In recent years, an increasing effort has been made towards a more efficient utilization of renewable agro-industrial residues, including sugarcane bagasse. Lignocellulosics are an abundant and inexpensive source of carbohydrates that can be used to produce high-value chemicals (Carvalho et al., 2002; 2005). Biotechnological production of xylitol could be economically attractive using hemicellulosic hydrolysates as potential substrates, instead of pure xylose, to reduce the cost of production (Gurpilhares et al., 2005; Mussato & Roberto, 2004; Pessoa Júnior et al., 1996). Hemicellulose is a plant cell wall polysaccharide and, in some plants, comprises up to 40% of the total dry material. As hemicellulose is abundant in nature and renewable, extensive research has been undertaken to convert hemicellulose to derived carbohydrates, particularly xylose. In Brazil, the bagasse is a particularly convenient source for carbohydrate conversion, because it is produced in large amounts (5.8×10^7 t in a year). Each ton of milled sugarcane gives 180-280 kg of bagasse residues (Pessoa Júnior et al., 1997). The advantages of using bagasse as a substrate are due to the great amounts of carbohydrates (cellulose and heteroxylan) content, low cost (agricultural and industrial residues), reduction of the environmental pollution and its availability all year in the sugar & alcohol industry (Toit et al., 1984; Zyl et al., 1988; Purchase, 1995; Rodrigues et al., 1998; Rasul & Rudolph, 2000). The sugar cane bagasse is composed of cellulose (35-42%); hemicellulose (30-35%); lignin (18-22%) and others (Purchase, 1995). Additionally, it is possible to hydrolyse hemicellulosic materials by several processes (enzymatic, physical and chemical) for producing monomer sugars with great purity and high yield (Singh & Mishra, 1995; Chong et al., 2004).

The hemicellulosic fraction can be easily hydrolysed by acid treatment. If cellulose and hemicellulose were utilized in an efficient hydrolysis process, the hemicellulose would be completly hydrolyzed to D-xylose (50-70% w w^{-1}) and L-arabinose (5-15% w w^{-1}), and the cellulose would be converted to glucose (Ladish, 1989; Cao et al., 1995; Puls & Schuseil, 1993). An acid hydrolysis under moderate conditions has several advantages, such as: (a) preventing xylose decomposition by furfural (an inhibiting microorganisms agent), (b) selective sub-products production by microorganisms (e.g. xylitol, arabitol), (c) promoting cellulose susceptibility for subsequent acid or enzymatic hydrolysis, and (d) avoiding occurrence of environmental problems due to usage of drastic chemical treatments used (Magee & Kosaric, 1985; Ghosh & Singh, 1993). The two-stage hydrolysis is possible due to the fact that cleavage of hemicellulose fraction is easier than cellulose. Cellulose hydrolysis is slower than hemicellulose, due to the glucose molecule's rigidity, joined in crystalline regions by hydrogen bonds between the hydroxyl groups and the hydrogen atoms of adjacent chains (Pessoa Júnior, 1991). On the other hand, amorphous cellulose and hemicellulose barely interfere in the molecule flexibility, making possible a faster hydrolysis when compared to crystalline cellulose. The resulting fiber (hydrolysed bagasse) can be further processed for the production of cellulose pulp (Caraschi et al., 1996).

As long as D-xylose has been utilized in bioconversion processes as the carbon source, the biotechnological process could lower the production costs by the direct use of hydrolysates under moderate temperatures. In this context, producing D-xylose from sugarcane bagasse contributes to reducing the environmental impact and also to reducing the bioprocess cost. In order to optimize the xylose production by hydrolysing sugarcane bagasse, response surface methodology (RSM) was used to maximize those conditions.

MATERIAL AND METHODS

Material

The sugarcane bagasse was obtained from the sugar industry, Usina Açucareira Ester SA, located in the district of Cosmópolis, SP. The bagasse was washed, dried at 60 °C until constant weight, and triturated in a mill. Soon afterwards, it was separated and selected by two sieves (32-20 mesh).

Hydrolysis of the sugarcane bagasse

Samples of the sugarcane bagasse (3.0 g, dry matter) were placed in 125-mL Erlenmeyer flasks and soaked in a H_2SO_4 solution in a ratio of 1:5 (w v^{-1}), respectively. The samples remained at room temperature overnight. After this period, the samples were hydrolysed in a static retort autoclave (250 L, vertical), and submitted to direct injection of saturated steam for 18, 30 and 50 min. The temperatures and concentrations of H_2SO_4 of the samples varied according to the experimental design (Table 1).

Table 1. Experimental range of the independent variables, with different levels, to study xylose production, during the hydrolysis of sugarcane bagasse

Independent Variable	Symbol	Range and levels				
		-1.414	-1	0	+1	+1.414
Temperature (°C)	A	111.5	114.0	120.0	126.0	128.5
H_2SO_4 (%,v v^{-1})	B	0.20	0.70	1.90	3.10	3.60

During the hydrolysis, the temperature was maintained constant by a system of electronic control. The hydrolysed bagasse was pressed (Carver pilot hydraulic press) to separate the hemicellulosic fraction. Then, the hydrolysate was neutralized with $Ca(OH)_2$ solution (10% w v^{-1}) to pH 5.5, and centrifuged at 2000 g for 15 min. The precipitate was discarded. The water content of the pressed fraction of hydrolysed and the bagasse was determined by drying them in a Fanem oven (model 515) until constant weight.

Xylose determination in the hydrolysate

D-xylose concentration was determined by high perfor-

mance liquid chromatography -HPLC (Shimadzu Co., model LC-10AD), using a refractive index (RI) detector RID-6A. The sugar was separated in a column RT-CH-CA Merck (300 mm x 6.5 mm) at 80 °C, using de-ionized water as eluent (flow = 0.5 mL min^{-1}), previously degassed with helium.

Experimental design and statistical analysis

A full 2^2 factorial design with three replicates at the centre point (Box et al., 1978; Barros Neto et al., 1996) was used to study the effect of the combination of temperature and sulphuric acid (H_2SO_4) concentration on xylose production. The independent variables were established with three levels codified as -1, 0, +1. The corresponding real values appear in Table 1. The centre points predict the experimental error and determine the precision of the polynomial equation.

The experimental data obtained were analyzed for regression and graphical analysis by the software Statistica version 5.0 (Statsoft, USA). The statistical analysis of the second order model equation was performed in the form of the variance (ANOVA). This analysis included the Fischer's F-test, correlation coefficient (R), determination coefficient (R^2), which measures the proportion of variance explained by the results obtained.

RESULTS AND DISCUSSION

The experimental results obtained by hydrolysis for xylose production from sugarcane bagasse are in Table 2. The xylose production increased with prolonging the time of hydrolysis. In the treatment 7 (120 °C and 3.6% of sulphuric acid), the xylose concentration enhanced with the increasing reaction time of the process: at 18 (17.43 mg g^{-1}), 30 (44.75 mg g^{-1}) and 50 min (80.52 mg g^{-1}). At 18 min of the reaction, treatment 4 had the highest value of xylose production (266.73 mg of xylose g^{-1} of dry bagasse), showing a interaction between the independent variables, temperature and H_2SO_4 concentration. This result represents 96% of the theoretical maximum and agrees with Purchase (1995), being superior to those by Toit et al. (1984) and Morjanoff & Gray (1987).

Table 2. The 2^2 full factorial design with codified values and experimental results obtained for xylose production by sugarcane bagasse hydrolysis

Treatment (assay)	Variables		Xylose production (mg g^{-1} of dry bagasse)		
	A	B	18 min	30 min	50 min
1	-1	-1	62.75	151.10	188.09
2	-1	1	254.63	266.90	264.39
3	1	-1	180.03	277.84	264.71
4	1	1	266.73	246.36	258.42
5	-1.414	0	140.32	211.41	253.44
6	1.414	0	260.03	269.21	250.46
7	0	-1.414	17.43	44.75	80.520
8	0	1.414	247.43	264.54	262.06
9	0	0	185.34	258.20	255.66
10	0	0	201.83	242.91	257.69
11	0	0	203.32	247.21	256.00

However, when the hydrolysis time was enhanced (30 and 50 min), the best yields were verified in treatment 3 (277.84 and 264.71 mg of xylose g^{-1} of dry bagasse). It is important to highlight that 18 min of hydrolysis was enough to promote the most important hydrolysis of ligno-cellulose materials, breaking down the bonds b-(1,4) among xylose units from the main chain of the polymeric xylan. Such conditions can be considered moderate if compared to those by other authors (Caraschi et al., 1996; Felipe et al., 1997; Parajó et al., 1998), reducing the time of the hydrolysis process and its cost. This fact can also be observed at treatments 2, 4 and 8, in which increasing the process time did not enhance the xylose production significantly.

Figure 1 shows the linear and quadratic effects and their interaction of the independent variables, at the 95% of confidence level, on the xylose production at 18 min. It can be noted that the sulphuric acid at linear and quadratic levels was significant, however only the linear effect of temperature was significant. The largest main effect observed in the xylose production was the variable sulphuric acid at linear level, followed by temperature (linear level). Moreover, the interactive effects between the independent variables had a negative effect, indicating that increasing the temperature and acid concentration reduces the xylose concentration.

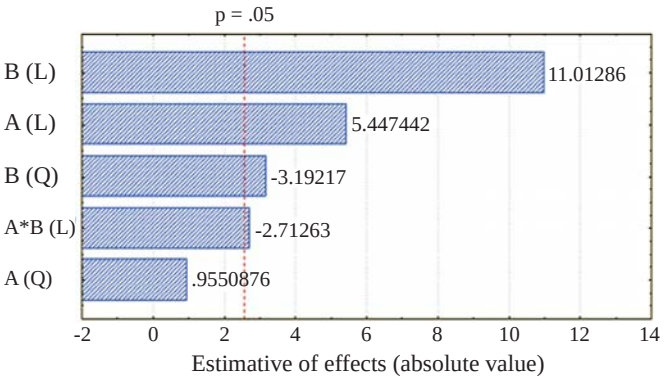

Figure 1. Pareto's chart to estimate the linear (L) and quadratic (Q) effects of the temperature (A) and H_2SO_4 concentration (B) on the xylose production from sugarcane bagasse, at 18 min of hydrolysis

The full quadratic models of the results were tested for adequacy by the analysis of variance (Table 3, 4 and 5). The analysis of variance (ANOVA) for xylose production at the reaction times 18, 30 and 50 min obtained the determination coefficients of 0.9716; 0.8042 and 0.7699, respectively. Although those coefficients (R^2) explain 97.16, 80.49 and 76.99% of the variance for the xylose production, at the respective times, other statistical parameters should be analyzed.

The R^2 for 18 min (Table 3) was highly significant ($p \leq 0.05$) and did not present evidence for lack of fit. Despite the coefficient being significant, the Fmodel should be at least four to five times the value of Ft (Box et al., 1978). Moreover, MS_{Lf}/MS_{Pe} should be lower than the $F_{3,2,}$ showing no lack of fit. This condition was fully satisfied at 18 min, Fmodel (34.21) and $F_{5,5}$ (5.05), indicating that the quadratic

model, at 95% of confidence level, is adjusted to describe the response surface in the studied region. Also, MS_{Lf}/MS_{Pe} (5.67) was lower than the $F_{3,2}$ (19.16). Since the xylose production had a high value of determination coefficient (0.97), and presented a Fmodel much greater than $F_{5,5}$, it was not necessary to adjust the model by ignoring the insignificant effects. That means the quadratic model, at 95% of confidence level, was useful to predict the process and the model could be used to foresee the response for production of xylose in that studied area. A regression analysis was carried out to attain a mathematical model that better described the relation among independent variables (temperature, sulphuric acid) and the studied response (xylose concentration).

Table 3. Analysis of variance for the regression model of xylose production, at 18 min of hydrolysis reaction, obtained from the response surface experiments

Xylose (mg g^{-1}) = 196.83 – 37.33A + 7.85A^2 + 75.48B – 26.02B^2 – 26.29AB R^2 = 0.9716					
Source	**SS**	**df**	**MS**	**Fmodel**	**M_{Lf}/M_{Pe}**
Regression	64804.83	5	12960.97	34.21	5.67
Residual	1894.17	5	378.834		
Lack of fit	1695.03	3	565.01		
Pure error	199.14	2	99.57		
Total	66699.00	10	-		

A – Hydrolysis temperature (°C); B – H_2SO_4 (%, v v^{-1}); R^2 – Determination coefficientSS – Sum of square; df – Degrees of freedom; MS – Mean of square; M_{Lf}/M_{Pe}- MS Lack of fit/MS Pure error; $F_{5,5}$ = 5.05, $F_{3,2}$ = 19.16

The mathematical model that better describes the xylose production, in the region studied, can be represented by the equation:

$$Y = 196.83 - 37.33A + 7.85A^2 + 75.48B - 26.02B^2 - 26.29AB$$

where Y represents the predicted value to xylose concentration (mg g^{-1}), and A, B represent the coded levels of variables temperature and sulphuric acid, respectively. The statistically insignificant term, A^2, was maintained in the model to minimize the error determination. On the other hand, the regressions corresponding to the hydrolysis time at 30 and 50 min were not significant ($p > 0.05$) and the residues presented an evident lack of fit. At 30 min (Table 4), the Fmodel (4.11) was lower than $F_{5,5}$ (5.05), and MS_{Lf}/MS_{Pe} (50.32) was greater than the $F_{3,2}$ (19.16), showing lack of fit. The longer the reaction time, the more inadjusted was the quadratic model.

Table 4. Analysis of variance for the regression model of xylose production, at 30 min of hydrolysis reaction, obtained from the response surface experiments

Xylose (mg g^{-1}) = 249.44 + 23.49A + 4.95A^2 + 49.39B – 37.88B^2 – 36.82AB R^2 = 0.8042					
Source	**SS**	**df**	**MS**	**Fmodel**	**M_{Lf}/M_{Pe}**
Regression	39059.93	5	7811.99	4.11	50.32
Residual	9511.5	5	1902.3		
Lack of fit	9387.15	3	3129.05		
Pure error	124.35	2	62.18		
Total	48571.43	10	-		

A – Hydrolysis temperature (°C); B – H_2SO_4 (%, v v^{-1}); R^2 – Determination coefficient; SS – Sum of square; df – Degrees of freedom; MS – Mean of square; M_{Lf}/M_{Pe} – MS Lack of fit/MS Pure error; $F_{5,5}$ = 5.05, $F_{3,2}$ = 19.16

At 50 min (Table 5), the Fmodel (3.15) was lower than $F_{5,5}$ (5.05), and MS_{Lf}/MS_{Pe} (2017.86) was a slightly greater than the $F_{3,2}$ (19.16), showing lack of fit. The analysis of variance demonstrated that this model is not adequate for describing the relationships between the response under study and the experimental factors. The regression coefficients at 30 and 50 min were not significant ($p > 0.05$) and the residues presented evident lack of fit, especially to the regression analysis at 50 min (Fmodel = 3.36 and $F_{5,5}$ = 5.05). Consequently, those conditions of hydrolysis were not adequate to predict the results in that studied area.

Table 5. Analysis of variance for the regression model of xylose production, at 50 min of hydrolysis reaction, obtained from the response surface experiments

Xylose (mg g^{-1}) = 256.45 + 8.30A + 5.82A^2 + 40.84B – 34.51B^2 – 20.0AB R^2 = 0.7699					
Source	**SS**	**df**	**MS**	**Fmodel**	**M_{Lf}/M_{Pe}**
Regression	22518.78	5	4503.75	3.15	2017.86
Residual	7145.6	5	1429.12		
Lack of fit	7143.24	3	2381.08		
Pure error	2.36	2	1.18		
Total	31049.76	10	-		

A – Hydrolysis temperature (°C); B – H_2SO_4 (%, v v^{-1}); R^2 – Determination coefficient; SS – Sum of square; df – Degrees of freedom; MS – Mean of square; M_{Lf}/M_{Pe} – MS Lack of fit/MS Pure error; $F_{5,5}$ = 5.05, $F_{3,2}$ = 19.16

The diagram of the response surface (Figure 2) indicates that xylose concentration is a function of the independent variables (temperature and sulphuric acid concentration) at

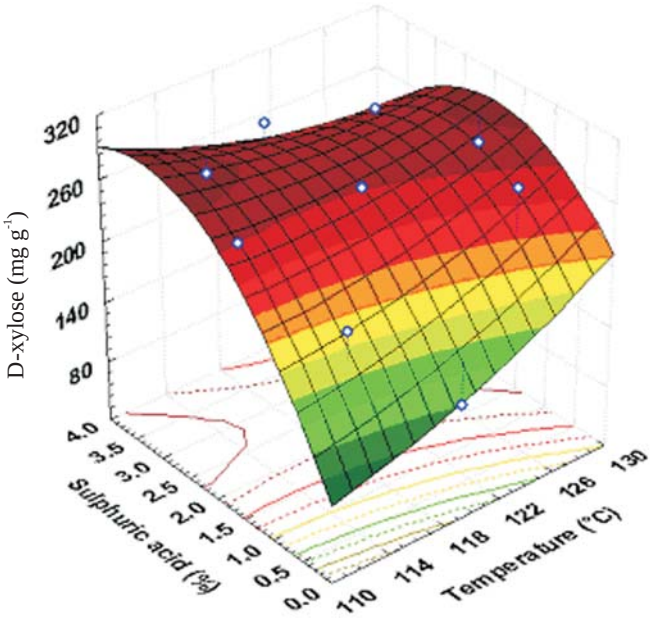

Figure 2. Response surface regarding the temperature effects and concentration of the sulphuric acid (H_2SO_4) on the xylose production from sugarcane bagasse at 18 min of hydrolysis

18 min of hydrolysis. Raising the temperature and concentration of the acid, the xylose concentration was increased. Nevertheless, at that time, the range was limited and demanded high temperature and high acid concentration. The effect of H_2SO_4 had more influence on xylose production than the temperature (Figure 3). Figure 3 shows that at concentrations higher than 3.10% (v v^{-1}) of sulphuric acid, raising temperature did not increase xylose production significantly. This fact is in accordance with Pessoa Júnior (1991), who observed that the temperature lessened its importance gradually with the increasing time of reaction, and it had little influence on recovering reducing sugars during sugarcane bagasse hydrolysis. This hydrolysis condition was used on further works to produce xylitol, using xylose from sugarcane bagasse (Paiva, 2002).

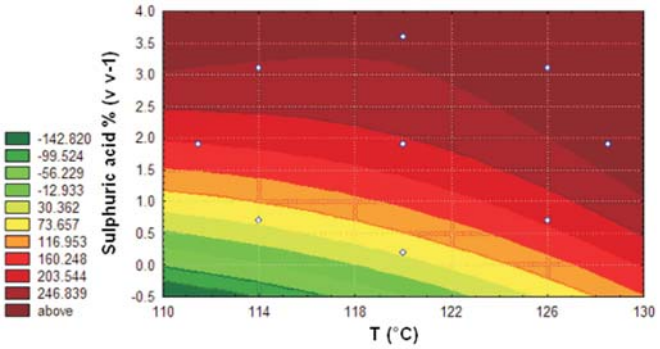

Figure 3. Contour curve of temperature (T) and sulphuric acid (H_2SO_4) concentrations on the xylose production from sugarcane bagasse at 18 min of hydrolysis

Conclusions

1. Sugarcane bagasse may be used to produce xylose as a carbon source for biotechnological process, instead of using commercial xylose.

2. Hemicellulosic hydrolysates obtained from industrial residues are environmentally interesting, and also can reduce the process cost, because they add high value to the product.

3. The combination of two independent variables (temperature and sulphuric acid) to hydrolyse sugarcane bagasse for xylose production showed itself to be effective in the results determined.

4. At concentrations of sulphuric acid higher than 3.10% (v v^{-1}), raising temperature had little influence on yield of xylose production.

5. The best condition observed to produce xylose (266.73 mg g^{-1}) from the bagasse, taking in account the process cost, was at 18 min of time reaction, at 126 °C and 3.10% (v v^{-1}) of sulphuric acid. This result represents 96.0% of the theoretical maximum.

Acknowledgements

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