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Dilute-Acid Hydrolysis of Sugarcane Bagasse at Varying Conditions

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Abstract

Sugarcane bagasse, a byproduct of the cane sugar industry, is an abundant source of hemicellulose that could be hydrolyzed to yield a fermentation feedstock for the production of fuel ethanol and chemicals. The effects of sulfuric acid concentration, temperature, time, and dry matter concentration on hemicellulose hydrolysis were studied with a 20-L batch hydrolysis reactor using a statistical experimental design. Even at less severe conditions considerable amounts (>29%) of the hemicellulose fraction could be extracted. The percentage of soluble oligosaccharides becomes very low in experiments with high yields in monosaccharides, which indicates that the cellulose fraction is only slightly affected. For the sugar yields, acid concentration appears to be the most important parameter, while for the formation of sugar degradation products, temperature shows the highest impact. It could be demonstrated that the dry matter concentration in the reaction slurry has a negative effect on the xylose yield that can be compensated by higher concentrations of sulfuric acid owing to a positive interaction between acid concentration and dry matter contents.

Index Entries: Sugarcane bagasse; hemicellulose; dilute-acid hydrolysis.

Introduction

Lignocellulosic material is an abundant source for fermentation feedstock because more than 70% of the dry mass consists of carbohydrates. The major part is cellulose, a β1→4 glucan, which can be converted into glucose, the most common carbon source in fermentations. Hemicelluloses are heteropolymers of pentoses and hexoses and make up 10–40% of

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lignocellulosic dry matter *(1)*. This fraction can be converted to monomeric sugars at temperatures below 200°C and low acid concentrations. Such conditions have also been described as pretreatment methods for enzymatic hydrolysis of cellulose *(2–4)*.

While most industrial fermentation processes are based mainly on hexoses, the use of hemicellulose as a source for carbohydrates has become a topic of interest over the past few years. However, most of the microorganisms used in traditional processes are not able to ferment the pentose sugars that are set free during hemicellulose hydrolysis. To overcome this obstacle, a number of strains have been developed that are able to ferment pentoses to valuable chemical products such as ethanol, organic acids, solvents, and xylitol *(5–8)*.

Sugarcane bagasse is the solid byproduct from sugar refining based on sugarcane. Currently, it is mainly used as a boiler fuel in order to provide energy for sugar mills. Possible biotechnologic applications of bagasse have been reviewed recently *(9)*. The hemicellulose contents is approx 25% and consists mainly of xylan. The aim of the present study was to optimize the extraction of hemicellulosic sugars from sugarcane bagasse in the monomeric form. The described process is carried out in a batch reactor and yields a liquid hydrolysate that contains the hemicellulose fraction and can be used for fermentation. The solid residue consists mainly of cellulose and lignin and either could be further refined or used as a fuel. It is known that during the hydrolysis of polysaccharides, the formation of the sugar degradation products furfural and 5-hydroxymethylfurfural (5-HMF) is taking place simultaneously. The kinetics of these reactions have been described by different models evaluated by Jacobsen and Wyman *(10)*. Degradation products do not only lower the yield on sugar monomers but also act as fermentation inhibitors *(11)*. To produce fermentable hydrolysates and to prevent high losses in yields, it is therefore necessary to choose reaction conditions that keep the generation of degradation products at a low level.

In the present study, the influence of acid concentration, temperature, reaction time, and dry matter contents of the reaction slurry on the yield of hemicellulosic sugars from sugarcane bagasse and the formation of furfural and 5-HMF were investigated using a statistical experimental design.

Materials and Methods

Sugarcane Bagasse

Dry sugarcane bagasse was provided by Kaset Thai Sugar and had a dry matter contents of 90%. Table 1 gives the composition.

Analytical Methods

The total hydrolysis of the carbohydrates and Klason lignin was determined according to a modified TAPPI standard method *(12,13)*. The determination of extractives was omitted, and the time for pressure cooking was

Table 1
Composition of Sugarcane Bagasse

Component	Quantity (g/100 g dry matter)
Glucan	40.19 ± 0.16
Xylan	22.54 ± 0.71
Galactan	1.40 ± 0.11
Arabinan	2.00 ± 0.15
Mannan	0.48 ± 0.11
Klason Lignin	25.15 ± 0.76

reduced to 20 min because the recovery of monosaccharides appeared to be higher at this reaction time. For the determination of soluble oligosaccharides, hydrolysate samples were subjected to a posthydrolysis for 20 min at 120°C and an acid concentration of 2.4% (w/w).

Sugars and sugar degradation products were analyzed by high-performance liquid chromatography (HPLC) using a Macherey-Nagel ET 300/7.8 Nucleogel SUGAR Pb Column and water as solvent, while a Merck Polyspher OA KC RT 300-700 Column with 0.01 *N* H_2SO_4 was used for sugars and organic acids. Both HPLC systems operated with a flux of 0.4 mL/min and refractive index detection.

Hydrolysis Experiments

Hydrolysis experiments were carried out in a 20-L batch hydrolysis reactor. The basic reactor is a cylindrical stainless steel tube with a 95-cm length and a 16.5-cm id. The heating is provided by electrical heating elements arranged around the reactor and surrounded by insulation. Temperature is measured with a Pt 100 inside the reactor, and pressure can be read from a manometer. It is possible to open the reactor on both ends for cleaning and filling purposes. On one end, a ball valve with 5-cm id is fixed. The reactor was filled with 0.45 kg of bagasse dry matter and the required amount of acid. Mixing of the reactor contents was provided by tumbling the reactor using an electric motor. The average time required to reach the reaction temperature was 11 min. The experiment was terminated by opening a ball valve to allow the reactor contents to expand into a 100-L container which was filled with 12 L of water to cool down the reaction mixture to a temperature below 70°C and to avoid losses owing to evaporation. The remaining contents in the reactor was washed out with 5 L of water, and this solution was mixed with the rest of the broth. The liquid phase was separated by pressing through a filter cloth, which left a solid press cake with approx 25% dry matter substance. Yields for sugars were calculated using the following formula:

$$Y = 100 \cdot \frac{c \cdot V}{M}$$

inwhich c is the concentration of the component in the liquid phase (g/L), V is the volume of the liquid phase (including the liquid bound to the press cake) (L), and M is the amount of bagasse dry matter in the experiment (g). The yield Y describes the amount of the substance in grams that can be achieved from 100 g of bagasse dry matter after a full extraction. This value seems to be more suitable for the comparison of the different experiments than the actual sugar concentrations, because experiments with high dry matter usually give hydrolysates with higher sugar concentrations although the actual yields may be low.

Experimental Design

Experiments were carried out according to a statistical experimental design that was created and evaluated with Statgraphics® 5.0. Four factors (acid concentration, temperature, reaction time, bagasse dry matter) were tested on two levels in an orthogonal 2^4 central composite design with 12 center points and 8 star points. The 36 experiments were carried out in fully randomized run order. The conditions of the experiments are listed together with the results in Table 2. This design allows estimation of the main effects and two factor interactions using analysis of variance (ANOVA). The star points are an additional set of points that make it possible to calculate a nonlinear response surface. In this case, the response surface is calculated using a quadratic polynomic model of the following form:

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \alpha_4 x_4 + \alpha_{12} x_1 x_2 + \alpha_{13} x_1 x_3 + \alpha_{14} x_1 x_4 + \alpha_{23} x_2 x_3 + \alpha_{24} x_2 x_4 + \alpha_{32} x_3 x_4 + \alpha_{11} x_1^2 + \alpha_{22} x_2^2 + \alpha_{33} x_3^2 + \alpha_{44} x_4^2 + \varepsilon$$

where y is the yield, x is the value of the respective effect, α is the coefficient, and ε is the random error. The subscripts 1, 2, 3, and 4 refer to acid, temperature, time, and dry matter.

Results and Discussion

The yields for the monosaccharides, 5-HMF, furfural, and acetic acid are shown in Table 2. These values were used to calculate the effects of the different parameters. The significance of the effects can be determined by ANOVA. The effects and their significance can be visualized in the standardized Pareto charts (Figs.1 and 2). Xylose and furfural were selected as examples because they are the most important sugar and the main degradation product. Acid concentration showed the highest effect on the xylose yield, followed by dry matter and time. For the formation of furfural, the temperature appeared to be most important, followed by acid concentration and time. It is notable that the dry matter contents had a negative effect for xylose as well as for the formation of furfural. However, its impact on the formation of furfural was much lower than on xylose yield. Figures 3–6 show response surfaces for xylose and furfural, which were calculated using the nonlinear model described in Material and Methods. From these graphs, it is obvious that the influence of dry matter concentration

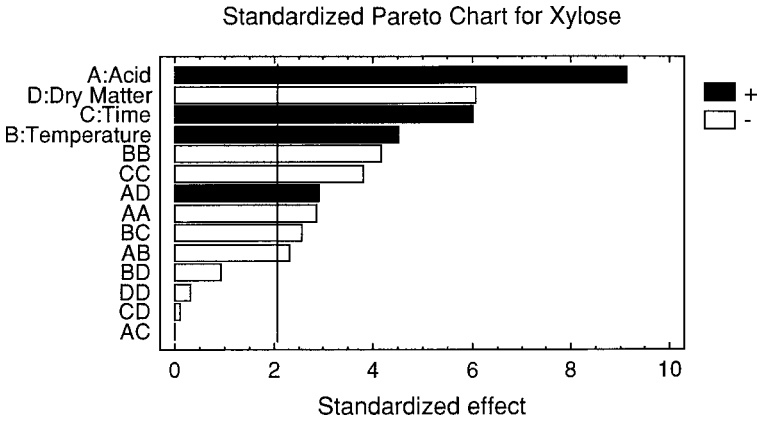

Fig. 1. Standardized Pareto chart for yields of xylose. Standardized effects are calculated by dividing the effect by its standard error. ■, Positive effects; □, negative effects. Effects below the line at 2.08 are not significant at the 95% confidence level. AA, BB, CC, and DD are quadratic effects. If these are significant, the respective effect is nonlinear.

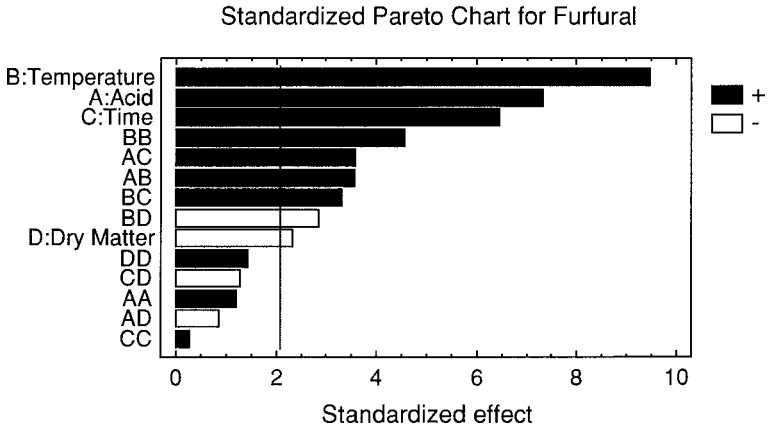

Fig. 2. Standardized Pareto chart for furfural.

on xylose yield was also dependent on acid concentration. Because there was a positive interaction between acid concentration and dry matter (Fig. 1), the negative effect was compensated at higher acid concentrations, depending also on temperature and time. Unlike in autohydrolysis processes *(14)*, in acid-catalyzed systems the sugar yields seem to be highly dependent on the dry matter contents in the reaction slurry in connection with the acid concentration. According to the model, maximum xylose yields of 22.95 g/100 g could be reached at 0.045 mol/L of sulfuric acid, 159°C, 17-min reaction time, and 20% dry matter. The same yield could be reached with 0.025 mol/L of acid, 177°C, 13-min reaction time, and 4% dry matter. The expected furfural yields, however, were 1.69 for the high-acid, high-dry matter conditions, and 2.47 for the low-acid, low-dry-matter conditions, respectively.

Table 2
Experimental Conditions and Yield of Monosaccharides (g/100 g of Dry Bagasse)[a]

Run	Acid (mol/L)	T (°C)	t (min)	DM (%)	Glucose (g/100 g DM)	Xylose (g/100 g DM)	Galactose (g/100 g DM)	Arabinose (g/100 g DM)	Mannose (g/100 g DM)	HMF (g/100 g DM)	Furfural (g/100 g DM)	Acetic Acid (g/100 g DM)	Soluble oligo-saccharides (g/100 g DM)
11	0.015	160	5	8	0.01 (1.40)	3.57 (11.96)	0.38 (0.86)	1.07 (1.56)	0.11 (1.04)	0.02 (0.06)	0.04 (0.53)	0.00	12.50
26	0.015	160	5	16	0.02 (1.05)	0.26 (5.13)	0.11 (0.56)	0.67 (1.05)	0.08 (0.23)	0.02 (0.05)	0.06 (0.38)	0.15	7.43
13	0.015	160	15	8	0.28 (1.76)	10.12 (15.96)	0.53 (1.04)	1.11 (1.66)	0.16 (0.96)	0.02 (0.07)	0.18 (0.83)	0.86	10.26
33	0.015	160	15	16	0.06 (1.23)	1.30 (10.49)	0.11 (0.65)	0.57 (1.09)	0.06 (0.39)	0.03 (0.00)	0.13 (0.89)	0.25	12.93
28	0.015	180	5	8	0.58 (1.25)	13.35 (8.74)	0.34 (0.58)	0.73 (1.11)	0.17 (2.58)	0.03 (0.04)	0.35 (0.93)	1.55	0.02
25	0.015	180	5	16	0.08 (1.37)	3.19 (12.73)	0.29 (0.73)	0.79 (1.23)	0.16 (0.33)	0.03 (0.05)	0.21 (0.75)	0.52	12.77
1	0.015	180	15	8	1.55 (2.19)	19.53 (20.92)	0.26 (0.97)	0.74 (1.25)	0.41 (0.60)	0.33 (0.14)	1.26 (2.82)	1.72	5.64
14	0.015	180	15	16	0.23 (1.57)	7.95 (15.54)	0.43 (0.94)	0.93 (1.36)	0.22 (0.79)	0.07 (0.04)	0.89 (1.39)	0.89	1.20
32	0.035	160	5	8	0.37 (1.01)	10.43 (5.55)	0.25 (0.67)	0.63 (0.97)	0.16 (2.60)	0.01 (0.04)	0.14 (0.94)	0.73	0.27
31	0.035	160	5	16	0.14 (0.98)	6.83 (6.27)	0.26 (0.62)	0.64 (0.95)	0.12 (2.34)	0.02 (0.00)	0.22 (0.90)	0.59	4.23
30	0.035	160	15	8	1.82 (1.48)	20.27 (9.08)	0.85 (0.76)	1.03 (1.16)	0.36 (3.13)	0.09 (0.03)	0.73 (1.49)	2.37	–7.62
19	0.035	160	15	16	2.13 (2.38)	19.87 (18.38)	0.86 (1.03)	1.19 (1.55)	0.35 (0.58)	0.11 (0.06)	1.39 (2.12)	2.65	0.60
6	0.035	180	5	8	2.23 (2.28)	18.69 (14.18)	0.81 (0.59)	0.85 (0.84)	0.40 (1.71)	0.07 (0.10)	1.30 (1.77)	2.11	–2.59
8	0.035	180	5	16	1.36 (1.94)	15.77 (16.14)	0.36 (0.84)	0.82 (1.14)	0.26 (0.95)	0.07 (0.10)	1.06 (1.86)	1.65	3.75
10	0.035	180	15	8	4.07 (4.39)	15.02 (14.40)	0.82 (0.83)	1.21 (1.20)	0.50 (0.37)	0.26 (0.22)	4.52 (2.85)	3.29	–3.16
22	0.035	180	15	16	2.01 (2.36)	15.09 (14.58)	0.47 (0.84)	0.96 (1.24)	0.40 (0.46)	0.10 (0.12)	2.40 (2.93)	2.50	1.41
Starpoints Acid:													
35	0.005	170	10	12	0.02 (1.00)	0.23 (6.07)	0.06 (0.62)	0.51 (0.88)	0.12 (0.39)	0.01 (0.00)	0.07 (0.67)	0.28	8.94
17	0.045	170	10	12	2.21 (2.45)	20.90 (19.02)	0.49 (1.07)	1.20 (1.59)	0.33 (0.64)	0.11 (0.12)	1.43 (2.22)	2.72	0.91
Starpoints Temperature:													
4	0.025	150	10	12	0.08 (1.24)	4.56 (9.41)	0.26 (0.61)	0.59 (0.77)	0.18 (1.86)	0.01 (0.04)	0.10 (0.69)	0.45	9.21
27	0.025	190	10	12	2.11 (1.77)	12.29 (6.22)	0.37 (0.53)	0.79 (0.85)	0.46 (1.99)	0.21 (0.14)	2.91 (2.60)	2.22	–5.22

[a]Values in parentheses indicate the results after posthydrolysis of the liquid phase. DM, dry matter.

Table 2
Experimental Conditions and Yield of Monosaccharides (g/100 g of Dry Bagasse)[a]

Run	Acid (mol/L)	T (°C)	t (min)	DM (%)	Glucose (g/100 g DM)	Xylose (g/100 g DM)	Galactose (g/100 g DM)	Arabinose (g/100 g DM)	Mannose (g/100 g DM)	HMF (g/100 g DM)	Furfural (g/100 g DM)	Acetic Acid (g/100 g DM)	Soluble oligo-saccharides (g/100 g DM)
Starpoints Time:													
36	0.025	170	0	12	0.04 (1.19)	1.13 (7.68)	0.08 (0.61)	0.45 (0.98)	0.16 (0.37)	0.01 (0.03)	0.07 (0.72)	0.13	10.03
24	0.025	170	20	12	1.43 (2.12)	16.92 (17.29)	0.75 (0.95)	1.03 (1.43)	0.31 (0.48)	0.27 (0.08)	1.01 (1.79)	1.94	2.78
Starpoints Dry Matter:													
23	0.025	170	10	4	2.50 (2.59)	21.90 (19.36)	1.00 (0.96)	1.26 (1.58)	0.47 (0.58)	0.35 (0.09)	1.16 (1.98)	3.28	–1.15
18	0.025	170	10	20	0.18 (1.46)	7.66 (15.61)	0.39 (0.90)	0.89 (1.38)	0.17 (0.48)	0.06 (0.03)	0.44 (1.18)	0.93	11.67
Center Points:													
2	0.025	170	10	12	1.07 (1.99)	17.45 (20.75)	0.29 (0.99)	0.67 (1.28)	0.28 (0.55)	0.05 (0.13)	0.57 (1.89)	1.42	7.99
3	0.025	170	10	12	0.84 (1.68)	16.11 (15.40)	0.24 (0.84)	0.64 (1.05)	0.23 (2.08)	0.04 (0.08)	0.49 (1.29)	1.14	4.31
5	0.025	170	10	12	0.71 (1.57)	15.38 (14.52)	0.29 (0.66)	0.73 (0.88)	0.23 (1.77)	0.02 (0.05)	0.44 (1.25)	1.00	3.38
7	0.025	170	10	12	0.78 (1.60)	15.91 (15.63)	0.33 (0.96)	0.80 (1.26)	0.21 (1.88)	0.04 (0.05)	0.50 (1.26)	1.47	4.53
9	0.025	170	10	12	0.77 (1.89)	15.44 (20.05)	0.36 (1.15)	1.01 (1.59)	0.21 (0.19)	0.06 (0.09)	0.50 (0.74)	1.29	7.50
12	0.025	170	10	12	0.78 (1.90)	14.64 (15.85)	0.42 (1.04)	1.04 (1.58)	0.18 (1.99)	0.03 (0.04)	0.49 (0.78)	1.19	5.78
15	0.025	170	10	12	0.90 (1.91)	15.66 (19.01)	0.67 (1.11)	1.08 (1.68)	0.19 (0.58)	0.10 (0.00)	0.57 (1.39)	1.56	6.94
16	0.025	170	10	12	0.97 (1.95)	15.91 (18.93)	0.60 (1.11)	1.03 (1.65)	0.17 (0.52)	0.05 (0.05)	0.55 (1.47)	1.53	6.94
20	0.025	170	10	12	0.81 (1.95)	15.12 (18.01)	0.47 (1.02)	1.07 (1.60)	0.20 (0.56)	0.07 (0.08)	0.48 (1.36)	1.50	6.89
21	0.025	170	10	12	0.83 (1.91)	15.70 (18.72)	0.49 (0.98)	0.95 (1.56)	0.17 (0.40)	0.04 (0.03)	0.51 (1.29)	1.36	6.67
29	0.025	170	10	12	0.77 (1.41)	15.51 (9.79)	0.48 (0.77)	0.80 (1.20)	0.22 (3.09)	0.04 (0.00)	0.49 (1.24)	1.17	–0.39
34	0.025	170	10	12	0.70 (1.73)	15.56 (17.94)	0.38 (0.89)	0.63 (1.28)	0.19 (0.56)	0.03 (0.00)	0.45 (1.63)	1.16	6.77

[a]Values in parentheses indicate the results after posthydrolysis of the liquid phase. DM, dry matter.

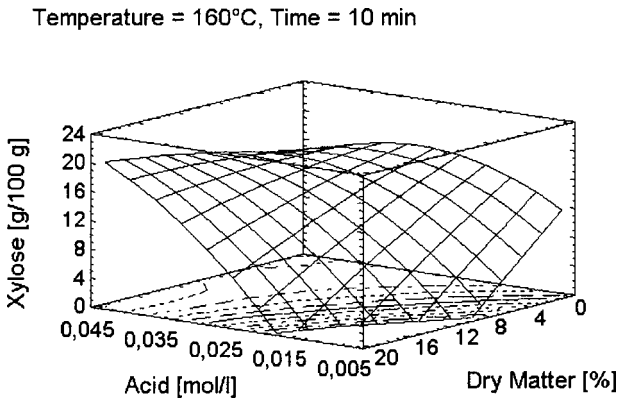

Fig. 3. Estimated response surface for xylose showing influence of acid concentration and dry matter at $T = 160°C$ and $t = 10$ min. Dotted lines are lines of equal yields. The adjusted correlation R^2 for the calculated model is 86.44%.

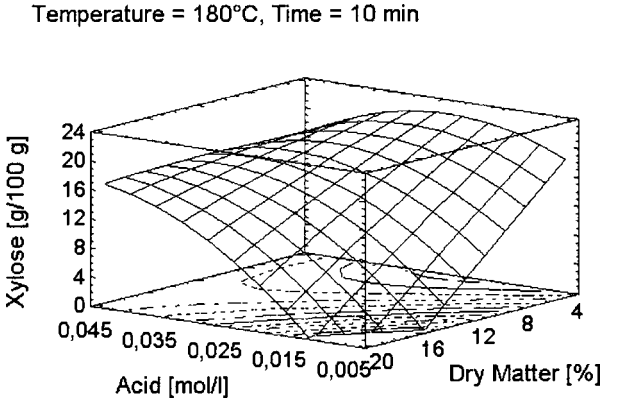

Fig. 4. Estimated response surface for xylose showing influence of acid concentration and dry matter at $T = 180°C$ and $t = 10$ min.

Since the interaction between acid concentration and dry matter was not significant for furfural (Fig. 2), losses owing to the formation of furfural formation are always lower at higher dry matter concentrations, if the other parameters are left constant. Higher dry matter concentrations usually also mean that higher amounts of raw material can be processed, which is an important aspect for industrial applications. From this point of view, it seems to be favorable to use processes operating with high acid concentrations and high dry matter. That temperature and reaction time also have a strong impact and show interactions still has to be considered. As a consequence, hydrolysis at high acid concentrations requires lower temperatures and longer reaction times in order to avoid excessive formation of degradation products and to obtain reasonable amounts of sugar. Thus, the advantages of high material input may be compensated by longer reaction times. An optimization will therefore have to consider all effects and is also dependent on the equipment.

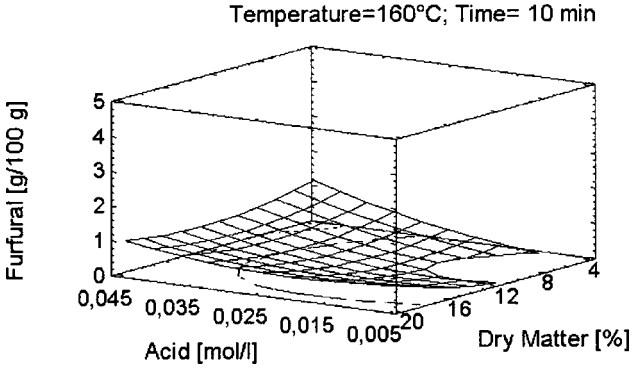

Fig. 5. Estimated response surface for furfural showing influence of acid concentration and dry matter at $T = 160°C$ and $t = 10$ min. Dotted lines are lines of equal yields. The adjusted correlation R^2 for the calculated model is 87.55%.

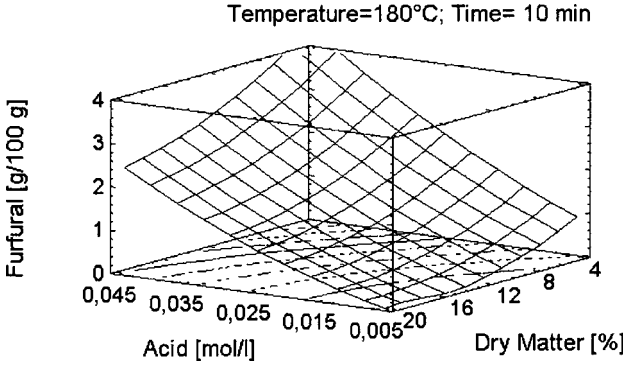

Fig. 6. Estimated response surface for furfural showing influence of acid concentration and dry matter at $T = 180°C$ and $t = 10$ min.

The values in Table 2 in parentheses represent the yields after posthydrolysis including the sugars that are dissolved as oligosaccharides. Comparison of these values with those before posthydrolysis reveals that some of them are lower. This is especially the case for hydrolysates with already high sugar yields and can be explained by the formation of furfural and 5-HMF from the monosaccharides. However, the values of these degradation products are in many cases lower than expected. This phenomenon has been observed previously and could be owing to polymerization of furfural and 5-HMF *(15)*. The soluble oligosaccharides are calculated by subtracting the sum of monosaccharides and degraded sugars before posthydrolysis from their amount after posthydrolysis. Apparently, at less severe conditions, considerable amounts of sugars are obtained as soluble oligosaccharides, while in experiments with high yields, this fraction represents only a very small part. This means that a satisfactory hydrolysis of bagasse hemicellulose to monosaccharides without excessive formation of degradation products can be achieved in a single-step reaction within the boundaries of the selected experimental design.

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