

1 Acid hydrolysis of wheat straw: A kinetic study

2
3 Esther Guerra-Rodríguez^a, Oscar M. Portilla-Rivera^b, Lorenzo Jarquín-Enríquez^b, Jose A. Ramírez^c, Manuel
4 Vázquez^{a,*}

5
6 ^aFood Science Area, Department of Analytical Chemistry, Faculty of Veterinary Science, University of Santiago
7 de Compostela (Campus Lugo), 27002 Lugo, Spain

8 ^bAgroindustrial engineering, Universidad Politécnica de Guanajuato, Cortazar. Gto. CP 38483, México

9 ^cDepartment of Food Science and Technology, U. A. M. Reynosa-Aztlán, Universidad Autónoma de
10 Tamaulipas, Apdo. Postal 1015, Reynosa, Tamaulipas, 88700 México

11
12
13
14 *Corresponding Author. Tel: +34-982-285900 ext 22420; Fax: +34-982-241835.

15 *E-mail addresses:* manuel.vazquezm@usc.es

16 17 18 **ABSTRACT**

19
20 Biotechnological xylitol production can be enhanced if the needed xylose solutions can be obtained
21 from hydrolysis of low-cost lignocellulosic wastes. The hydrolysis of wheat straw to obtain xylose solutions has
22 a double consequence, the elimination of a waste and the generation of a value-added product. The objective of
23 this work was to study the xylose production from wheat straw by sulphuric acid hydrolysis at 130°C. Several
24 acid concentrations (1, 2, 3, 4 or 5%) were evaluated. Kinetic models were developed to explain the variation
25 with time of xylose, glucose, arabinose, furfural, 5-(hydroxymethyl)furfural and acetic acid in the hydrolysates.
26 Optimal conditions found were 2% H₂SO₄ at 130°C for 29 min, which yielded a solution with xylose, 18.9 g/L;
27 glucose, 3.5 g/L; arabinose, 3.1 g/L; furfural, 0.6 g/L; HFM, 0.3 g/L and acetic acid, 2.3 g/L. In these conditions,
28 99% of the hemicelluloses and 11% of the glucon were hydrolysed.

29
30 *Keywords:* acid hydrolysis; straw; wheat; HMF; furfural; xylose; glucose; lignocellulosic wastes

31

32 **Nomenclature**

33

34 [] concentration

35 α_x ratio for the xylan fractions in raw material (g of susceptible xylan/ g of total xylan).

36 α_G ratio for the glucan fractions in raw material (g of susceptible glucan/ g of total glucan).

37 α_A ratio for the glucan fractions in raw material (g of susceptible araban/ g of total araban).

38 α_F ratio for the furfural fractions in raw material (g of susceptible furfural/ g of total furfural).

39 α_H ratio for the 5-hydroxymetil-2-furaldehyde fractions in raw material (g of susceptible 5-hydroxymetil-2-furaldehyde / g of total 5-hydroxymetil-2-furaldehyde).

41 α_{Ac} ratio for the acetic acid fractions in raw material (g of susceptible acetic acid/ g of total acetic acid).

43 a regression parameter

44 A arabinose concentration (g/L)

45 A_0 initial arabinose concentration (g/L)

46 A_n araban concentration (g/L)

47 Acn_0 potential acetic acid concentration (g/L)

48 AcH acetic acid concentration (g/L)

49 C acid concentration (%)

50 CX_{n_0} initial composition of xylan

51 F furfural concentration (g/L)

52 F_0 potential concentration of furfural (g/L)

53 G glucose concentration (g/L)

54 G_0 initial glucose concentration (g/L)

55 G_n glucan concentration (g/L)

56 HMF 5-hydroxymetil-2-furaldehyde

57 k_1 rate of the generation reaction (min^{-1})

58 k_2 rate of the decomposition reaction (min^{-1})

59 M monomer concentration (g/L)

60 M_0 initial monomer concentration (g/L l)

61 n regression parameter

62 P polymer concentration (g/L)

63 P_0 initial polymer concentration (g/L)

64 t time (min)

65 WSR water / solid ratio

66

67

68

69 1. Introduction

70

71 Wheat straw, a waste in the wheat seed production, is an abundant, renewable and low-cost
72 lignocellulosic material, which is commonly used as feedstuffs. The structure of wheat straw is composed of
73 cellulose, hemicellulose, and lignin. Cellulose is a crystalline glucose polymer and hemicellulose is a complex
74 amorphous polymer. The most abundant building block of hemicellulose is xylan, a xylose polymer. Cellulose
75 consists of a linear polysaccharide. Cellulose is a crystalline material while hemicellulose is amorphous because
76 of its branched nature. Hemicellulose is relatively easy to hydrolyze to its monomer sugars compared to
77 cellulose. Therefore the hydrolysis of wheat straw could be a good alternative for the xylose production [1].

78 Xylose can be used as a carbon and energy source in fermentation processes. Among others, the
79 bioconversion from xylose to xylitol is of interest as it is a polyol with important applications as a sweetener.
80 Xylitol has important advantages over glucose or saccharose, such as anticarcinogenicity, low caloric value and
81 negative heat of dissolution [2-4]. Economic interest in xylitol production by fermentation can be enhanced if
82 the required xylose solutions can be obtained from the hydrolysis of low-cost lignocellulosic wastes. The
83 hydrolysis of wheat straw to obtain xylose solutions has a double consequence, the elimination of a waste and
84 the production of a value-added product that enhances the economics of the process [5].

85 Dilute acids can be used as catalysts of a partial hydrolysis called prehydrolysis. This is a hydrolysis of
86 the hemicellulosic fraction. The cellulose and lignin fractions remain almost unaltered and they can be used for
87 further processing. H_2SO_4 [6-7], HCl [8], HF [9], H_3PO_4 [10], HNO_3 [11] or CH_3COOH [12] are acids
88 commonly employed as catalysts, being H_2SO_4 the most used.

89 The above acids release protons that break the heterocyclic ether bonds between the sugar monomers in
90 the polymeric chains formed by the hemicelluloses and the cellulose. The breaking of these bonds releases
91 several compounds, mainly sugars such as xylose, glucose and arabinose. Other compounds released are
92 oligomers, furfural and acetic acid. A quantitative hydrolysis of the hemicelluloses can be performed almost
93 without damage to the cellulose because the bonds in hemicelluloses are weaker than in cellulose. Therefore, a
94 solid waste formed by cellulose and lignin is obtained in the prehydrolysis of wheat straw. This by-product can
95 be use for the production of glucose solutions to be used in the production of lactic acid or ethanol by
96 fermentation or for the production of paper pulp [13].

97 Few studies dealing with the acid hydrolysis of wheat straw exists. Acetic acid was used to pretreat and
98 fractionate wheat straw. A 25% acetic acid solution at 160°C could removed all xylan and 26.2% of lignin in the
99 wheat straw [12]. Sulphuric acid was used for the hydrolysis of wheat straw at 121°C as a pretreatment for the
100 enzymatic hydrolysis of the cellulose fraction [14] for the bioethanol production [15,17].

101 The hydrolysates obtained in the prehydrolysis of wheat straw can be used after neutralization for
102 conversion to xylitol [4] or single cell protein [18] due to the high xylose content. However, furan derivatives
103 such as furfural and 5-hydroxymethyl-furfural (HMF), as well as aliphatic acids such as acetic acid are
104 inhibitory compounds present in lignocellulose hydrolysates. For the fermentation process, the presence of these
105 inhibitory compounds in the hydrolysates can hinder or prevent a subsequent fermentation step. Therefore,
106 hydrolysates with low concentrations of inhibitors are required.

107 This work deals with the acid hydrolysis of wheat straw using sulphuric acid at 130°C. Kinetic models
108 were developed to explain the variation with time of the main products generated. The hydrolysis was optimised
109 to obtained xylose solutions with low concentration of growth inhibitors.

111 2. Materials and methods

112
113 The raw material used, wheat straw, was collected in a local industry (Pitita cereals, Dozón, Spain). It
114 was air dried, milled, screened to select the fraction of particles with a size lower than 0.5 mm, homogenized in
115 a single lot and stored until needed.

116 Analyses of the main fractions (cellulose, hemicelluloses and Klason lignin) were carried out using a
117 quantitative acid hydrolysis under standard conditions [19]. The main composition of the wheat straw is shown
118 in Table 1.

119 Treatments were performed at 130°C in media containing 1, 2, 3, 4 or 5 g H₂SO₄ / 100 g liquor using a
120 charge of 1 g wheat straw / 12 g liquor on dry basis. Samples were collected at several reaction times in the
121 range 0-180 min.

122 The experiments were performed in 5 sets. The operational conditions of the sets are shown in Figures
123 1-2. At given reaction times, samples of liquors were taken from the reaction media and analysed. The samples
124 were diluted with water (1/10 v/v), centrifuged to separate the water-insoluble phenolic fraction and analysed by
125 HPLC for glucose, xylose, arabinose, acetic acid, furfural and HMF. The HPLC analyses were carried out using
126 a Transgenomic ION-300 column (oven temperature = 45°C) with isocratic elution (flow rate= 0.4 ml/min;
127 mobile phase: H₂SO₄ 0.0025 M). RI detector was used for all compounds excepting furfural and HMF that were
128 detected spectrophotometrically by DAD.

129 All experiments were carried out in triplicate and means are given. Non-linear regression analyses of
130 experimental data were performed with a commercial optimisation routine using Newton's method (Solver,
131 Microsoft Excel 2003, Microsoft Corporation, Redmond, WA, USA) by minimizing the sum of the squares of
132 deviations between experimental and calculated data according the philosophy reported elsewhere [20].

134 3. Results

136 3.1. Raw material composition and potential concentrations

137 The composition of the wheat straw used in this study is shown in Table 1. The main fractions of wheat
138 straw were in the same range as other herbaceous materials, such as rice, barley straw [19] and sorghum straw
139 [21]. For example, the glucan content in wheat straw determined in our study was 34.5%. It compares very well
140 with 35.5% found by Wang et al. [12]. The high content of xylan (20.2%) makes this waste adequate for xylose
141 production.

142 Using the value of water/solid ratio and the composition of raw material, the potential concentration of
143 each sugar can be calculated assuming a total conversion of polysaccharides to sugar without degradation. This
144 is an ideal situation because it is known that hemicelluloses are degraded to furfural at the same time that this is
145 released. On the other hand, it is not possible, in acid hydrolysis, to obtain the total and simultaneously

146 conversion of cellulose and hemicelluloses. For comparative purposes and kinetic calculations, the maximum
147 concentrations for each sugar were calculated using the following equation [22]:

$$148 \quad P_0 = F \frac{CPn_0}{WSR} \rho \quad (1)$$

149
150 where P_0 is the maximum (potential) concentration of each sugar (in g/L), F is the stoichiometric factor due to
151 the hydration of molecules during the hydrolysis (F_{pentoses} is 150/132 and F_{hexoses} is 180/162), CPn_0 is the
152 composition of raw material for the polysaccharide Pn (in g of polysaccharide/g of raw material, on dry basis),
153 WSR is the water/solid ratio used (12 g/g) and ρ is the density of hydrolysates (1025 g/L). Applying Eq. (1), it
154 was obtained the potential composition of the liquors (29.9 g glucose/L; 19.7 g xylose/L and 3.3 g arabinose/L).
155 The potential concentration of acetic acid generated by the hydrolysis of acetyl groups was not determined
156 because the content of acetyl groups was not determined in the sorghum straw.

157 In the acid hydrolysis, furfural is generated by dehydration of pentoses (xylose and arabinose) and
158 HMF by dehydration of hexoses (glucose in this case). Using the stoichiometric factors ($F_{\text{furfural}} = 96/132$ and
159 $F_{\text{HMF}} = 126/162$) was also obtained the potential concentration of furfural (14.6 g furfural/L) and HMF (22.9 g
160 HMF/L). Obviously, the conditions to obtain the potential concentrations for furfural and HMF also lead to
161 obtain zero concentration of monosaccharides.

162 In the acid hydrolysis with dilute acids, it is known that the main fraction affected is the
163 hemicelluloses, remaining the cellulose almost unaltered. Therefore, the HMF concentration expected that can
164 be obtained from the dehydration of glucose should be negligible.

165

166 3.2. Composition of hydrolysates

167 Hydrolysates were obtained using H_2SO_4 at 130°C. Figure 1 shows concentration of xylose, glucose,
168 arabinose. The xylose concentration reached up to 19.7 g/L. It was obtained in the experiment performed using
169 1% H_2SO_4 for 60 min, corresponding to 100% of the potential concentration predicted.

170 It was observed that the xylose concentration reached a maximum value and then decreased with the
171 reaction time and with the acid concentration. This suggests that xylose undergoes decomposition reactions
172 promoted by the sulphuric acid concentration, and they are conducting probably to the furfural synthesis.

173 During the hydrolysis of wheat straw, other sugars are released to liquors, mainly glucose and
174 arabinose. Glucose proceeds from the cellulosic fraction or from some heteropolymers of the hemicellulosic
175 fraction while arabinose proceeds only from the hemicellulosic fraction. The glucose concentration was affected
176 by the sulphuric acid concentration. The maximum value was 7.3 g/L in the experiments performed with 5%
177 H_2SO_4 at 130 °C for 180 min, corresponding to 37% of the potential glucose predicted. In experiments
178 performed in severe conditions, an increase in the glucose concentration was observed over a long time. This
179 fact suggests that glucose release is promoted by the acid concentration.

180 Arabinose is presented as furanose, thus it was hydrolysed more quickly than the pyranoses glucose
181 and xylose (Fendel et al., 1984) as can be observed in Fig. 1. The occurrence of arabinose showed a similar
182 pattern than xylose. The maximum concentration (3.3 g/L) was obtained using the lowest concentration of acid
183 (1%) for 180 min. The increase in the concentration of sulphuric acid leads to a decrease in the arabinose

184 concentration, suggesting degradation reactions toward furfural. As in the case of xylose, a quantitative
185 conversion was obtained since the potential concentration was reached.

186 Figure 2 shows the furfural, HMF and acetic acid concentrations. Furfural was generated as a
187 degradation product from pentoses such as xylose and arabinose. The furfural concentration increased with the
188 reaction time and the concentration of the catalyst, sulphuric acid. The higher value reached was 4.0 g furfural/L
189 in the experiment with 4-5% H₂SO₄ at 130 °C for 180 min.

190 HMF was generated as a degradation product from glucose. The higher value reached was 0.3 g
191 HMF/L in the experiment with 1% H₂SO₄ at 130 °C for 60 min. the concentration of HMF decreased for long
192 time of treatment. This effect was stronger at high acid concentrations. This suggests that further reactions
193 occurred. HMF can be converted to 2,5-dimethylfuran (DMF), a liquid transportation fuel with 40 percent
194 greater energy density than ethanol [1].

195 The acetic acid derives from the hydrolysis of the acetyl groups bound to the hemicellulosic monomers.
196 Acetic acid at relative high concentrations is an inhibitor of microorganism growth. Ferrari et al. [23] reported
197 that 10.5 g acetic acid/l hindered the growth of *Pichia stipitis*. In our study, 2.3-2.6 g acetic acid/L was the
198 maximum reached in all experiments. Through material balances, the initial acetyl groups can be determined. It
199 was 2 g acetyl groups/100 g dry wheat straw. This value was slightly lower than that obtained for sugar cane
200 bagasse, 3.2-3.5 g acetyl groups/100 g [7].

201 202 4. Discussion

203
204 Usually the models proposed in the literature consider a sequence of pseudo-homogeneous irreversible
205 first-order reactions. The first model used successful was proposed by Saeman [24] based on the hydrolysis of
206 cellulose to yield glucose and then toward decomposition products. The Saeman model was designed for the
207 hydrolysis of cellulose from fir wood using sulphuric acid. Later the model was also applied to the hydrolysis of
208 the hemicellulosic fraction [22,25]. Therefore, it can be generalized like in Eq. (1):



211
212 Where k_1 is the rate of the generation reaction (min^{-1}) and k_2 is the rate of the decomposition reaction
213 (min^{-1}). Solving the differential equations, the following model predicts the concentration of monomers:

214
215
$$M = M_0 \cdot e^{-k_2 t} + P_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 t} - e^{-k_2 t}) \quad (2)$$

216
217 Where M and P are concentrations of monomer and polymer expressed in g/L, t is time and subscript 0 indicates
218 initial conditions. P₀ was calculated as described in section 3.1.

219 The fitting of experimental data to Eq. (2) frequently was not enough successful. Therefore, the kinetic
220 model was modified to include the existence of two fractions, one easy to hydrolyse and the other difficult to
221 hydrolyse. This fact was previously described by other authors [5]. It is frequent to find that one fraction does
222 not react in some experimental conditions [19,20]. The parameter α represents the ratio between the fractions (g

223 of susceptible polymer/ g of total polymer) in the raw material. Eq. (2) was modified to include α as is shown in
224 Eq. (3).

225

$$226 \quad M = M_0 \cdot e^{-k_2 \cdot t} + \alpha \cdot P_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \quad (3)$$

227

228 4.1. Kinetic modelling of xylose concentration

229 Xylose is the main product of the hydrolysis of wheat straw. The Eq. (3) adapted for xylose could be as
230 following:

$$231 \quad X = X_0 \cdot e^{-k_2 \cdot t} + \alpha_x \cdot Xn_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \quad (4)$$

232 Where X is the xylose concentration (g/l); X_0 is the initial xylose concentration (determined by
233 regression, g/L); Xn_0 is the xylan concentration corresponding with the quantitative conversion to xylose (29.20
234 g/L); k_1 is the rate of the generation reaction from xylan to xylose (min^{-1}); k_2 is the rate of the decomposition
235 reaction from xylose to furfural (min^{-1}); α_x is the ratio for the xylan fractions in raw material (g of susceptible
236 xylan/ g of total xylan).

237 Data was fitting applying Eq. (4) and Fig. 1 shows the experimental and predicted data for xylose
238 concentrations. The fitting was performed separately for each set. Table 2 shows the kinetic and statistical
239 parameters of the fitting. The statistical parameters r^2 and F-test probability corroborate that the two-fraction
240 model fits very well.

241 Comparing the values of k_1 with k_2 , it can be observed that the kinetic coefficients of generation
242 reactions of xylose are around 30-fold higher than those of the degradation reactions. Generalizing, the values of
243 kinetic coefficient increase with the concentration of catalyst, k_1 slightly and k_2 strongly.

244 The value of α was in the range 0.182-0.634. The average, 0.306 g/g, is lower that values reported for
245 dilute-acid hydrolysis of other materials. For example, Kim and Lee [26] reported values of α in the range 0.58-
246 0.80 g/g for oak hydrolysis. Eken-Saraçoğlu et al. [27] found values of 0.84 g/g for corn cob and 0.86 g/g for
247 sunflower seed hulls. Using bagasse waste, Nee and Yee [28] obtained a value of 0.65 g/g. For sugar cane
248 bagasse, it was obtained values in the range 0.554-0.998 g/g [7].

249 It is normal to find that α also varies with the operational conditions. Hydrolysing *Pinus pinaster* [29]
250 at atmospheric pressure with dilute sulphuric acid, α was in the range 0.57-0.63 g/g, however, it was 0.86-0.87
251 g/g if the hydrolysis is at higher pressure [30].

252 For wheat straw, we have also found that α varies with the concentration of the catalyst, decreasing
253 with the increasing of the acid concentration. At time 0, the concentration of xylose increased with the acid
254 concentration. The values of M_0 predicted by the model also increased from 7.55 g/L with 1% of sulphuric acid
255 concentration to 16.52 g/L using 5% of sulphuric acid. This suggests an almost instant hydrolysis of a part of the
256 easy fraction. Therefore, in this case the decreasing of the values of α with the acid concentration could be
257 associated with a quicker hydrolysis of the easy fraction more than a decreasing of the amount of easy fraction.

258 It is common to model the effect of the acid concentration on k_1 as follow [31,32]:

259

260 $k_1 = a \cdot C^n$ (5)

261

262 Where a and n are the regression parameters and C is the acid concentration in % (w/w). Using the
 263 values previously obtained for k_1 and applying a non-linear regression analysis to the model of Eq. (5), k_1
 264 correlated with H_2SO_4 concentration as shown in Eq. (6).

265

266 $k_1 = 0.00420C$ (6)

267

268 This equation can be considered well fitted ($r^2 = 0.9467$ and the value of F-test probability = 0.9616).

269 The effect of acid concentration on k_2 was also modelled using the same equation. In this case, the
 270 equation best fitted was:

271 $k_2 = 0.00059C^{1.34}$ (7)

272

273 The regression parameter a for k_1 was more than 10-folds higher than that for k_2 . Both values of the
 274 regression parameter n (1 for k_1 and 1.34 for k_2) were in the range reported in the literature for similar
 275 lignocellulosic materials: $n = 1.55$ [27], $n = 0.80$ [33] and $n = 0.66$ [26].

276 Knowing these parameters is possible to predict the xylose concentration for any time and acid
 277 concentration in the range of study.

278

279

280 4.2. Kinetic modelling of glucose concentration

281 Glucose is a by-product obtained in the acid hydrolysis of wheat straw. The glucose released in the
 282 hydrolysis can proceed from both hemicellulosic heteropolymers and cellulose. The glucose from cellulose is
 283 not usually hydrolysed in the range of operational conditions commonly used for the acid hydrolysis. Therefore,
 284 it is probable that the released glucose proceed almost quantitatively from hemicelluloses.

285 From glucose, the reaction can lead to decomposition products, mainly HMF. This is formed in acid
 286 medium due to the release of 3 molecules of water from hexose.

287 The potential concentration of glucose was 29.9 g/L. It was also considered that there is a glucan
 288 fraction that it is susceptible to react and other not susceptible. The parameter α_G represents the ratio between
 289 the glucan fractions (g of susceptible glucan/ g of total glucan) in the raw material. Consequently, the glucose
 290 concentration can be calculated by Eq. (8).

291

292

293 $G = G_0 \cdot e^{-k_2 \cdot t} + \alpha_G \cdot Gn_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t})$ (8)

294

295 Where G is the glucose concentration (g/l); G_0 is the initial glucose concentration (determined by
 296 regression, g/L); Gn_0 is the glucan concentration corresponding with the quantitative conversion to glucose

297 (29.20 g/L); k_1 is the rate of the generation reaction from glucan to glucose (min^{-1}); k_2 is the rate of the
298 decomposition reaction from glucose to HMF (min^{-1}).

299 Table 3 shows the kinetic and statistical parameters fitting the model of Eq. (8) for glucose generated.
300 Fig. 1 shows the experimental and predicted data in these hydrolyses. The values of r^2 and F-test probability
301 showed a good agreement between experimental and predicted data.

302 The kinetic parameters of glucose released (k_1) decreased with the increase of the acid concentration.
303 This can be associated with the increase of the initial glucose concentration (G_0) promote by the increase of the
304 acid concentration more than a low catalytic effect at high acid concentrations.

305 Kinetic parameters of decomposition reactions of glucose (k_2) was 0. It can be supposed that this is due
306 to the high energy of activation of the reactions of glucose degradation to HMF. The decomposition reaction
307 occurred but it was at so low speed that it was not detected by the significant digits used for k_2 in the modelling.

308 The parameter α_G was affected slightly by acid concentration. Only 11-18% of glucan was susceptible
309 to hydrolysis. This fraction proceeds from hemicellulosic heteropolymers. It can be concluded that 18%
310 represents the glucose in the hemicelluloses of wheat straw. This value compared very well with that obtained
311 for sugar cane bagasse, 18.2% [7].

312 Using the values previously obtained for k_1 and applying a non-linear regression, k_1 correlated with
313 H_2SO_4 concentration as shown in Eq. (9).

314

$$315 \quad k_1 = 0.02930C^{-0.63} \quad (9)$$

316

317 This equation can be considered well fitted ($r^2 = 0.9104$ and the value of F-test probability = 0.8726).
318 Since k_2 was 0, it was not modelled in function of the acid concentration.

319

320

321 4.2. Kinetic modelling of arabinose concentration

322 The arabinose released in the hydrolysis proceeds from the hemicellulosic heteropolymer of wheat
323 straw, accompanying xylose. The potential concentration was 3.3 g/L. Consequently, the arabinose
324 concentration can be calculated by Eq. (10).

325

$$326 \quad A = A_0 \cdot e^{-k_2 t} + \alpha_A \cdot An_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 t} - e^{-k_2 t}) \quad (10)$$

327 Where A is the arabinose concentration (g/l); A_0 is the initial glucose concentration (determined by
328 regression, g/L); An_0 is the glucan concentration corresponding with the quantitative conversion to glucose
329 (3.24 g/L); k_1 is the rate of the generation reaction from araban to arabinose (min^{-1}); k_2 is the rate of the
330 decomposition reaction from glucose to furfural (min^{-1}).

331 Table 4 shows the kinetic and statistical parameters fitting the model of Eq. (10). Fig. 1 shows the
332 experimental and predicted data in these hydrolyses. Overall, the values of r^2 and F-test probability showed a
333 good agreement between experimental and predicted data. However for 5% sulphuric acid the model did not fit
334 well (r^2 was 0.7106 and F-test probability was 0.6646). With 4-5% of sulphuric acid a quick decrease of
335 arabinose concentration with the time was observed. The values of k_1 fitted did not show a clear trend but the

336 values of k_2 increased with the acid concentration, showing a reaction toward furfural. A_0 was in the range 2.19-
337 2.38 g/L. The modelling of the effect of the acid concentration did not fit well.

338

339 4.3. Kinetic modelling of furfural concentration

340 In the hydrolysis of wheat straw, furfural (a decomposition product of pentoses), HMF (a
341 decomposition product of hexoses), and phenolic compounds (decomposition products of lignin) are generated.
342 Dilute-acid hydrolysis affects hemicelluloses and, only in strict conditions, cellulose. Therefore, the amount of
343 phenolic compounds in the hydrolysates of wheat straw is negligible, the amount of HMF expected should be
344 low and the main decomposition product should be furfural. All these products can continue the reactions
345 toward other products or polymers. Furfural is the triple dehydration product of xylose. Based on furfural results
346 from Fig. 2, a similar model to those used for pentoses and hexones can be considered. Eq. (11) expresses the
347 furfural concentration (F) as a function of time (t).

348

$$349 \quad F = F_0 \cdot e^{-k_2 t} + \alpha_F \cdot F n_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 t} - e^{-k_2 t}) \quad (11)$$

350

351 Where F is the furfural concentration (g/L); F_0 is the initial glucose concentration (determined by
352 regression, g/L); $F n_0$ is the furfural concentration corresponding with the quantitative conversion from pentoses
353 (14.29 g/L); k_1 is the rate of the generation reaction from pentoses to furfural (min^{-1}); k_2 is the rate of the
354 decomposition reaction from furfural to polymers (min^{-1}).

355 Table 5 shows the kinetic and statistical parameters obtained in the fitting of the furfural generated in
356 the hydrolysis. Fig. 2 shows the comparison between experimental and predicted data.

357 It can be observed that all parameters were affected by H_2SO_4 concentration. F_0 varied from 0 g/L using
358 2% H_2SO_4 to 0.17 g/L using 5% H_2SO_4 . The same behaviour was observed for the hydrolysis of sorghum straw
359 but in that case the highest concentration reached was 3.33 g/L [21]. Highest F_0 values were obtained for sugar
360 cane bagasse [7].

361

362 4.3. Kinetic modelling of HMF concentration

363 HMF is a decomposition product of hexoses. The concentration of glucose released was low. Then the
364 expected HMF concentration should be very low. Based on HMF results from Fig. 2, a similar model to those
365 used for HMF can be considered including the reaction toward other products. Eq. (12) expresses the HMF
366 concentration (H) as a function of time (t).

367

$$368 \quad H = H_0 \cdot e^{-k_2 t} + \alpha_H \cdot H n_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 t} - e^{-k_2 t}) \quad (12)$$

369

370 Where H is the HMF concentration (g/L); H_0 is the initial glucose concentration (determined by
371 regression, g/L); $H n_0$ is the HMF concentration corresponding with the quantitative conversion from glucose
372 (23.36 g/L); k_1 is the rate of the generation reaction from glucose to HMF (min^{-1}); k_2 is the rate of the
373 decomposition reaction from HMF to polymers or 2,5-dimethylfuran (min^{-1}). Table 6 shows the kinetic and

374 statistical parameters obtained in the fitting of the HMF generated in the hydrolysis. Fig. 2 shows the
375 comparison between experimental and predicted data.

376 It can be observed that all parameters were affected by H₂SO₄ concentration. H₀ varied from 0.03 g/L
377 using 2% H₂SO₄ to 0.17 g/L using 5% H₂SO₄. The values of k₁ and k₂ increased with H₂SO₄ concentration,
378 being very high the values of k₂ respect to k₁. This suggests that the HMF release reacts toward polymerization
379 products or 2,5-dimethylfuran. Anyway, the HMF concentration was very low.

380

381 4.4. Kinetic modelling of acetic acid concentration

382 The acetic acid is generated for the hydrolysis of the acetyl groups present in the hemicellulosic
383 heteropolymers. Based on the experimental data, degradation reactions of the acetic acid was not observed in
384 accordance with the results for sorghum straw and other lignocellulosic materials [19-21]. Therefore, the model
385 of Eq. (13) expresses the acetic acid concentration (Ac) as a function of time (t) in the hydrolysis of wheat
386 straw.

387

$$388 \quad Ac = Ac_0 \cdot e^{-k_2 t} + \alpha_{Ac} \cdot Acn_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 t} - e^{-k_2 t}) \quad (13)$$

389 Where Ac₀ is the initial acetic acid concentration (g/L); Acn₀ is the potential concentration of acetyl
390 groups; k₁ is the rate of acetic acid generation (min⁻¹) and k₂ is the rate of the decomposition reaction (min⁻¹).

391 The Ac₀ was introduced as a regression parameter. Acn₀ is not easy to determine, therefore it was
392 included in the same regression parameter than α_{Ac}.

393 Table 7 shows the kinetic and statistical parameters obtained in the fitting of the acetic acid generated
394 in the hydrolysis of wheat straw. The values of r² showed that only the equations obtained for 1% and 2%
395 H₂SO₄ were well fitted. Fig. 2 shows the agreement between experimental and predicted data.

396 Ac₀ varied from 1.23 g/L using 2% H₂SO₄ to 2.33 g/L using 6% H₂SO₄. Ac₀ was clearly affected by
397 H₂SO₄ concentration. The bad regression could be due to the low acetic acid concentration, with a quick and
398 stable release to give values around 2.4 g/L.

399

400

401 4.5. Overall optimisation

402 It is important to obtain sugar solutions with low concentrations of inhibitor (furfural, HMF and acetic
403 acid) if the hydrolysates of wheat straw are going to be used as carbon source in fermentation media.

404 For comparative purposes, the optimum values are those that result in a high concentration of xylose
405 and a low concentration of potential microorganism growth inhibitors like furfural, HFM and acetic acid. Fig. 1-
406 2 show that the optimum could be using a reaction time around 15-30 min.

407 Using the models, the optimum for xylose was predicted using 29.3 min and 2% H₂SO₄. using theses
408 conditions, a hydrolysates with the following composition can be obtained: xylose, 18.9 g/L; glucose, 3.5 g/L;
409 arabinose, 3.1 g/L; furfural, 0.6 g/L; HFM, 0.3 g/L and acetic acid, 2.3 g/L.

410 Furthermore, the glucose concentration is low, indicating a small degradation of the cellulosic fraction.
411 This is favourable if it is desired a later use of the solid residue. For instance, applications for the solid residue

412 are the production of cellulosic paste, the generation of glucose solutions by enzymatic hydrolysis or the
413 production of HFM as precursor of 2,5-dimethylfuran (DMF), a liquid fuel [1].

414

415 **Conclusion**

416 The kinetic models developed allow the prediction of the reaction time for the optimal values of the
417 hydrolysis of wheat straw. The operational conditions 2% H₂SO₄ at 130 °C for 29.3 min were selected because it
418 resulted in solutions with high concentration of fermentable sugars (25.5 g/L) and low concentrations of growth
419 inhibitors (less than 0.9 g/L for furfural-HMF and 2.3 g/L for acetic acid). In these conditions, approximately
420 99% of the hemicellulosic sugars were hydrolysed with a small concentration of by-products and only 12%
421 degradation of the glucan fraction.

422

423 **Acknowledgements**

424

425 The Authors are grateful to MICINN (Spain) for the financial support of this work (project AGL2006-
426 08250/ALI) and the FEDER funds of the European Union.

427

428 **REFERENCES**

429

430 [1] Huber GW, Iborra S, Corma A. Synthesis of Transportation Fuels from Biomass: Chemistry, Catalysts, and
431 Engineering. *Chem. Rev.* 2006;106(9):4044-4098. doi:10.1021/cr068360d.

432 [2] Kim JH, Ryu YW, Seo JH. Analysis and optimization of a two-substrate fermentation for xylitol production
433 using *Candida tropicalis*. *J. Ind. Microbiol. Biotechnol.* 1999;22(3):181-186.

434 [3] Parajó JC, Domínguez H, Domínguez JM. Production of xylitol from concentrated wood hydrolysates by
435 *Debaryomyces hansenii*: effect of the initial cell concentration. *Biotechnol. Lett.* 1996;18:593-598.

436 [4] Silva SS, Felipe MGA, Mancilha IM. Factors that affect the biosynthesis of xylitol by xylose-fermenting
437 yeasts. A review. *App. Biochem. Biotechnol.* 1998;70-72:331-340.

438 [5] du Toit PJ, Olivier SP, van Bijon PL. Wheat straw as a possible source of fermentable carbohydrates. I.
439 Characterization of bagasse with regard to monosaccharide, hemicellulose, and amino acid
440 composition. *Biotechnol. Bioeng.* 1984;26:1071-1078.

441 [6] Nguyen QA, Tucker MP, Keller FA, Eddy FP. Two-stage dilute-acid pretreatment of softwoods. *Appl.*
442 *Biochem. Biotechnol.* 2000;84-86:561-576.

443 [7] Aguilar R, Ramirez JA, Garrote G, Vázquez M. Kinetic study of the hydrolysis of sugar cane bagasse. *J.*
444 *Food Eng.* 2002;55:309-318.

445 [8] Herrera A, Téllez-Luis SJ, Ramírez JA, Vázquez M. Production of xylose from sorghum straw using
446 hydrochloric acid. *J. Cereal Sci.* 2003;37(3):267-274.

447 [9] Franz R, Erckel R, Riehm T, Woernle R, Deger HM. Lignocellulose saccharification by HF. In: *Energy from*
448 *biomass*. Ed. Applied Science Publishers. London. pp. 873-878; 1982.

449 [10] Vázquez M, Oliva M, Téllez-Luis SJ, Ramírez JA. Hydrolysis of sorghum straw using phosphoric acid:
450 evaluation of furfural production. *Biores. Technol.* 2007;98:3053-3060.

451 [11] Rodríguez-Chong A, Ramírez JA, Garrote G, Vázquez M. Hydrolysis of sugar cane bagasse using nitric
452 acid: A kinetic assessment. *J. Food Eng.* 2004;61(2):143-152.

- 453 [12] Wang L, Luo Z, Xiu S, Shahbazi A. Pretreatment and Fractionation of Wheat Straw with Acetic Acid to
454 Enhance Enzymatic Hydrolysis and Ethanol Fermentation. *Energy Sources Part A*. 2011;33(13):1230-
455 1238.
- 456 [13] Grethlein HE, Converse AO. Common aspects of acid prehydrolysis and steam explosion for pretreating
457 wood. *Biores. Technol.* 1991;36:77-82.
- 458 [14] Zhu S, Wu Y, Yu Z, Zhang X, Wang C, Yu F, Jin S. Production of ethanol from microwave-assisted alkali
459 pretreated wheat straw. *Process Biochem.* 2006;41(4):869–873.
- 460 [15] Saha B, Iten L, Cotta M, Wu Y. Dilute acid pretreatment, enzymatic saccharification and fermentation of
461 wheat straw to ethanol. *Process Biochem.* 2005;40(12):3693–3700.
- 462 [16] Sun F, Chen H. Enhanced enzymatic hydrolysis of wheat straw by aqueous glycerol pretreatment. *Biores.*
463 *Technol.* 2008;99(14):6156–6161.
- 464 [17] Xu F, Shi YC, Wu XR, Theerattananon K, Staggenborg S, Wang DH. Sulphuric acid pretreatment and
465 enzymatic hydrolysis of photoperiod sensitive sorghum for ethanol production. *Bioprocess Biosystems*
466 *Eng.* 2011;34(4):485-492.
- 467 [18] Nigam JN. Single cell protein from pineapple cannery effluent. *World J. Microbiol. Biotechnol.*
468 1998;14(5):693-696.
- 469 [19] Garrote G, Domínguez H, Parajó JC. Mild autohydrolysis: an environmentally friendly technology for
470 xylooligosaccharide production from wood. *J. Chemical Technol. Biotechnol.* 1999;74:1101-1109.
- 471 [20] Garrote G, Domínguez H, Parajó JC. Kinetic modeling of corncob autohydrolysis. *Process Biochem.*
472 2001;36:571-578.
- 473 [21] Téllez-Luis SJ, Ramírez JA, Vázquez M. Mathematical modelling of hemicellulosic sugar production from
474 sorghum straw. *J. Food Eng.* 2002;52:285-291.
- 475 [22] Gámez S, Ramírez JA, Garrote G, Vázquez M. Study of the hydrolysis of sugar cane bagasse using
476 phosphoric acid. *J. Food Eng.* 2006;74(1):78-88.
- 477 [23] Ferrari MD, Neirótti E, Albornoz C, Saucedo E. Ethanol production from Eucalyptus wood hemicellulose
478 hydrolysate by *Pichia stipitis*. *Biotechnol. Bioeng.* 1992;40:753-759.
- 479 [24] Saeman JF. Kinetics of wood saccharification. Hydrolysis of cellulose and decomposition of sugars in
480 dilute acid at high temperature. *Ind. Eng. Chem.* 1945;37:43-52.
- 481 [25] Herrera A, Téllez-Luis SJ, González-Cabrales JJ, Ramírez JA, Vázquez M. Effect of the hydrochloric acid
482 concentration on the hydrolysis of sorghum straw at atmospheric pressure. *J. Food Eng.*
483 2004;63(1):103-109.
- 484 [26] Kim SB, Yum DM, Park SC. Step-change variation of acid concentration in a percolation reactor for
485 hydrolysis of hardwood hemicellulose. *Biores. Technol.* 2000;72:289-294.
- 486 [27] Eken-Saraçoğlu N, Ferda D, Dilmaç G, Çavuşoğlu H. A comparative kinetic study of acidic hemicellulose
487 hydrolysis in corn cob and sunflower seed hulls. *Biores. Technol.* 1998;65:29-33.
- 488 [28] Nee CI, Yee WF. Hydrolysis of pentosans in bagasse pith. *J. App. Chem. Biotechnol.* 1976;2:283-287.
- 489 [29] Parajó JC, Santos V, del Río F. Hidrólisis de la fracción hemicelulósica de la madera de pino. I. Cinética y
490 distribución de productos en operación a presión atmosférica. *Afinidad* 1995;52:162-170.
- 491 [30] Parajó JC, Santos V, del Río F. Hidrólisis de la fracción hemicelulósica de la madera de pino. II. Operación
492 a presiones superiores a la atmosférica. *Afinidad* 1995;52:267-274.

- 493 [31] Ranganathan S, McDonald DG, Bakhshi NN. Kinetic studies of wheat straw hydrolysis using sulphuric
494 acid. *Canadian J. Chem. Eng.* 1985;63:840-844.
- 495 [32] Brennan A, Hoagland W, Schell DJ. High temperature acid hydrolysis of biomass using an engineering-
496 scale plug flow reactor: results of low solids testing. *Biotechnol. Bioeng. Symp.* 1986;17:53-70.
- 497 [33] Veeraraghavan S, Chambers RP, Myles M, Lee YA. Kinetic model and reactor development in
498 hemicellulose hydrolysis. In: *AICHE Nat. Meeting, Orlando, USA.* 1982.
499

500 Table 1
501 Main components of wheat straw used in this study
502

Components	Percentage dry weight
Glucan	34.5 ± 3.3
Xylan	20.2 ± 2.1
Araban	3.4 ± 0.2
Klason lignin	18.2 ± 1.5
Extracts	6.4 ± 1.0
Others	15.3 ± 1.0

503

504 Table 2

505 Kinetic and statistical parameters of xylose released for the H₂SO₄ hydrolysis of wheat straw at 130°C

506

Operational set	α_x (g/g)	X_0 (g/L)	k_1 (min ⁻¹)	k_2 (min ⁻¹)	r^2	F test prob
1% H ₂ SO ₄	0.634	7.55	0.1068	0.00026	0.9932	0.9930
2% H ₂ SO ₄	0.294	14.22	0.1122	0.00124	0.9758	0.9756
3% H ₂ SO ₄	0.227	16.10	0.1139	0.00302	0.9942	0.9852
4% H ₂ SO ₄	0.195	16.18	0.1173	0.00389	0.9903	0.9929
5% H ₂ SO ₄	0.182	16.52	0.1252	0.00494	0.9909	0.9937

507

508 Table 3

509 Kinetic and statistical parameters of glucose released for the H₂SO₄ hydrolysis of wheat straw at 130°C

510

Operational set	α_G (g/g)	G_0 (g/L)	k_1 (min ⁻¹)	k_2 (min ⁻¹)	r^2	F test prob
1% H ₂ SO ₄	0.118	0.96	0.0279	0.00000	0.9817	0.9602
2% H ₂ SO ₄	0.114	1.85	0.0228	0.00000	0.9619	0.9942
3% H ₂ SO ₄	0.106	2.56	0.0154	0.00000	0.9539	0.9519
4% H ₂ SO ₄	0.140	2.78	0.0105	0.00000	0.9609	0.9594
5% H ₂ SO ₄	0.180	2.88	0.0086	0.00000	0.9831	0.9826

511

512

513

514 Table 4

515 Kinetic and statistical parameters of arabinose released for the H₂SO₄ hydrolysis of wheat straw at 130°C

516

Operational set	α_A (g/g)	A_0	k_1 (min ⁻¹)	k_2 (min ⁻¹)	r^2	F test prob
1% H ₂ SO ₄	0.319	2.19	0.0615	0.00000	0.8885	0.9331
2% H ₂ SO ₄	0.309	2.25	0.0892	0.00042	0.8564	0.8431
3% H ₂ SO ₄	0.305	2.38	0.1030	0.00199	0.9199	0.9227
4% H ₂ SO ₄	0.316	2.31	0.0960	0.00222	0.9100	0.9121
5% H ₂ SO ₄	0.225	2.35	0.1636	0.00193	0.7106	0.6646

517

518

519

520

521

522 Table 5

523 Kinetic and statistical parameters of furfural released for the H₂SO₄ hydrolysis of wheat straw at 130°C.

524

Operational set	α_F (g/g)	F ₀	k ₁ (min ⁻¹)	k ₂ (min ⁻¹)	r ²	F test prob
1% H ₂ SO ₄	0.746	0.00	0.0007	-0.00062	0.9943	0.9453
2% H ₂ SO ₄	0.731	0.00	0.0020	0.00192	0.9973	0.9877
3% H ₂ SO ₄	0.698	0.04	0.0037	0.00251	0.9990	0.9990
4% H ₂ SO ₄	0.720	0.10	0.0051	0.00446	0.9978	0.9958
5% H ₂ SO ₄	0.720	0.17	0.0048	0.00454	0.9945	0.9942

525

526

527 Table 6

528 Kinetic and statistical parameters of HMF released for the H₂SO₄ hydrolysis of wheat straw at 130°C.

529

Operational set	αH (g/g)	H ₀ (g/L)	k ₁ (min. ⁻¹)	k ₂ (min. ⁻¹)	r ²	F test prob
1% H ₂ SO ₄	0.091	0.03	0.0046	0.02059	0.9911	0.9913
2% H ₂ SO ₄	0.106	0.08	0.0092	0.06199	0.9841	0.9925
3% H ₂ SO ₄	0.164	0.12	0.0116	0.14199	0.9582	0.9531
4% H ₂ SO ₄	0.260	0.14	0.0119	0.26800	0.8995	0.9359
5% H ₂ SO ₄	1.000	0.17	0.0109	1.08496	0.8463	0.9469

530

531

532

533 Table 7

534 Kinetic and statistical parameters of acetic acid released for the H₂SO₄ hydrolysis of wheat straw at 130°C.

535

Operational set	$\alpha_{Ac}A_{cn0}$ (g/L)	A_{c0} (g/L)	k_1 (min. ⁻¹)	k_2 (min. ⁻¹)	r^2	F test prob
1% H ₂ SO ₄	1.13	1.23	0.0590	0.00000	0.9687	0.9676
2% H ₂ SO ₄	0.63	1.86	0.0587	0.00000	0.9426	0.9400
3% H ₂ SO ₄	0.43	2.12	0.1010	0.00065	0.8622	0.8535
4% H ₂ SO ₄	0.14	2.31	0.1500	0.00000	0.6122	0.5330
5% H ₂ SO ₄	0.19	2.33	0.0994	0.00027	0.5823	0.4914

536

537

538

539 **Legends of figures**

540

541

542 Figure 1. Experimental and predicted dependence of the xylose, glucose and arabinose concentration on time at
543 several H₂SO₄ concentrations.

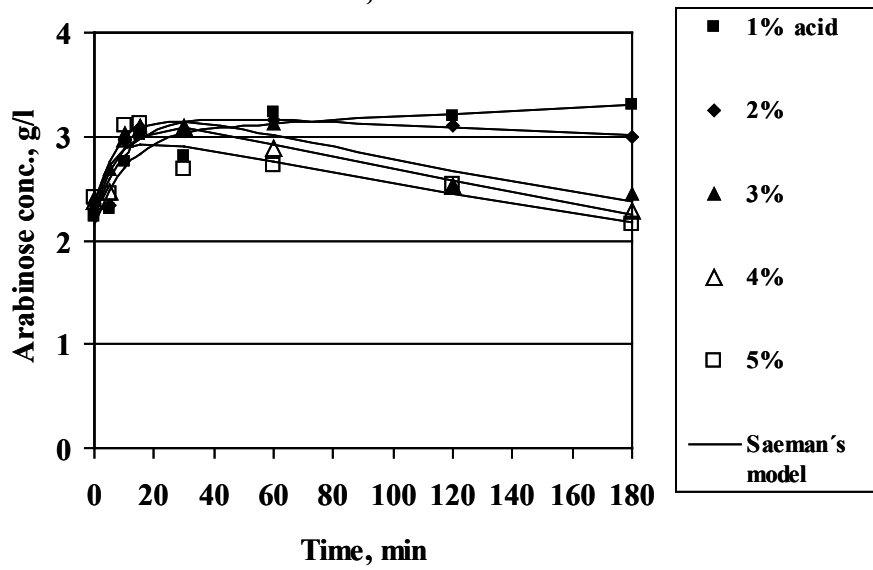
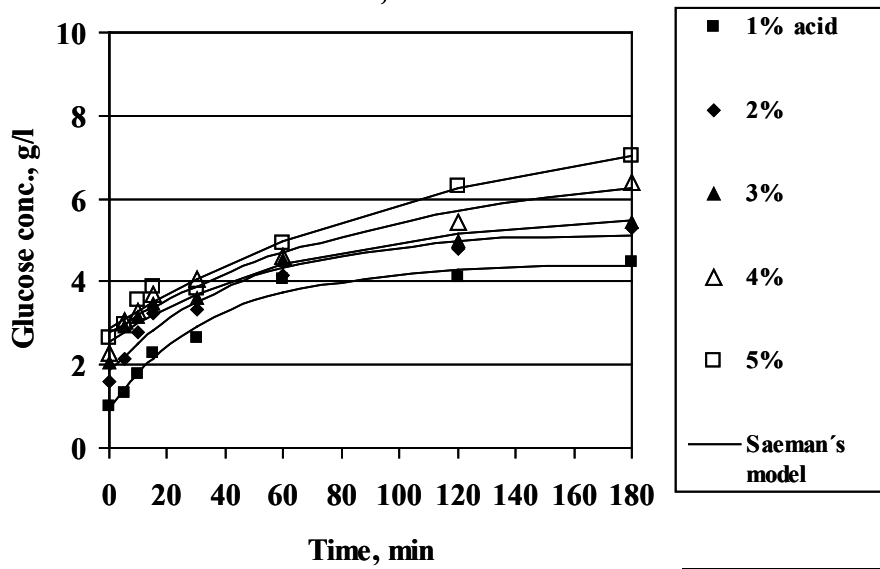
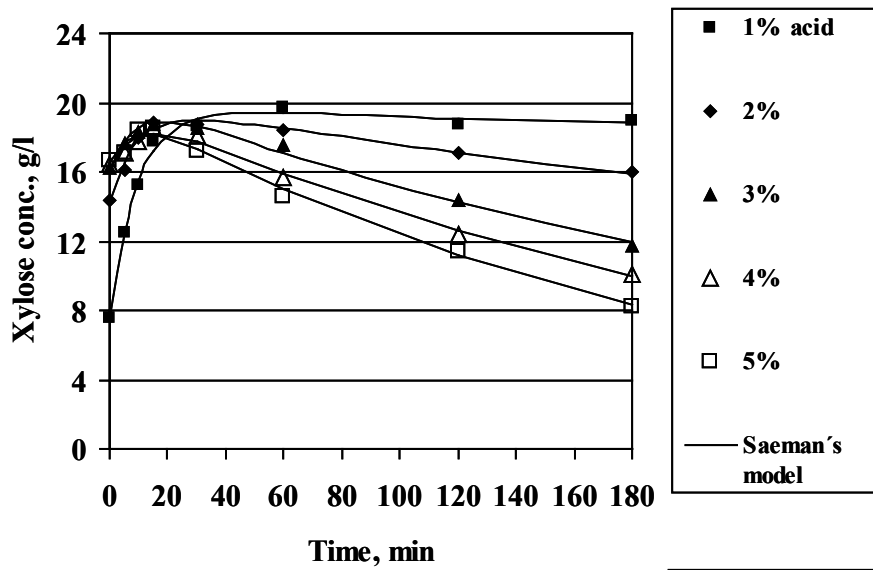
544 Figure 2. Experimental and predicted dependence of the furfural, HMF and acetic acid concentration on time at
545 several H₂SO₄ concentrations.

546

547

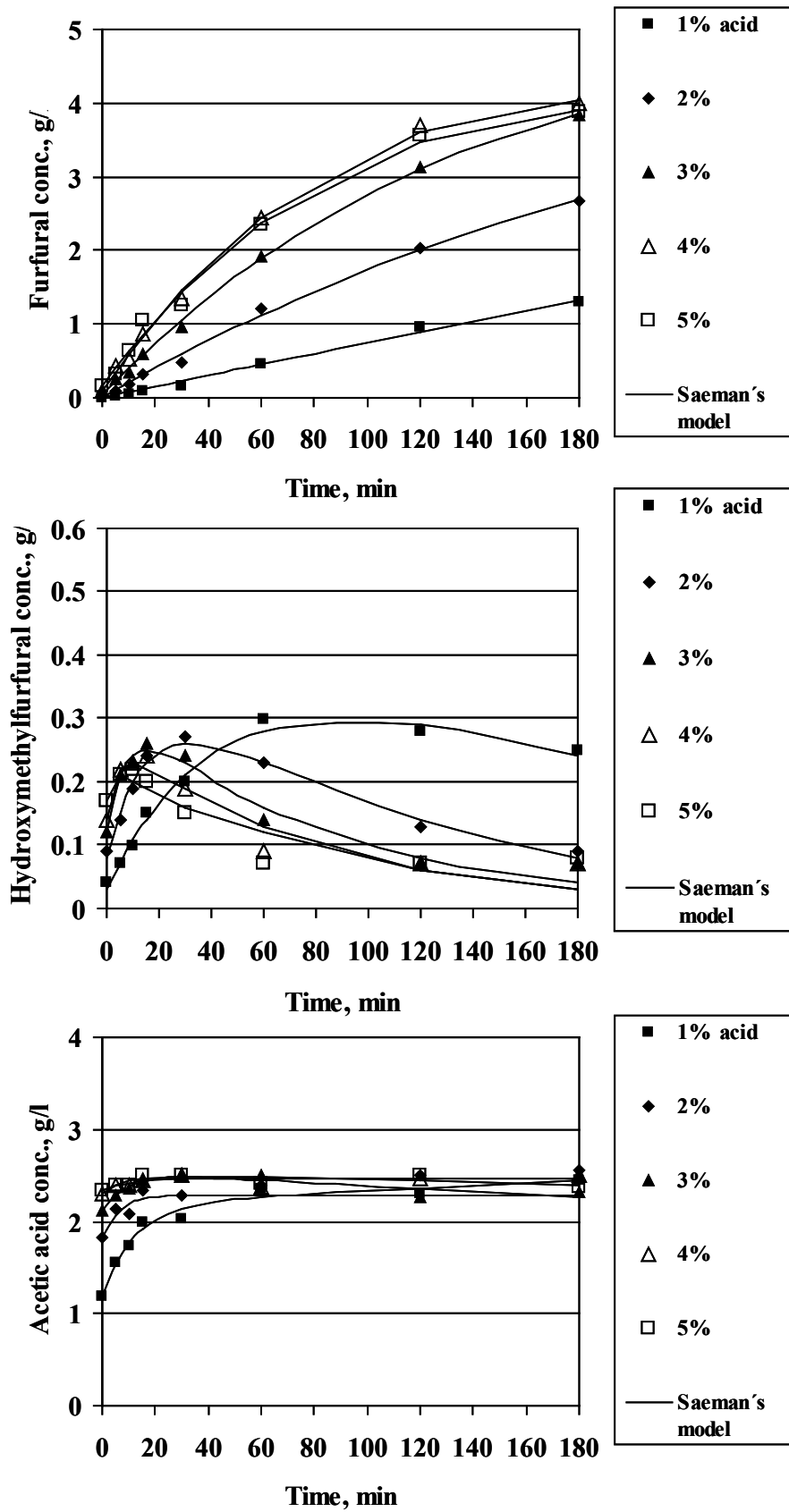
548

549



550
551
552

Figure 1



553

554

Figure 2