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Manufacture of Xylose-Based Fermentation Media from Corncobs by Posthydrolysis of Autohydrolysis Liquors

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Abstract

Milled corncob samples were mixed with water and heated to obtain a liquid phase containing oligosaccharides, sugars, and acetic acid as main reaction products (autohydrolysis reaction). To hydrolyze the sugar oligomers to the correspondent monomers, sulfuric acid was added to the autohydrolysis liquors to reach 0.5–2 wt% of solution, and the reaction media were heated at 101.5–135°C. With this operational procedure, sugar solutions suitable as fermentation media (containing xylose as the major component) were obtained. The kinetics of the posthydrolysis step was characterized on the basis of experimental data concerning the time courses of the concentrations of xylooligosaccharides, xylose, furfural, and acetic acid. The concentrations of other reaction byproducts (glucose or arabinose) were also measured.

Index Entries: Autohydrolysis; corncob; fermentation media; furfural; oligosaccharides; posthydrolysis; xylose.

Introduction

Lignocellulosic materials can be selectively fractionated by means of hydrothermal treatments, which allow a substantial solubilization of hemicelluloses *(1)* leaving the cellulose almost untouched in solid phase. During these treatments, a part of lignin (typically in the range of 10–20% of the initial amount) can also be solubilized. The solid residue from treatments, containing both cellulose and insoluble lignin, can be further fractionated (e.g., with alkalis, acids, oxidizing agents, or organosolvents) to isolate the cellulosic fraction.

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The hydrothermal treatments usually have been applied to the processing of xylan-rich materials such as hardwoods or agricultural byproducts, in order to obtain liquors containing xylan-degradation products (xylooligosaccharides, xylose, and furfural). Under selected conditions, the joint contribution of xylose and xylooligosaccharides can account for most of the initial xylan *(2–5)*.

The manufacture of xylose-based fermentation media (to be used for the production of compounds such as ethanol or xylitol) is a potential utilization of autohydrolysis liquors. Because the yeast cannot metabolize sugar oligomers, the liquors must be subjected to a posthydrolysis stage with acids or enzymes before fermentation, in order to convert the oligomers into monomeric sugars (mainly xylose). Acid posthydrolysis presents operational and economical advantages over enzymatic hydrolysis because of the faster kinetics and the lower cost of the catalyst. Under selected conditions, the acid posthydrolysis of oligomers can be carried out with minimal decomposition to furfural *(6)*. On the other hand, acetic acid (generated from the acetyl groups contained in the raw materials and oligomers) and other sugars (such as arabinose and glucose) are byproducts from both autohydrolysis and posthydrolysis.

The fermentation of solutions obtained from direct treatment of lignocellulosic materials with mineral acids (prehydrolysis reactions) is hindered (or even prevented) by the presence of reaction byproducts from other fractions of the raw material (e.g., lignin or acetyl groups) as well as by sugar decomposition products. As a result, physicochemical *(7–9)* and/or biologic detoxification *(10,11)* has been reported to be necessary for a satisfactory fermentation of hydrolysates. Since higher severity of the chemical treatments results in increased inhibitory potential of hydrolysates *(12)*, mild operational conditions are desirable. The approach described herein (with no acid added in the first treatment of the raw material) presents several comparative benefits with respect to prehydrolysis, including limited solubilization of lignin and decreased generation of furfural.

The present study deals with the manufacture of xylose solutions suitable as fermentation media from a xylan-rich feedstock through autohydrolysis (carried out under defined operational conditions) followed by posthydrolysis of the liquors obtained in the first step. In the study of the posthydrolysis kinetics, the effects of temperature (in the range of 101.5–135°C), catalyst concentration (in the range of 0.5–2 wt% of sulfuric acid) and reaction time (in the range of 1.02–8.20 h) on the concentrations of xylooligomers, xylose, furfural, and acetic acid were assessed by means of kinetic models. The concentrations of other reaction byproducts (glucose or arabinose) were also measured.

Materials and Methods

Raw Material

Milled corncob samples were subjected to nonisothermal autohydrolysis treatments under reported conditions using a water-to-substrate ratio

of 8 g/g and a maximum temperature of 202°C *(13)*. The composition of autohydrolysis liquors was as follows: 25.42 g/L of xylooligosaccharides (as xylose equivalent), degree of xylooligosaccharide acetylation of 0.224 acetyl groups/xylose unit, 1.82 g/L of arabinose oligomers (as arabinose equivalent), 2.03 g/L of glucose oligomers (as glucose equivalent), 3.05 g/L of xylose, 1.75 g/L of arabinose, 0.55 g/L of glucose, 0.55 g/L of furfural, and 1.75 g/L of acetic acid.

Posthydrolysis of Autohydrolysis Liquors

Sulfuric acid was added to autohydrolysis liquors to reach the desired concentration (0.5, 1, 1.5, or 2 wt% of solutions), and the reaction media were kept at 101.5, 115, 125, or 135°C in stirred-batch reactors with agitation and temperature controls. Time zero was set when the preset temperature was reached, and a sample was then withdrawn to measure the initial composition. Additional samples were withdrawn at selected reaction times and analyzed. The duration of each experiment (in the range of 1.02–8.20 h) was selected long enough to ensure the complete conversion of xylooligosaccharides.

Analysis of Samples

Samples from the reaction media were filtered through cellulose acetate membranes (pore diameter of 0.45 μm) and analyzed by high-performance liquid chromatography (Hewlett Packard, Waldbronn, Germany) with refractive index (RI) and diode array detection (DAD) detection using an Aminex HPX 87H column eluted with 1.8×10^{-4} *M* H_2SO_4 at 45°C.

Fitting of Data

The experimental data were fitted to the proposed models by minimization of the sums of the deviation squares using commercial software dealing with the Newton´s method.

Results and Discussion

Autohydrolysis

Autohydrolysis was carried out on the basis of previously reported results *(13,14)*. The raw material was contacted with water at room temperature in a Parr reactor and heated following the standard heating profile to reach 202°C. Then, the reactor was cooled and the liquors were recovered by filtration. The compositional data of the liquors cited in the previous section showed that 75.7% of the initial xylan was recovered as xylooligomers and xylose, whereas the furfural concentration accounted for only 2.3% of the initial xylan.

Posthydrolysis of Autohydrolysis Liquors

Figures 1 and 2 show the experimental data describing the dynamics of the concentrations of xylooligomers, xylose (the corresponding hydro-

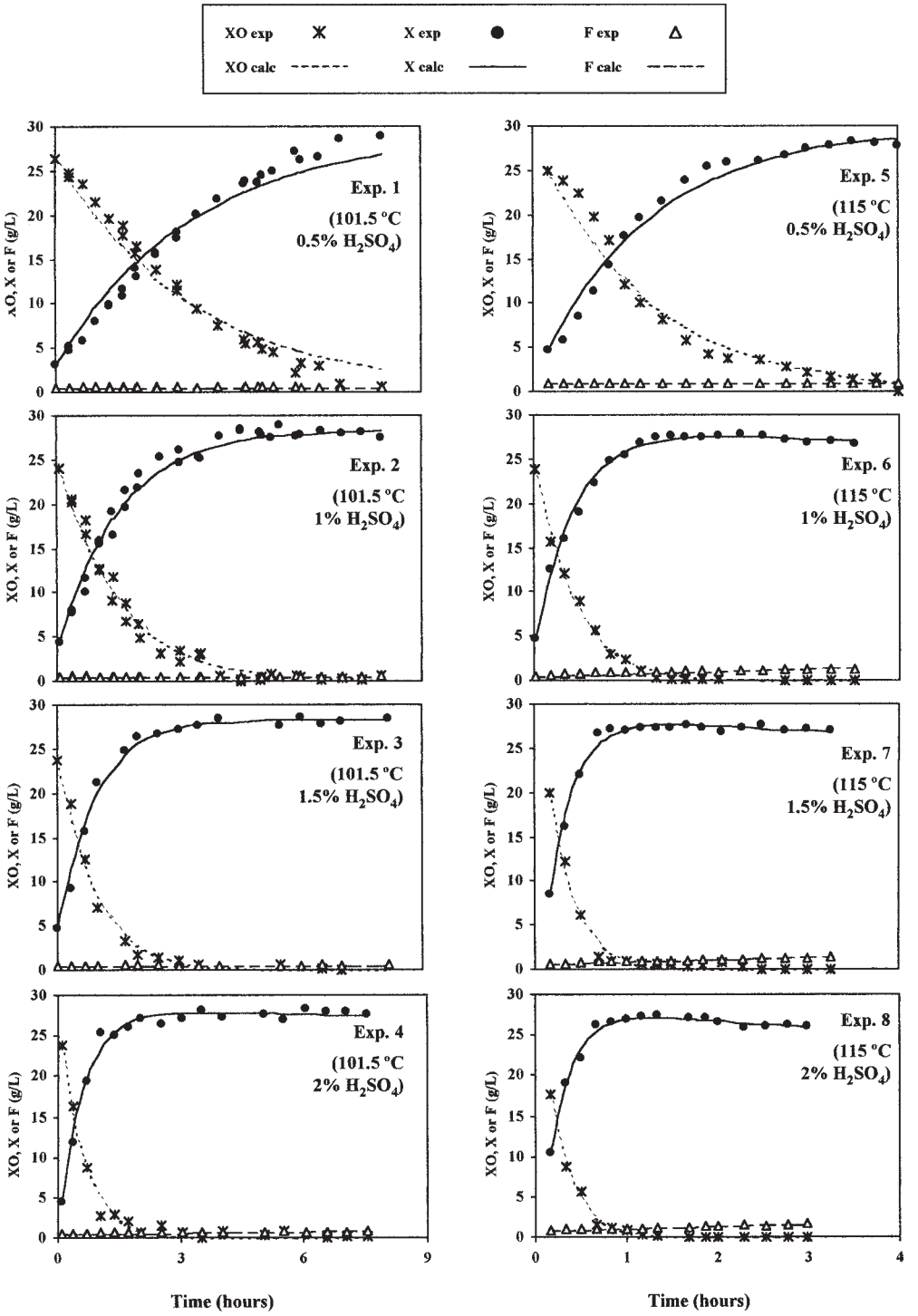

Fig. 1. Experimental and calculated time courses of concentrations of xylooligomers (XO), xylose (X), and furfural (F) corresponding to experiments 1–8 (operation at 101.5 or 115°C with 0.5, 1, 1.5, or 2% sulfuric acid).

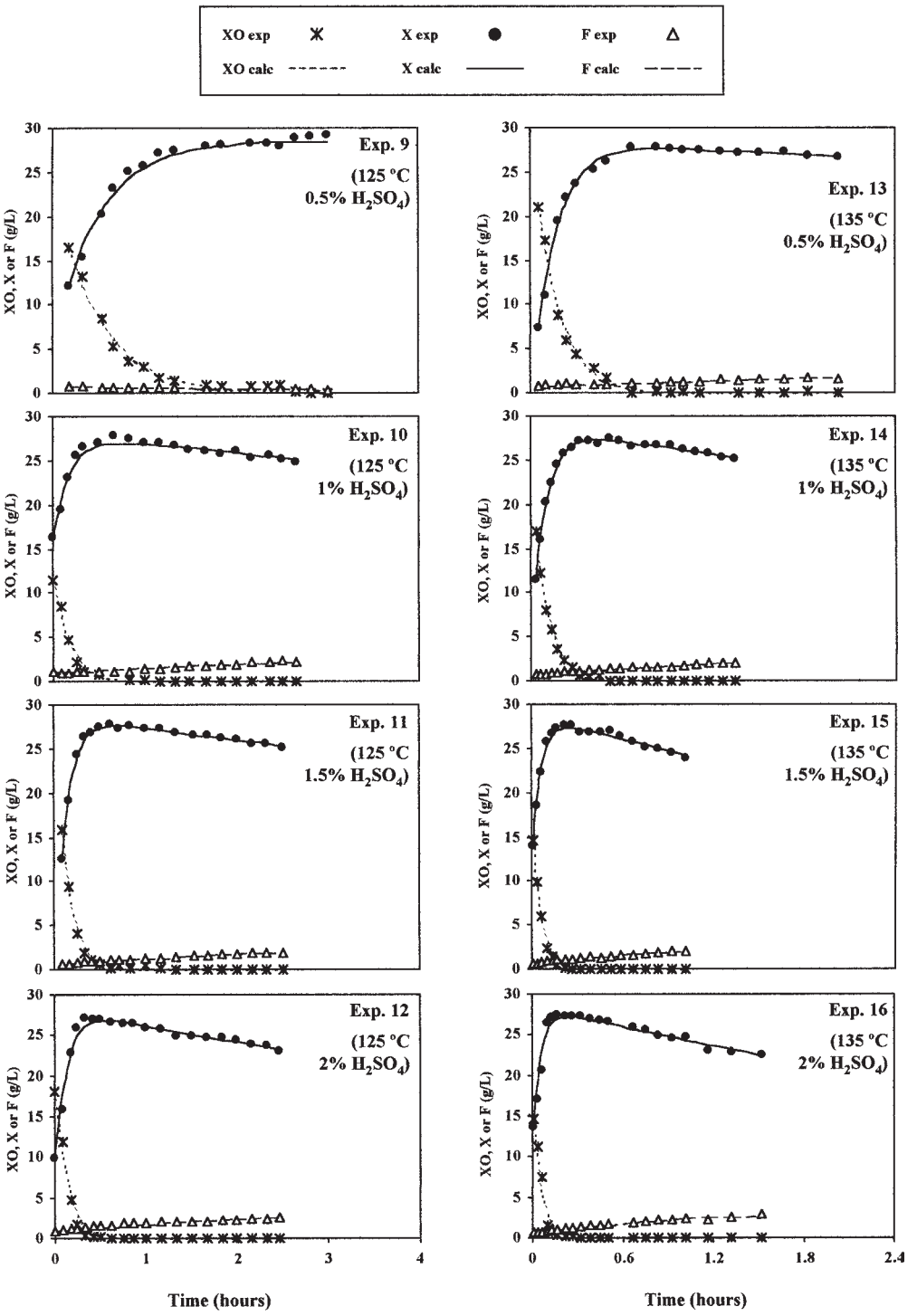

Fig. 2. Experimental and calculated time courses of concentrations of xylooligomers (XO), xylose (X), and furfural (F) corresponding to experiments 9–16 (operation at 125 or 135°C with 0.5, 1, 1.5, or 2% sulfuric acid).

lysis product), and furfural (produced by xylose dehydration) in posthydrolysis experiments carried out in the presence of sulfuric acid. The data establish the kinetic pattern of xylooligomer degradation. The main trends governing the xylooligosaccharide hydrolysis were similar in all cases, with a first reaction stage characterized by a fast reduction in xylooligosaccharide concentration and a proportional increase in the concentration of xylose. The furfural concentration was negligible for practical purposes in experiments carried out under mild conditions (reduced temperature, acid concentration, and/or reaction time) and increased steadily with the severity of the operation.

Besides xylooligomers, other sugar oligomers (made up of arabinose and glucose units) are also generated during the hydrothermal processing of corncobs. During the posthydrolysis stage, both kinds of oligomers decompose to give the corresponding monomers. Thus, the arabinose concentration increased markedly in the first reaction stages of posthydrolysis to reach a plateau dependent on temperature (3.6 g/L at 101.5°C, 3.4 g/L at 115°C, and 3.3 g/L at 125 or 135°C). The fact that the maximum arabinose concentration decreased with temperature suggests that some arabinose was decomposed to furfural, but this effect had little importance for modeling the overall behavior of the hemicellulosic fraction of corncobs. Similarly, the glucose concentration increased with the reaction time owing to the hydrolysis of glucose-containing oligomers, reaching a maximum concentration in the range of 2.4–2.6 g/L for all the posthydrolysis conditions considered. The fact that the experiments carried out at high temperature led to maximal glucose concentrations near the lower limit of its variation range suggests that some decomposition to hydroxymethylfurfural may occur, a well-known reaction *(15)*.

Operational Conditions Leading to Maximal Xylose Concentrations in Posthydrolysis Experiments

Owing to the dilution effect caused by the addition of sulfuric acid to the autohydrolysis liquors, the potential xylose concentration (defined as the one resulting from the stoichiometric conversion of xylooligosaccharides into xylose) varied in the range of 29.05–29.25 g/L for solutions containing 0.5–2% sulfuric acid. Considering this fact, the maximal xylose concentrations achieved in the set of experiments shown in Figs. 1 and 2 (27.0–29.2 g/L) corresponded to percentages of xylooligosaccharide conversion into xylose in the range of 93.0–99.9%. Under the operational conditions leading to maximal xylose concentrations, the content in residual xylooligomers was negligible, whereas the concentrations of glucose (in the range of 1.59–2.58 g/L) and arabinose (in the range of 2.79–3.57 g/L) were favorable for the manufacture of xylose-based fermentation media. On the other hand, under the same conditions, the concentration of acetic acid (a potential inhibitor of the microbial metabolism) was in the range of 4.02–4.52 g/L, whereas the concentration of furfural (a compound with

toxic effects when present in concentrations above a given threshold value) varied in the range of 0.32–1.37 g/L.

To provide comparative data for assessing the possible inhibition effects derived from the presence of furfural and acetic acid in the posthydrolysis liquors, it must be considered that the inhibition of the microbial metabolism depends on a variety of factors, including the nature of the fermentation medium, the type of microorganism and the fermentation process considered, and the possible adaptation of the microorganism to the environment. Typically, the inhibition by acetic acid occurs at concentrations ranging from 4 *(16)* up to 10 g/L *(17,18)*. The results obtained in our study for the acetic acid concentration are near the lower limit of this range, but it must be considered that the acetic acid concentration could be reduced during a possible concentration of posthydrolysis liquors before fermentation. On the other hand, it can be noted that the acetic acid is useful as a buffering agent in fermentation processes, and as a possible additional carbon source for microorganisms *(19)*. According to reported data, the furfural causes inhibition in the range of 1–5 g/L *(20,21)*, but this limit was reached in the present study only under harsh operational conditions.

Kinetic Modeling of Xylooligomer Degradation During Posthydrolysis

According to the ideas we have explained, the degradation of xylooligomers goes through sequential stages of hydrolysis to xylose and dehydration of this sugar to furfural. Since material balances showed that some furfural was consumed during the reaction, an additional reaction step (furfural conversion into degradation products) was necessary for the kinetic interpretation of data. The resulting mechanism is as follows:

$$\mathrm{XO} \xrightarrow{k_1} \mathrm{X} \xrightarrow{k_2} \mathrm{F} \xrightarrow{k_3} \mathrm{DP}$$

in which XO is xylooligomers, X is xylose, F is furfural, and DP is degradation products. The kinetic coefficients k_1, k_2, and k_3 were assumed to correspond to first-order reactions.

The experimental results were fitted to the equations derived from this kinetic model (see ref. *22* for equations) by minimization of the deviation squares to find the optimal values of the kinetic parameters k_1, k_2, and k_3, following the procedure reported elsewhere *(22)*. The values of the kinetic coefficients k_1 and k_2 obtained by data analysis are given in Table 1. With this information, the time course of the compounds participating in the reaction can be directly calculated (see solid and dotted lines in Figs. 1 and 2). The close correspondence between experimental and calculated data observed in all the experiments confirmed the suitability of the models for a quantitative interpretation of the experimental results.

As expected, the results achieved for the kinetic coefficients increased with both temperature and acid concentration. In the case of k_3, the results

Table 1
Values Obtained for Regression Parameters and Statistical R^2 Corresponding to Fitting of Experimental Data to Proposed Models

Experiment no.	Regression parameter k_1 (h^{-1})	k_2 (h^{-1})	k_3 (h^{-1})	k_a (h^{-1})	R^2 for variable[a] XO	X	F	AcH
1	0.286	0.000	0.000	0.563	0.956	0.956	<0.7	0.992
2	0.662	0.000	0.000	1.07	0.978	0.977	<0.7	0.974
3	1.08	0.000	0.000	1.75	0.988	0.987	<0.7	0.972
4	1.67	0.005	0.045	2.68	0.984	0.982	<0.7	0.979
5	0.822	0.000	0.000	1.09	0.958	0.978	<0.7	0.990
6	2.33	0.017	0.000	1.71	0.994	0.992	0.710	0.953
7	3.57	0.020	0.084	3.73	0.985	0.985	<0.7	0.991
8	3.97	0.032	0.189	4.07	0.994	0.991	<0.7	0.990
9	2.04	0.000	0.241	3.30	0.986	0.984	0.956	0.987
10	5.71	0.038	0.109	3.81	0.986	0.967	0.891	0.986
11	7.76	0.051	0.235	6.98	0.994	0.994	0.975	0.992
12	7.69	0.079	0.321	7.72	0.982	0.973	0.920	0.950
13	6.39	0.034	0.047	3.38	0.994	0.993	0.896	0.982
14	10.7	0.093	0.360	6.92	0.998	0.996	0.960	0.990
15	16.5	0.168	0.923	12.0	0.992	0.984	0.956	0.983
16	18.3	0.158	0.574	12.5	0.977	0.936	0.977	0.955

[a]XO = concentration of xylooligomers (g/L); X = concentration of xylose (g/L); F = concentration of furfural (g/L); AcH = concentration of acetic acid (g/L).

were significantly affected by the experimental error owing to the little importance of furfural decomposition.

To provide a combined interpretation of the effects caused by catalyst and temperature on the kinetic coefficients, reported studies *(23–25)* proposed an Arrhenius-type dependence on temperature with a preexponential factor dependent on the catalyst concentration. This approach leads to the following equation:

$$k_1 = k_{10} \cdot \exp\left(-\frac{E_a}{R \cdot T}\right) = a \cdot C^n \cdot \exp\left(-\frac{E_a}{R \cdot T}\right)$$

in which k is the kinetic coefficient, k_{10} is the preexponential factor, E_a is the activation energy, a and n are empirical parameters, and C is the molar catalyst concentration.

Figure 3 shows the fair interpretation of data resulting from this previous equation. The value of the parameter n (1.057) resulting from data analysis was close to 1, similar to that reported in the literature for the generation of xylose in acid-catalyzed media *(24,26)* and for the generation of glucose from cellulose *(25)*. To limit the number of regression parameters

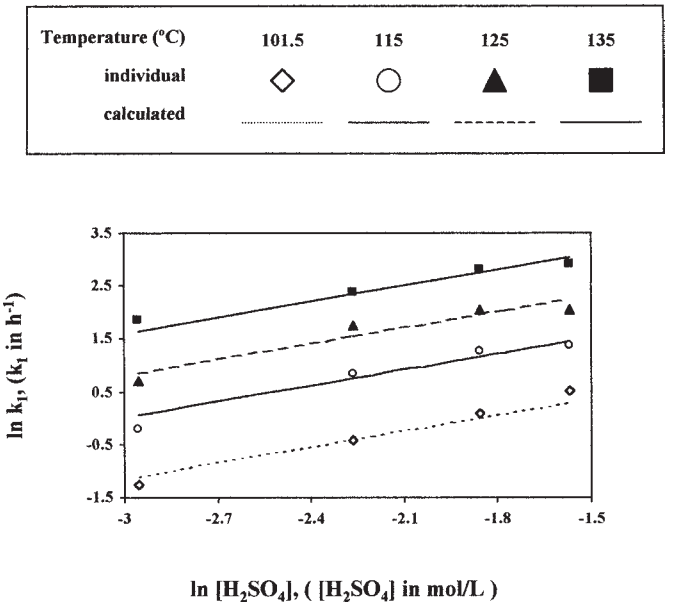

Fig. 3. Dependence of kinetic coefficient k_1 on catalyst concentration and temperature.

involved in the model, it was assumed that $n = 1$, and the whole set of k_1 values was recalculated, considering both a and E_a as regression parameters. This operational procedure led to $\ln a = 35.3$ (a being expressed in L/[h·mol H_2SO_4]), and $E_a = 104.1$ kJ/mol, with $R^2 = 0.983$.

The same operational procedure was followed to establish the dependence of k_2 on the catalyst concentration and temperature. In the analysis of data, experiments 1–5 and 9 were omitted, because the low severity led to values of this parameter near zero. When the rest of the experiments were fitted, the regression analysis led to $\ln a = 37.92$, (a being expressed in $h^{-1}\cdot$[mol of H_2SO_4/L]$^{-n}$), $n = 1.17$, and $E_a = 127.8$ kJ/mol, with $R^2 = 0.970$.

Modeling of Acetic Acid Generation in Posthydrolysis Experiments

The xylooligosaccharides coming from autohydrolysis liquors are substituted with acetyl groups, which are hydrolyzed to acetic acid in the posthydrolysis step according to the following reaction:

$$\mathrm{Ac} \xrightarrow{k_a} \mathrm{AcH}$$

in which Ac is the acetyl groups and AcH is acetic acid. Assuming that this reaction follows a irreversible, first-order kinetics with a kinetic coefficient k_a, the time course of the acetic acid concentration is given as follows:

$$\mathrm{AcH} = \mathrm{AcH_o} + [\mathrm{AcH_{max}} - \mathrm{AcH_o}] \cdot [1 - \exp(-k_a \cdot t)]$$

in which AcH_o is the initial concentration of acetic acid (g/L), AcH_{max} is the concentration corresponding to the total conversion of acetyl groups into

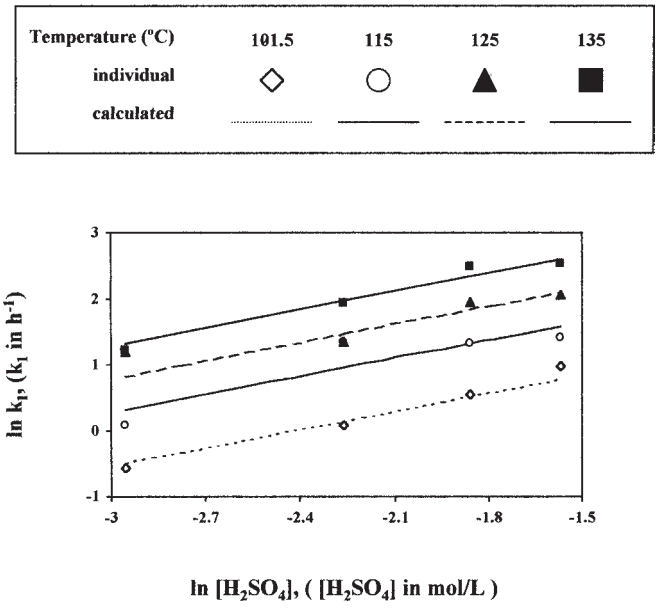

Fig. 4. Dependence of kinetic coefficient k_a on catalyst concentration and temperature.

acetic acid, and k_a is the kinetic coefficient. The data obtained by regression analysis are given in Table 1. The dependence of k_a on both catalyst concentration and temperature was described by the same equation already applied to k_1 and k_2 (see Fig. 4). This operational procedure led to the following results: $\ln a = 23.58$ (a being expressed in $h^{-1} \cdot [\text{mol of } H_2SO_4/L]^{-n}$), $n = 0.907$, and $E_a = 66.41$ kJ/mol, with $R^2 = 0.95$. As can be seen from Fig. 5, the experimental and calculated data showed a close interrelationship in all the assays performed.

Conclusion

The generation of xylose solutions to be used as fermentation media from corncobs through sequential stages of autohydrolysis and posthydrolysis shows favorable features in terms of substrate conversion, reaction selectivity, and low inhibitor concentration. Starting from autohydrolysis liquors obtained under selected operational conditions, and based on the knowledge of the posthydrolysis kinetics, operational conditions leading to high xylose yield with little decomposition to furfural have been identified. The kinetic modeling of the posthydrolysis step was carried out by means of a mechanism describing the xylooligomer degradation involving three sequential reactions, which allowed reliable interpretation of data. The acetic acid generated from the acetyl groups bound to xylooligosaccharides was modeled on the basis of a simple reaction with first-order kinetics. A further generalization of the dependence of the kinetic coefficients participating in the kinetic models on both temperature and catalyst

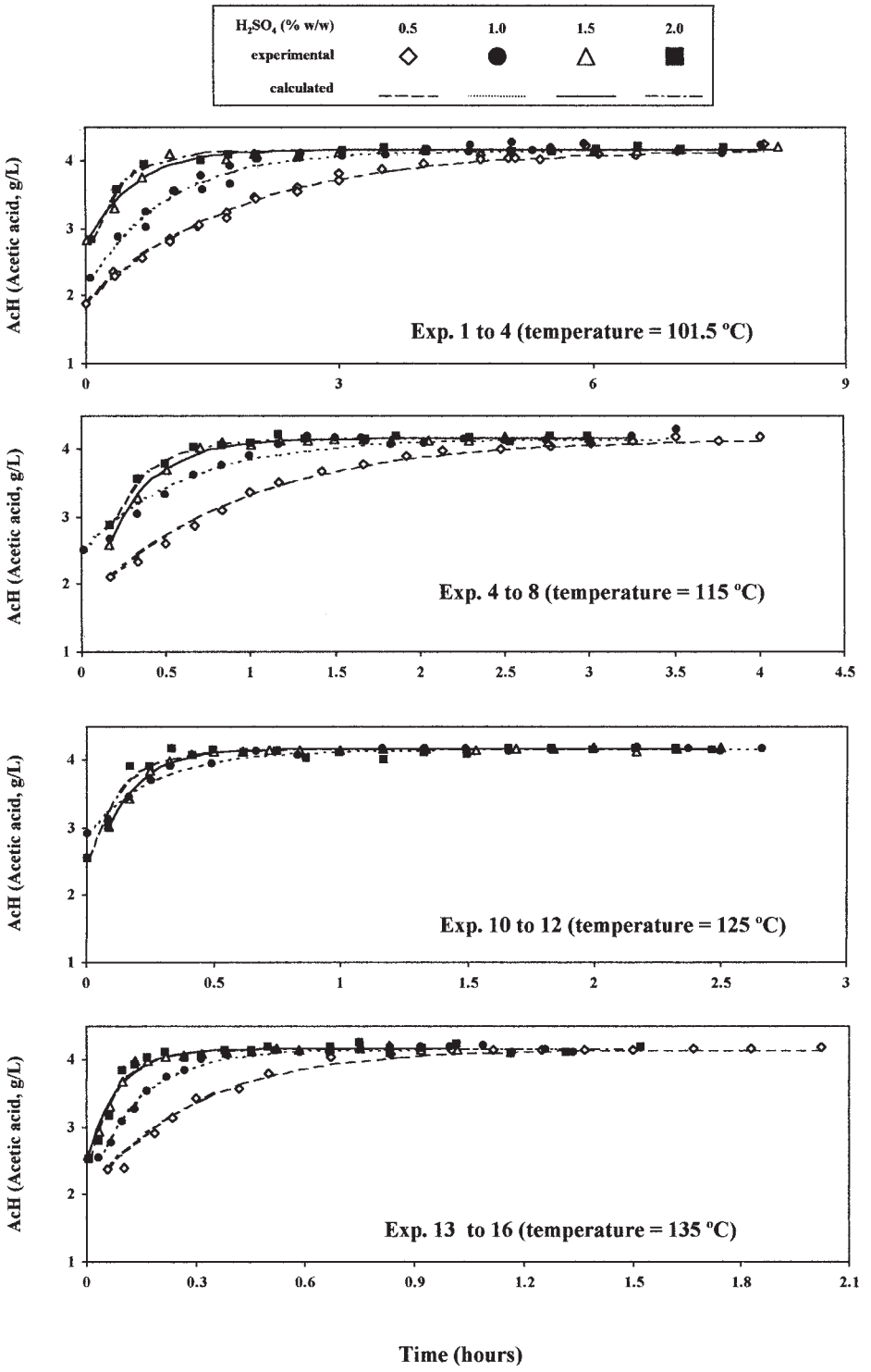

Fig. 5. Experimental and calculated time courses of acetic acid concentration (AcH) in experiments 1–16.

concentration allowed the development of a calculation procedure leading to the prediction of the concentrations of xylooligomers, xylooligomer decomposition products, and acetic acid in the whole range considered for the operational variables. The concentrations of other reaction byproducts (such as glucose and arabinose) were also determined. The possibility of making suitable xylose-based fermentation media from hydrolysates was supported by the compositional results achieved for the posthydrolyzed liquors.

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