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Study of the hydrolysis of sugar cane bagasse using phosphoric acid

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Abstract

In the present work, samples of sugar cane bagasse were hydrolysed with phosphoric acid under mild conditions (H_3PO_4 2–6%, time 0–300 min and 122 °C) to study the feasibility of using the liquid phase as fermentation media. Solid yield, sugar concentrations and decomposition product concentrations were measured. The composition of hydrolysates, their purity and the ratio sugars/inhibitors were analyzed. Kinetic models were developed to describe the course of products of the acid hydrolysis. The course of xylose, glucose, arabinose, acetic acid and furfural were satisfactorily described by the models. The optimal conditions selected were 122 °C, 4% H_3PO_4 and 300 min. Using these conditions, 17.6 g of xylose/l; 2.6 g of arabinose/l; 3.0 g of glucose/l, 1.2 g furfural/l and 4.0 g acetic acid/l were obtained. The efficiency in these conditions was 4.46 g sugars/g inhibitors and the mass fraction of sugars in dissolved solids in liquid phase was superior to 55%.
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1. Introduction

Sugar cane bagasse (SCB), a waste in the process of sugar extraction, is an abundant and low-cost lignocellulosic material (Ferrara, Bon, & Neto, 2002; Takahashi, Lima, Takahashi, & Alterthum, 2000). An adequate use of the SCB gives an added value for this material and a solution for the removal of this abundant waste, solving a problem of the sugar industry and increasing the economical yield of the process. Therefore, a double effect is obtained, economic and ecologic. The most frequent use for SCB is the energy production by its combustion (Kilicaslan, Sarac, Özdemír, & Ermiş, 1999; Neureiter, Danner, Thomasser, Saidi, & Braun, 2002). This provokes a problem of pollution, increasing the emissions of CO_2. Other alternative uses of SCB are the production of chemical compounds such as furfural or hydroxymethylfurfural (HMF) (Almazán, González, & Gálvez, 2001; Gong, Chen, & Chen, 1993), the production of paper paste (Caraschi, Campana, & Curvelho, 1996; Nagieb, Abd-El-Sayed, E-I-Sakhawy, & Khalil, 2000; Shukry, Hassan, Yousef, & Fadel, 2002) or the biotechnological production of ethanol (David, Fornasier, Greindl-Fallon, & Vanlautem, 1985; Grethlein & Converse, 1991; Laser et al., 2002).

SCB is formed by three main fractions (cellulose, hemicelluloses and lignin). Approximately a 25–33% of SCB are hemicelluloses (du Toit, Olivier, & van Bijon, 1984; Rodríguez-Chong, Ramírez, Garrote, & Vázquez, 2004; Sasaki, Adschiri, & Arai, 2003). In the SCB

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materials, the main hemicellulosic polymers are arabinoxylans, with a high content of xylose and lower of arabinose (Aguilar, Ramírez, Garrote, & Vázquez, 2002; Neureiter et al., 2002; Sun, Sun, Sun, & Su, 2004). On the basis of its composition, the SCB is a raw material very attractive for the production of fermentative media based on xylose. The main application of xylose is its bioconversion to xylitol, a functional sweetener with important technological properties (Rodrigues, Felipe, Roberto, & Vitolo, 2003). Xylitol can be also used for treatment of diabetics (Rodrigues, Silva, & Felipe, 1998). The biotechnological production of xylitol is a cheap alternative to the chemical way (Carvalho et al., 2002). The economic interest in xylitol production can be enhanced if the needed xylose solutions can be obtained from the hydrolysis of low-cost lignocellulosic wastes, such as the SCB.

To obtain sugar solutions of xylose from the SCB, treatments that solubilize the sugars from the hemicellulosic fraction and let the other fractions, celluloses and lignin, the most unaltered as possible in the solid phase, should be selected. Nowadays, there is not any treatment that is able to operate such a fractioning. One alternative is the hydrolysis with dilute acids (called acid hydrolysis or prehydrolysis). This consists in the hydrolysis of the hemicellulosic fraction with diluted acids (sulphuric, hydrochloric or acetic acid are habitual, typically 1–10% weight) using a moderate temperature (in the range 100–150 °C). The acid medium hydrolyses the polysaccharides. But in these relatively moderate operational conditions, it does not attack the cellulosic fraction in an appreciable extension.

Selecting the operational conditions, the liquid phase of the hydrolysis will be constituted by sugar (xylose, glucose and arabinose) and products of decomposition of the hemicelluloses (such as oligomers from the polymers and acetic acid generated from the hydrolysis of acetyl groups linked to sugars) and/or the decomposition of the monosaccharides released (such as furfural, product of dehydration of pentoses, and HMF, product of dehydration of hexoses).

The hydrolysates obtained after the acid hydrolysis need to be processed if they are going to be used as fermentation media. In general the following operations are needed (in this sequence): concentration, detoxification, neutralization and supplementation with nutrients.

The concentration of hydrolysates by evaporation is usual to increase the sugar concentration. In this operation, besides water, small amounts of growth inhibitors such as acetic acid, furfural and HMF are removed (Carvalho et al., 2002; Martínez et al., 2002).

A detoxification operation by adsorption on charcoal can remove the growth inhibitors cited. In this operation, phenolic compounds proceeding from lignin can also be removed (Gong et al., 1993; Rodrigues et al., 2003).

In the operation of neutralization, it is usual to add chemicals that neutralize the acids of the hydrolysates, forming salts (Alves, Felipe, Silva, Silva, & Prata, 1998; Alves, Vitolo, Felipe, & Silva, 2002). These salts have low solubility and are normally removed by filtration. For example, hydrolysates containing sulphuric acid are neutralized with calcium carbonate, forming calcium sulphate.

Finally, the processed hydrolysates are supplemented with several nutrients to be a favorable fermentation medium. These nutrients contribute the nitrogen and micronutrients needed for the growth of the microorganisms.

The acid most used is H_2SO_4 (Aguilar et al., 2002; Alves et al., 1998; Alves et al., 2002; Gong et al., 1993; Neureiter et al., 2002), although other acids can be used such as HCl (Bustos, Ramírez, Garrote, & Vázquez, 2003) or HNO_3 (Rodríguez-Chong et al., 2004).

The interest in the use of H_3PO_4 is that after neutralization of hydrolysates with NaOH, the salt formed is sodium phosphate. This salt can remain in the hydrolysates because it is used as nutrient by microorganisms. Therefore, an operation of filtration is not needed with the consequent advantage: improve the economic of the process (avoid the filtration to remove the salts and decrease the amount of nutrient needed for fermentation) and is friendly with the environment (the salt formed is not a waste).

This work deal with the study of the hydrolysis of SCB with phosphoric acid in autoclave (122 °C), to obtain fermentative media with high sugar concentrations and low growth inhibitor concentrations. Kinetic models were developed to explain the time course of the xylose, glucose, arabinose, acetic acid and furfural generated.

2. Materials and methods

2.1. Raw material

The raw material used in experiments was SCB from a local industry (Ingenio Azucarero de Mante, Tamaulipas, México). The SCB were air dried, milled, screened to select the fraction of particles with a size lower than 0.5 mm, homogenized in a single lot and stored until needed.

2.2. Analysis of samples

Aliquots of the homogenised lot are analyzed for moisture determination (drying at 105 °C to constant weight). Analyses of the main fractions (cellulose, hemicelluloses and Klason lignin) were carried out using a quantitative acid hydrolysis under standard conditions (Garrote, Domínguez, & Parajó, 1999a).

2.3. Acid hydrolysis of samples

Treatments were performed at 122 °C in media containing 2, 4 or 6 g H_3PO_4/100 g, values selected according the literature (Téllez-Luis, Ramírez, & Vázquez, 2002). All experiments were performed using a water/solid ratio (WSR) of 8 g water/g sugar cane bagasse on dry basis. Samples were collected at several reaction times in the range 0–300 min and centrifuged. The pellets were washed twice with sterile water and the supernatant used to determine the composition of the hydrolysates (glucose, xylose, arabinose and acetic acid) by HPLC. The analyses were carried out using a Transgenomic ION-300 column (oven temperature = 45 °C) with isocratic elution (flow rate = 0.4 ml/min; mobile phase: H_2SO_4 0.005 N) and a refraction index detector. Furfural was determined by spectrophotometry at 280 nm (Téllez-Luis et al., 2002). The pellets were used for determining the solubilized fraction (SF) by the difference from the original weight, drying at 105 °C, to constant weight. The SF was calculated as grams of solubilized solids recovered after acid hydrolysis per 100 g of raw material, on dry basis.

2.4. Statistical analysis

All experimental data were carried out in triplicate and averages are given. Non-linear regression analyses of experimental data were performed with a commercial optimization routine dealing with the Newton's method (Solver, Microsoft Excel 2000, Microsoft Corporation, Redmond, WA, USA) by minimizing the sum of the squares of deviations between experimental and calculated data according the philosophy reported previously (Garrote, Domínguez, & Parajó, 2001a).

3. Results and discussion

3.1. Raw material composition

The composition obtained for the SCB was (average values of three replicates, error lower than 1% in all compounds, weight percent on dry basis): Glucan, 38.9%; Xylan, 20.6%; Araban, 5.56%; Klason lignin, 23.9%; others (by difference), 11.0%. These values are in the range found for this kind of materials (Laser et al., 2002; Neureiter et al., 2002; Sasaki et al., 2003).

3.2. Potential concentration

Using the value of WSR and the composition of raw material, can be calculated the potential concentration of each sugar, assuming a total conversion of polysaccharides to sugar without degradation.

This is an ideal situation because it is known that main fraction affected by acid hydrolysis, hemicelluloses, usually is degraded to compounds such as furfural. On the other hand, it is not possible, in acid hydrolysis, to obtain the total and simultaneously conversion of cellulose and hemicelluloses. For comparative purposes and kinetic calculations, the maximum concentrations for each sugar were calculated using the following equation:

$$S_P = f \cdot \frac{CPn_0}{WSR} \cdot \rho \qquad (1)$$

where S_P is the maximum (potential) concentration of each sugar (in g/l), f is the stoichiometric factor due to the hydration of molecules during the hydrolysis ($f_{pentoses}$ is 150/132 and $f_{hexoses}$ is 180/162), CPn_0 is the composition of raw material for the polysaccharide Pn (in g of polysaccharide/g of raw material, on dry basis), WSR is the water/solid ratio used (8 g/g) and ρ is the density of hydrolysates (in g/l).

Applying Eq. (1), it is obtained the potential composition of liquors in glucose ($G_0 = 54.0$ g glucose/l), xylose ($X_0 = 29.3$ g xylose/l) and arabinose ($Ar_0 = 7.90$ g arabinose/l).

The potential concentration of acetic acid generated by the hydrolysis of acetyl groups was not determined because the content of acetyl groups was not determined in the SCB. In the acid hydrolysis, furfural is generated by dehydration of pentoses (xylose and arabinose) and HMF by dehydration of hexoses (glucose in this case). Using the stoichiometric factors ($f_{furfural} = 96/132$ and $f_{HMF} = 126/162$) was also obtained the potential concentration of furfural ($F_0 = 19.0$ g of furfural/l) and HMF ($HMF_0 = 30.3$ g of HMF/l). Obviously, the conditions that lead to obtain these potential concentrations for furfural and HMF also lead to obtain zero concentration of sugars.

3.3. Composition of hydrolysates

Fig. 1 shows the concentrations of the sugars determined in the hydrolysates for different hydrolysis times (xylose, arabinose and glucose concentrations).

The highest xylose concentration was 17.6 g/l in the experiment carried out at 4% H_3PO_4 during 300 min. Xylose concentration was always increasing with time except when the highest concentration of phosphoric acid was used (6% H_3PO_4). In this case xylose concentration decreased from 14.7 g/l at 180 min to 13.6 g/l at 300 min.

The rate of xylose release increased with the phosphoric acid concentration. For example, xylose concentrations in hydrolysates at 60 min of reaction were 6.1, 7.3 and 8.6 g/l using H_3PO_4 concentrations of 2%, 4% and 6%, respectively. This effect was also observed in the kinetic coefficients that are shown later.

Arabinose concentrations (see Fig. 1) increased quickly up to values around 3 g/l (2.8 g/l at 300 min using 2% phosphoric acid, 3.0 g/l at 180 min using 4% and 2.7 g/l at 60 min using 6%). Using the lowest
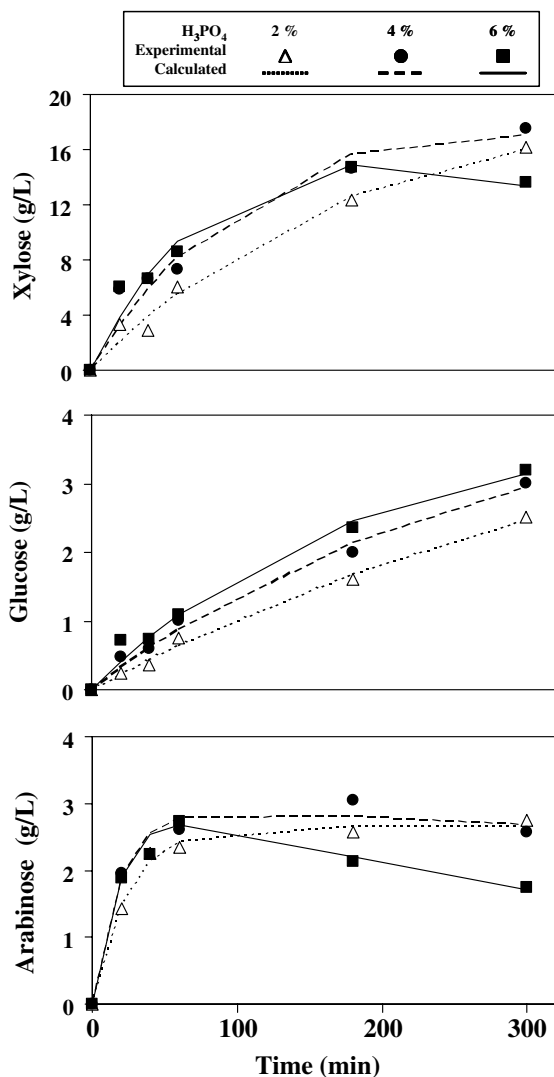
Fig. 1. Experimental and predicted dependence of the sugars released with the time during the hydrolysis using 2%, 4% or 6% H_3PO_4 at 122 °C.

concentration of acid, 2% H_3PO_4, it was observed an initial fast increase in the concentration (2.2 g/l at 40 min) and then a slow increase. Using 4% H_3PO_4, it was also observed an initial fast increase in the concentration (2.0 g/l at 20 min), with maximum value at 180 min (3.0 g/l) and then a decrease at long time (2.6 g/l at 300 min). Using the highest concentration, the maximum value was obtained at 60 min (2.7 g/l) and then decreased to 1.8 g/l at 300 min. This behavior is typical of a compound in degradation.

Arabinose is present as furanose that is more susceptible to hydrolysis than the piranose xylose (Fengel & Wegener, 1984). This implies a faster present in the hydrolysates than the piranoses.

It was observed that the decomposition reactions are more important in arabinose than in xylose, obtaining less than 40% of the potential concentration of arabinose. In the case of the xylose, it was only observed

decomposition reactions using the highest concentration of phosphoric acid and long times, decreasing the xylose concentration only 8% on the maximum; meanwhile in the case of the arabinose this decrease was general, being the decrease of arabinose concentration up to 36% of the maximum. This observation let us to deduce that the formation of furfural is mainly due to the decomposition of arabinose.

The glucose concentration increased with reaction time and phosphoric acid concentrations, obtaining values at 300 min of 2.5, 3.0 and 3.2 g/l using H_3PO_4 concentrations of 2%, 4% and 6%, respectively. A decreased in the concentration of glucose was not observed. This fact implies that degradation reactions to HMF were not effective. HMF is a growth inhibitor of microorganism with a lethal effect much more important than furfural (Larsson et al., 1999). The low glucose concentrations (lower than 6% of the potential concentration) also imply that the glucan fraction was practically unaltered (an average of 98% from the initial glucan remained in the solid phase) or more probable, the glucose obtained came from the hemicellusic fraction.

For comparatives purposes, the concentration of pentoses (*P*, sum of arabinose and xylose) and the concentration of total sugars (*S*, sum of arabinose, glucose and xylose) were calculated. The conclusion of the analysis of both *P* and *S* is the same. In the first 60 min, the concentration of *P* and *S* increased with time and phosphoric acid concentration. Over 60 min, the rate of release is lower, being more drastic using high acid concentration. It was observed only a decrease of *P* and *S* using the most drastic conditions (300 min, 6% acid). The maximum values were obtained at 300 min using 4% H_3PO_4 (20.1 g pentoses/l and 23.1 g sugars/l). Comparing these results obtained at 122 °C with whose reported at 100 °C (Gámez, Ramírez, Garrote, & Vázquez, 2004), higher values of pentoses and total sugars at 122 °C was obtained and lower acid concentration was needed for this maximum (4% at 122 °C vs. 6% at 100 °C).

3.4. Inhibitors composition

Acetic acid is generated in the hydrolysis of the acetyl groups of the hemicelluloses (Fig. 2). The acetic acid concentration increased quickly with time and phosphoric acid concentration during the first 60 min. After that, the rate of release was very low but the acetic acid concentration was not decreased indicating that no decomposition reactions occur. The highest value was 4.0 g acetic acid/l in the experiment carried out at 4% H_3PO_4 during 300 min. Using concentrations of phosphoric acid of 2% or 6%, the trend was the same than using 4%, but the maximum values were close to 3 g acetic acid/l.

Acetic acid can be an inhibitor of microbial growth from 4 to 10 g/l (Lawford & Rousseau, 1998) because
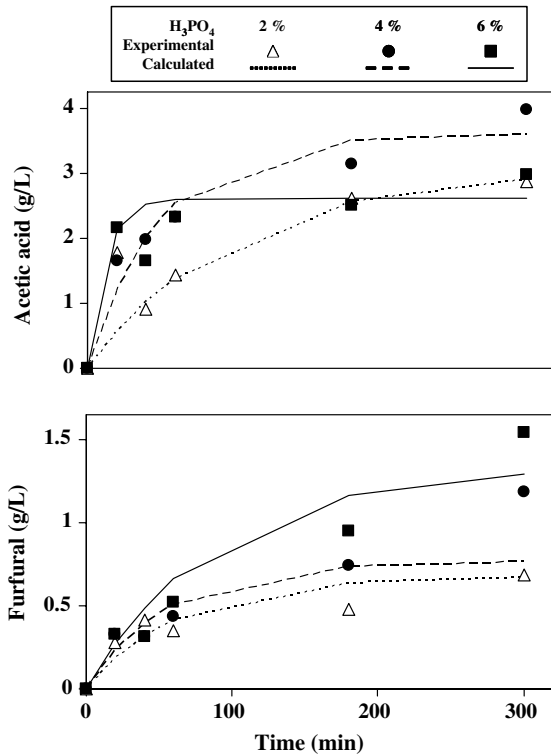
Fig. 2. Experimental and predicted dependence of the decomposition products with time during the hydrolysis using 2%, 4% or 6% H₃PO₄ at 122 °C.

Table 1
Values of solubilized fraction SF

Time (min)	2% H_3PO_4	4% H_3PO_4	6% H_3PO_4
0	0	0	0
20	19.5 ± 0.3	24.5 ± 0.2	25.4 ± 0.9
40	26.2 ± 0.1	31.1 ± 0.2	34.0 ± 0.0
60	27.5 ± 0.4	31.9 ± 0.3	33.5 ± 0.9
180	36.5 ± 0.8	35.0 ± 0.3	31.7 ± 0.5
300	38.6 ± 0.6	24.4 ± 0.5	24.9 ± 0.3

rial. The economics of this process can be enhanced if the hydrolysates (liquid phase) have the lowest amount of other compounds that are not sugars. This decreases the cost of downstream treatments.

Above the volumetric composition of liquors were discussed. Base on material balances, the composition of hydrolysates can be expressed using other units, such as mass fraction of solubilized solids. Using these new units, it is possible to know what the fraction represent each compound in the liquid phase (excluding water). For convenience, the compounds studied were grouped as follow: total sugars (S), inhibitors (I = acetic acid + furfural) and others. The fraction others is calculated by difference and included mainly the extracts of the raw material (11%) and the solubilized lignin. The mass fractions of sugar (F_S), inhibitors (F_I) and others (F_O) were calculated as follows:

$$F_S = S \cdot \left(WSR + \frac{SF}{100} \right) \cdot \frac{100}{SF} \cdot \frac{1}{\rho} \qquad (2)$$

$$F_I = I \cdot \left(WSR + \frac{SF}{100} \right) \cdot \frac{100}{SF} \cdot \frac{1}{\rho} \qquad (3)$$

$$F_O = 1 - F_S - F_I \qquad (4)$$

The use of the hydrolysates as fermentation media needs a previous detoxification treatment to remove the growth inhibitor. After the fermentation, purification of products and waste treatments are also needed. It is evident that if the amount of inhibitors in hydrolysates is small, the global process will be more profitable.

Fig. 3 shows the variation of the three mass fractions with the reaction time. It was observed a similar behaviour for F_S and F_I, both opposing to F_O. F_S and F_I increased with reaction time, quickly at 20 min and then slowly. F_S and F_I also increased with phosphoric acid. The maximum values were $F_S = 0.57$ g sugars/g solubilized solids and $F_I = 0.14$ g inhibitors/g solubilized solids, both at 300 min and 6% H_3PO_4.

The trend of F_O was opposed, decreasing with reaction time and acid concentration, being the minimum value (then economic optimum) of $F_O = 0.29$ g others/g solubilized solids at 300 min using 6% acid.

The conditions that permit to obtain the maximum sugar concentrations (300 min using 4% H_3PO_4) have also values close to the optimum of mass fractions,

it goes through the cellular membranes and decrease intracellular pH, which affect to the metabolism of the microorganism (Maiorella, Blanch, & Wilke, 1983; van Zyl, Prior, & Du Preez, 1991). In our study, the maximum acetic acid concentrations were in the lower limit of the toxic effect.

The furfural concentration also increased with time and phosphoric acid (Fig. 2), being the maximum values 0.68, 1.2 and 1.5 g furfural/l at 300 °C using H₃PO₄ concentrations of 2%, 4% and 6%, respectively. The values obtained are relative low, 4%, 6% and 8% of the potential concentration of furfural. The average furfural concentration was 0.5 g/l, a 2.6% of the potential furfural concentration. This shows that the decomposition of pentoses to furfural is low and confirms the selectivity of this treatment using phosphoric acid.

3.5. Purity of hydrolyzates

Table 1 shows the variations of the solubilized fraction, SF, in the experiments performed. The SF values increased quickly during the first times of reaction. Then, using 2% acid it increased slowly up to 39 g/100 g, using 4% it remain around 35 g/100 g and using 6% decreased to 25 g/100 g.

The main fraction affect by the acid hydrolysis is the hemicelluloses, but other fractions are also partially solubilized, such as lignin or extracts presents in this mate-
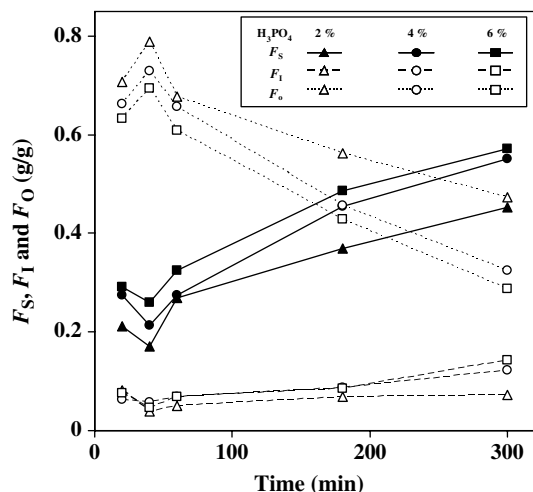
Fig. 3. Variation of the mass fractions of sugar (F_S), inhibitors (F_I) and others (F_O) with time during the hydrolysis with 2%, 4% or 6% H_3PO_4 at 122 °C.

F_S = 0.55 g /g, F_I = 0.12 g/g and F_O = 0.33 g/g, therefore the economics of the process is feasible.

3.6. Optimization of the composition of fermentation media

The main use of hydrolysates is for fermentation process. Then it is required that the sugar solutions have high sugar concentrations (carbon source for microorganism growth) and low concentration of growth inhibitors (acetic acid and furfural). To compare the different experiments, the efficiency as catalyst can be defined as the ratio E:

$$E = \frac{S}{I} \qquad (5)$$

Fig. 4 shows the variation of E with the catalyst concentration and reaction time. A small increase of E was observed in the experiments using 2% acid (E = 6.0 g sugars/g inhibitors at 300 min), while using 4% acid the maximum value was 5.1 g/g a 180 min and then decreased to 4.5 g/g at 300 min. Using 6% acid the trend was the same that using 4%, decreasing from 5.6 g/g at 180 min to 4.1 g/g at 300 min.

Comparing these results with those reported using H_3PO_4 at 100 °C (Gámez et al., 2004), it was observed that the decomposition reaction of xylose and mainly
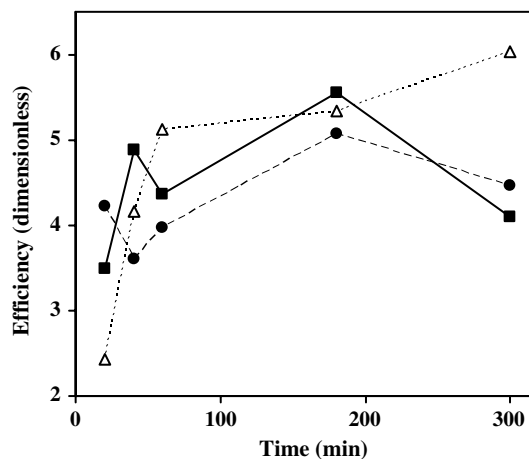

Fig. 4. Variation of the efficiency (E) with the reaction time during the hydrolysis with 2%, 4% or 6% H_3PO_4 at 122 °C.

arabinose have an important influence in the values of E. Using 2% acid, E always increased with time, while using 4% or 6% a decrease at 300 min was observed.

In Table 2 it was compared the results in the same operational conditions using different strong acids. Lower values of E were obtained with those acids, in the range 2.07–4.03 g/g, while in the conditions of this work E was 4.47 g/g.

3.7. Kinetic models

The treatments of dilute acids (typically strong acid at concentration under 10%) on lignocellulosic materials have the target of solubilizing the hemicelluloses to obtain a liquid phase with high concentration of sugars. The collateral effects are the partial solubilization of cellulose, extracts (previously called fraction of others) and lignin. Selecting the operational conditions, it is possible to maximize solubilizing the hemicelluloses and to minimize the collateral effects. For this selecting, the mathematical modeling of reaction of hydrolysis is very useful. The main reaction is the hydrolysis of hemicelluloses, therefore kinetic models should be developed for this fraction.

The modelling of the hydrolysis of a polysaccharide is very complicate. Multiple factors related with the lignocellulosic material (size, particle shape, structure, accessibility of proton to heterocyclic ether bond, etc.) and

Table 2
Comparison of the values of efficiency E obtained using different acids

Acid	Concentration (wt.%)	Temperature (°C)	Time (min)	E (g/g)	Reference
H_2SO_4	4	122	300	3.75[a]	Aguilar et al. (2002)
HCl	4	122	300	2.07	Bustos et al. (2003)
HNO_3	4	122	300	4.03	Rodríguez-Chong et al. (2004)
H_3PO_4	4	122	300	4.47	This work

[a] No arabinose was measured.

with the reaction medium (type of acid, concentration, temperature, time, etc.) affect. Other factors can also interfere in the determination of the reaction (Carrasco & Roy, 1992; Conner, 1984; Springer & Harris, 1985), such as the amount of wet in the raw material, interferences of other compounds, presence of different kind of bonds (sugar–sugar, sugar–acetyl groups, etc.), protector effect of the structured of the intact cell, etc. A deep analysis is reported by Gámez et al. (2004).

By the middle of the past century, the acid hydrolysis of cellulose was studied (Saeman, 1945). The main difficulty to study this lineal homopolymer is the presence of two fractions, one is crystalline and the other is amorphous, with different susceptibility to acid hydrolysis.

In the case of the hemicelluloses, the problem is more difficult to study than that of the cellulose, due to the presence of a heterogenic nature with several sugar monomers (xylose, arabinose and glucose) and other compounds such as acetyl groups and uronic acids. All these compounds are linked forming a ramified polymer with an unknown structure.

The analytical methods used (habitually HPLC) measuring sugar and other compound concentration (glucose, xylose, arabinose and acetic acid) and these are expressed as homopolymers in the origin lignocellulosic material (glucan, xylan, araban and acetyl groups). Glucan fundamentally corresponds to cellulose, xylan, araban and acetyl groups are linked in the hemicelluloses, forming the arabanoxylan, the main heteropolymer in the agricultural materials (Garrote, Domínguez, & Parajó, 1999b).

The solution of compromise between the complexity of a rigorous model and the search of equations that modelling the empirical data in a simple and satisfactory way have conducted to the general use of pseudohomogeneous, irreversible and first order kinetics that make easy the calculations without to sacrifice the theory basis.

It is important to remark that the practical application of the study of kinetic models is the optimization of the process but also the performance of economic estimations (Maloney, Chapman, & Baker, 1985).

The simplified models for the study of the kinetics of hydrolysis process using acids began with the work of Saeman for the hydrolysis of douglas fir wood using sulphuric acid (Saeman, 1945). In this research the hydrolysis of cellulose was studied establishing the following model:

$$\text{Cellulose} \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} \text{Decomposition products}$$
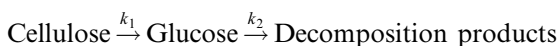
where k_1 (min^{-1}) is the rate for release of glucose from cellulose and k_2 (min^{-1}) is rate for glucose decomposition.

This model considers the hydrolysis of cellulose to release glucose that in severe conditions is decomposed. Both reaction (release and decomposition) was considered irreversible and first order reactions. The model of Saeman was also applied to the hydrolysis of the hemicellulosic fraction (Grant, Han, Anderson, & Frey, 1977; Téllez-Luis et al., 2002).

The model of Saeman can be applied to other polysaccharides; therefore the model can be generalized for any polymer:

$$\text{Polymer} \rightarrow \text{Monomer} \rightarrow \text{Decomposition products}$$
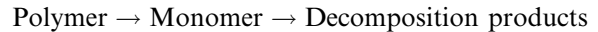
The generalized polymer could be cellulose, xylan, araban, etc. Solving the differential equations for an isothermal reaction, the following model predicts the concentration of monomers:

$$M = M_0 \cdot e^{-k_2 \cdot t} + P_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \tag{6}$$

where M and P are concentrations of monomer and polymer expressed in g/l, t is time and subscript 0 indicates initial conditions. Assuming $M_0 \cong 0$, Eq. (6) can be simplified to:

$$\frac{M}{P_0} = \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \tag{7}$$

Other possibility is that only one fraction of the polymer reacts in the operational conditions studied. This alternatives have being considered in the model called two-fraction model where it is considered a susceptible or fast fraction and a less susceptible or slow fraction. The ratio between the two fractions is the measured for the parameter α (mass fraction of the susceptible polymer in the raw material). The simplest case for the model of two-fraction is that the lesser susceptible fraction do not reacts, remaining always in the solid phase. Then, the equation that governs the kinetics is:

$$\frac{M}{P_0} = \alpha \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \tag{8}$$

In this work, the models cited above have been applied to the different fractions affected by the hydrolysis using phosphoric acid (glucan, xylan, araban, acetyl groups and furfural).

3.8. Kinetic modeling of xylose concentration

Xylose is the main product obtained in the acid hydrolysis of SCB. It is accepted that the hydrolysis of xylan is better modelled using the two-fraction model than the Saeman model (Garrote et al., 2001a; Kim & Lee, 1987). In some case, the fast fraction is the 100% of the initial xylan (Garrote, Domínguez, & Parajó, 2004). In hydrolysis of SCB using sulphuric acid it was found that the model of two-fraction fit better the experimental data, but with a great dispersion of data that hinder the analysis (Lavarack, Griffin, & Rodman, 2000).

In our study, the model that fitted better was the model of Saeman (Eq. (7)). Table 3 shows the results of the fit-

Table 3
Results of fitting the kinetic models

	2% H$_3$PO$_4$	4% H$_3$PO$_4$	6% H$_3$PO$_4$
Xylan			
$k_1 \times 10^3$ (min^{-1})	3.64	5.89	7.36
$k_2 \times 10^3$ (min^{-1})	1.12	1.92	3.52
r^2	0.98	0.96	0.97
Araban			
$k_1 \times 10^3$ (min^{-1})	40.8	51.5	50.3
$k_2 \times 10^3$ (min^{-1})	0.00	0.34	2.11
α_A (g/g)	0.34	0.37	0.39
r^2	0.99	0.98	0.999
Glucan			
$k_1 \times 10^3$ (min^{-1})	2.38	3.84	4.90
$k_2 \times 10^3$ (min^{-1})	0.000	0.003	0.305
α_G (g/g)	0.09	0.08	0.08
r^2	0.99	0.99	0.99
Acetyl groups			
$k_1 \times 10^3$ (min^{-1})	10.1	20.4	84.5
Ac_0 (g/l)	3.06	2.60	3.62
r^2	0.99	0.94	0.96
Furfural			
$k_1 \times 10^3$ (min^{-1})	15.7	18.0	11.4
F_0 (g/l)	0.68	0.76	1.33
r^2	0.95	0.96	0.90

ting of the kinetic parameters of the Saeman model for xylan and the statistical value r^2. Fig. 1 shows the comparison between experimental data and calculated with the Saeman model for the xylose concentration.

The values of k_1 and k_2 increased with acid concentration. The values of k_1 are higher than those for k_2, but the ratio k_1/k_2 decreased with the time. Values of k_1/k_2 were 3.3, 3.1 and 2.1 for acid concentrations of 2%, 4% and 6%, respectively, indicating that the decomposition reactions of xylose are more important using higher acid concentration. In treatments using sulphuric acid (Aguilar et al., 2002), the values of k_1 were slightly higher, while the values of k_2 were similar (the ratio k_1/k_2 was in the range 48–90). Treatments using hydrochloric acid gave ratios k_1/k_2 in the range 23–147 (Bustos et al., 2003) and using nitric acid in the range 19–46 (Rodríguez-Chong et al., 2004). Comparing these ratios, it is possible to deduce that the decomposition reactions of xylose are faster using phosphoric acid.

3.9. Kinetic modeling of arabinose concentration

Arabinose is a sugar formed from arabinoxylans, hemicellulosic heteropolymers found in agricultural materials like SCB. In arabinoxylans, it is higher the amount of xylose than arabinose. In the case of SCB, it was found that the content of arabinose is in the range 9–16% in weight (Sun et al., 2004).

The maximum concentration obtained of arabinose was 3.0 g/l and the potential concentration was 7.9 g/l,

this indicates that a fraction of araban does not react or that the decomposition reactions are very important.

The results fitting the Saeman model were unsatisfactory, with an average values for r^2 of 0.83. However, the model of two-fraction with one that does not react gave a better fitting. The parameter α_A is the ratio between susceptible araban and total araban. Eq. (8) was modified for adapting it to araban.

$$Ar = \alpha_A \cdot Arn_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \quad (9)$$

Arn_0 is 7.90 g/l. Fig. 1 shows the comparison between experimental data of arabinose concentration and those calculated fitting Eq. (9). Table 3 shows the values of the kinetic parameters k_1, k_2 and α_A, as well as the values of r^2 (in the range 0.98–0.999).

The value of α_G varied from 0.34 g/g to 0.39 g/g, with a slightly increase with the acid concentration, but the small differences can be due to the low values obtained that are more affected by the experimental error. In SCB hydrolysis with other acids, α_A shows a similar trend, with an average values at 122 °C of 0.53 g/g in experiences carried out with HCl (Bustos et al., 2003) and 0.39 g/g with HNO$_3$ (Rodríguez-Chong et al., 2004), close to the average value of this work, 0.37 g/g. No special influence of acid concentration was found.

The values of k_1 were higher than those found for xylose (from 6.8 to 11.2 times higher), confirming the faster susceptibility of arabinose to acid hydrolysis. Kinetic coefficients for arabinose decomposition (probably to furfural) were in the range 0–2.11 × 10^{-3} min^{-1}. The ratio k_1/k_2 decreased with the acid concentration, with values of 151 and 24 at acid concentrations of 4% and 6% of phosphoric acid, respectively.

3.10. Kinetic modeling of glucose concentration

During the acid hydrolysis, low concentrations of glucose are released to the reaction medium. The glucose can proceed from the hydrolysis of some hemicellulosic polymer or from cellulose. The operational conditions selected does not attack cellulose because is a polymer very resistant to dilute acids. Experimental evidence of the presence of glucose in hemicellulosic polymers in SCB has been made (Sun et al., 2004), with mass fractions of glucose in hemicelluloses in the range 0.04–0.20 (especially in water soluble hemicelluloses).

The potential concentration of glucose (Gn_0) was determined as it was done for xylose, resulting Gn_0 = 54.0 g/l, while the experimental maximum concentration was 3.2 g/l. The continuous increase indicates that decomposition reactions do not occur. Then the better model to apply is the model of two-fraction with one that does not react. In this case, the reactive fraction is the hemicellulosic glucan and the fraction that does not react is the cellulosic glucan. Both fractions are

related by the parameter α_G, defined as the glucan fraction susceptible of hydrolysis (g of hydrolysable glucan/total glucan). Eq. (8) was adapted for glucan as follows:

$$G = \alpha_G \cdot Gn_0 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \qquad (10)$$

Fig. 1 shows the comparison between experimental values of glucose and calculated fitting the equation. Table 3 shows the values for the kinetic parameters k_1, k_2 and α_G, as well as values for r^2 that was close to 0.99.

The value of α_G varied from 0.08 g/g to 0.09 g/g, with an average value of 0.083 g/g. This value corresponds with a 3.2% of the raw material and is the hemicellulosic glucan. Influence of acid concentration was not observed.

The values of k_1 and k_2 increase with the phosphoric acid concentration. The rate of glucose release is a little lower than the rate of xylose release, finding that the ratio $k_{1\,GLUCOSE}/k_{1\,XYLOSE}$ is constant with a value of 0.66. The values of the ratio k_1/k_2 of glucose were 1280 and 16.1 using H_3PO_4 concentrations of 4% and 6%, respectively, indicating that the decomposition reactions of glucose are negligible. This is very interesting because the main decomposition product of glucose is HMF, a great microbial growth inhibitor, and the main application of the hydrolysates is its use as fermentation media.

3.11. Kinetic modelling of acetic acid concentration

Some hemicellulosic monomers like xylose can have bonded acetyl groups. In acid media, acetyl groups are hydrolyzed to acetic acid. The results showed that the acetic acid concentration increased until a constant value. The following simple model, according with the literature, was applied (Garrote, Domínguez, & Parajó, 2001b; Lawford & Rousseau, 1998; Maloney et al., 1985):

Acetyl groups $\overset{k_1}{\rightarrow}$ Acetic acid

Based on this reaction model and solving differential equation leads to Eq. (11), this expresses the acetic acid concentration (AcH) as a function of time (t).

$$\frac{AcH}{Ac_0} = 1 - e^{-k_1 \cdot t} \qquad (11)$$

where Ac_0 is the potential concentration of acetyl groups and k_1 is the rate of acetic acid generation (min^{-1}). The Ac_0 was expressed as acetic acid and introduced as a regression parameter. Table 3 shows the coefficients obtained in the fitting the experimental data. Fig. 2 shows the comparison between experimental values of acetic acid and calculated fitting the Eq. (11).

Ac_0 varied between 3.6 g/l and 2.6 g/l, with an average value of 3.1 g/l. An influence of phosphoric acid concentration was not observed. The values of acetic acid obtained are relatively low for the use the hydrolysates as fermentation media. The values of k_1 increased with

the phosphoric acid concentration and were higher than the values found for release of xylose or glucose.

3.12. Kinetic modelling of furfural concentration

Furfural is generated as a decomposition product of pentoses in the hydrolysis of SCB. Based on the experimental data of furfural concentration, the model to use can be similar than the model used for acetic. Eq. (11) expresses the furfural concentration (F) as a function of time (t).

$$\frac{F}{F_0} = 1 - e^{-k_1 \cdot t} \qquad (12)$$

where F_0 is the potential concentration of furfural and k_1 is the rate of furfural generation (min^{-1}). F_0 was introduced only as a regression parameter. Table 3 shows the kinetic and statistical parameters obtained in the fitting of furfural generated in the hydrolysis. Fig. 2 shows the comparison between experimental and predicted data. The value of k_1 increased with the concentration of acid. F_0 also increased with the concentration of acid, in the range 0.68–1.33 g/l, values enough low to do not hide fermentations in the hydrolysates.

3.13. Dependence of the kinetic parameters with phosphoric acid concentration

To model the effect of the acid concentration on the kinetics, a potential variation of the kinetic coefficients with the acid concentration was supposed (Téllez-Luis et al., 2002):

$$k_i = a_i \cdot C^n \qquad (13)$$

where k_i is the kinetic coefficient (with $i = 1$ for release reactions and $i = 2$ for decomposition reaction), a_i and n are regression parameters and C is the concentration of phosphoric acid (in mass fraction). This behaviour is frequently found in this kind of hydrolysis (Garrote et al., 2001b; Maloney et al., 1985).

In Table 4 is shown the values of the parameters a_i and n as well as the values of the statistical parameter r^2, which values show the good agreement of the kinetic coefficients of Eq. (13). This table only shows the parameters for k_1 (reactions of releases) because the coefficient k_2 (reactions of decomposition of sugar) was zero

Table 4
Effect of H_3PO_4 concentration on regression parameter of rates of releases

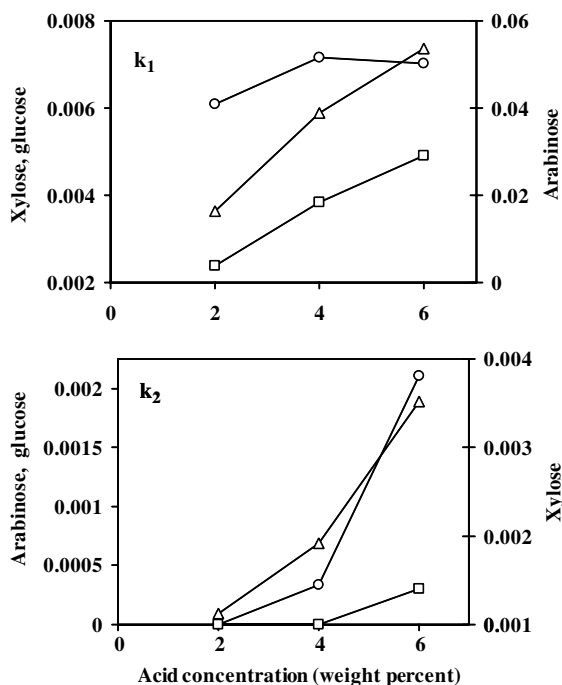| Fraction	$\ln a_1$ (a_1 in min^{-1})	n (dimensionless)	r^2
Xylan	−3.07	0.65	0.996
Araban	−2.37	0.22	<0.80
Glucan	−3.44	0.66	0.999
Acetyl groups	1.41	1.50	<0.80
Furfural	−5.00	−0.24	0.94

Fig. 5. Variation of the kinetic coefficients k_1 (release) and k_2 (decomposition) for xylan (triangle), glucan (square) and araban (circle).

at 2% acid for araban and glucan. In the case of xylose, the fitting for k_2 was satisfactory ($\ln a_2 = -2.87$, $n = 1.01$ and $r^2 = 0.97$). To complete this analysis, Fig. 5 shows the variation of the kinetic coefficients k_1 and k_2 for xylose, arabinose and glucose. It can be observed the general increase of the coefficients with the phosphoric acid concentration.

4. Conclusions

The acid hydrolysis in autoclave with H_3PO_4 is an adequate method to fractionate SCB. The main fraction affected is the hemicellulosic, releasing sugars and some derivates such as acetic acid and furfural. The hydrolysates obtained are suitable to be used as fermentation media due to the main compounds are sugars (xylose, glucose, arabinose) and the low concentrations of growth inhibitors. In the selected conditions, more than 55% of solubilized solids are sugars. The kinetic models developed will be used for future technical and economic studies of optimisation.

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