ETHANOL-WATER FRACTIONATION OF SUGAR CANE BAGASSE CATALYZED WITH ACIDS

CRISTINA M. AREA, FERNANDO E. FELISSIA* and MARIA E. VALLEJOS

Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Programa de Investigación de Celulosa y Papel, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Felix de Azara 1552 (3300) Posadas, Misiones, Argentina

**Programa de Investigación de Celulosa y Papel, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Felix de Azara 1552 (3300) Posadas, Misiones, Argentina*

Received May 21, 2009

The conditions for maximizing bagasse ethanol-water fractionation (in cellulose, hemicelluloses and lignin) were established by the evaluation of different variables (time, temperature, catalyst). To this end, the organic compounds of the fibrous material and liquors were quantified. Acetic (2 g/L) and sulfuric (0.5, 1.0 and 2.0 g/L) acids were used as catalysts. An alkaline extraction was applied as a pre-treatment, using 3.6 g/L NaOH, at 100 ºC, for 1 h. The effects of different conditions of the organosolv treatment on the kinetics, composition of the fibrous material and residual liquor, upon the fractionation of sugar cane bagasse used in this work, were investigated. The kinetic parameters were determined for delignification under six different conditions. The organic compounds of the fibrous material were quantified and lignin (by UV-visible spectroscopy) and carbohydrates, organic acids, furfural and HMF (by HPLC chromatography) were evaluated in residual liquors. The incorporation of sulfuric acid at 160 ºC produced delignification degrees similar to those obtained by the addition of acetic acid at 180 ºC, or in the fractionation without catalyst (85-86%). The delignification values showed that fractionations could be stopped at 90 or 120 min, since, after 90 min, the delignification percentage decreases by only about 1%. The incorporation of sulfuric acid extracted a large amount of xylose and produced further degradation of cellulose. Fractionations with acetic acid did not differ largely from the auto-catalyzed ones.

Keywords: organosolv, fractionation, kinetics, sugar cane bagasse, acetic acid, sulfuric acid

INTRODUCTION

The sugar industry could become a modern and efficient agro-industry by diversifying its production into large amounts of by-products and energy. Sugar cane bagasse, the main by-product of the sugar cane industry, is a fibrous residue of sugar cane resulting from conventional milling. Sugar mills generate approximately 270 kg of bagasse (50% moisture) per metric ton of sugar cane. About 50% of this amount is sufficient to supply the energy required by the sugar and ethanol plants.[1] The remaining bagasse is usually stock-piled, constituting an environmental problem, due to the risk of spontaneous combustion of stored bagasse.[2] However, out of the about 130 sugar cane derivatives known, over half are derived from bagasse.[3,4]

Bagasse fractionation, performed for separating its main components (cellulose, hemicelluloses and lignin) and for obtaining higher added-value products, has been tested under different conditions and processes. Literature references to fractionations

involve: acid hydrolysis with hydrochloric, sulfuric,[5-7] nitric[8] and phosphoric acid;[9] hot water treatment[10] at 230-280 ºC; sequential alkaline extraction with sodium hydroxide and hydrogen peroxide,[11] treatments with ammonia[12] and supercritical CO_2 in solvent mixtures,[13,14] as well as with ultrasound, sodium hydroxide and hydrogen peroxide.[15] Most of the cited studies followed exclusively the extraction of hemicelluloses and cellulose fractions and did not evaluate the quantity or quality of the lignin fraction. Organosolv lignins present potential uses in the manufacture of phenol-formaldehyde resins (as a source of phenols) and of biodegradable polyurethanes.

The organosolv processes use neutral or acid solvents, either with or without acids as catalysts. When using ethanol-water mixtures, the deacetylation of the hemicelluloses from the fibrous material generates acetic acid, which acidifies the medium throughout the process, called "auto-catalyzed" process,[16-18] of which Alcell is an example for the pulp production using an ethanol-water mixture.[19]

It has been reported that, in the presence of low acid concentrations and low temperatures, the alcoholic solutions assure the best conditions for maximum delignification, with low cellulose degradation and lower formation of condensation products.[20] Both sulfuric and hydrochloric acids have been used as catalysts in the ethanol-water fractionation of wheat straw.[21,22] Although acetic acid is a natural catalyst of the process, few works have reported its use in ethanol-water fractionation.[18]

The kinetics of organosolv delignification has been explained by a model with two simultaneous pseudo-first-order reactions: a faster or dominant reaction and a slower, residual one.[20,23,24] The acid-catalyzed process rate seems to be controlled by the hydrolysis of the α-ether linkage of lignin, although other complex reactions, such as condensation, partial hydrolysis of the β-ether linkage, formaldehyde release and rearrangement of the free radicals also take place.[23] The importance of the β-ether linkage hydrolysis is under discussion, while the increase in the free phenolic hydroxyl groups in lignin with acid concentration might be taken as an evidence of it.[17]

Ethanol-water delignification is an interesting process for treating sugar cane bagasse, as it allows the use of resources already existing in the sugar mill (ethanol and bagasse). Although the kinetics of the ethanol-water organosolv pulping of sugar cane bagasse is thoroughly described in literature,[16,20-24] few investigations have made a characterization of the residual liquor, as the authors' aim is mainly pulp production. Such a characterization would permit to determine the type and quantity of the by-products that could be recovered from the residual liquor. In addition, the optimization of the ethanol-water process, for improving the product yields, product performance and operating costs has not been solved yet.

In the present work, the conditions for maximizing bagasse ethanol-water fractionation (in cellulose, hemicelluloses and lignin) were established by the evaluation of different variables (time, temperature, catalyst). Experiments were performed at temperatures lower than those usually applied in conventional organosolv processes, in an attempt to simplify the operation and to allow the utilization of more inexpensive equipments. To evaluate the performance, the organic compounds present in residual liquors and in the fibrous material were quantified.

This information is essential for the technical and economic feasibility of the production of non-conventional by-products from sugar cane bagasse, by using environmentally compatible and economically competitive technologies.

MATERIALS AND METHODS

Sugar cane bagasse and ethanol were supplied by a local mill (San Javier Sugar Mill, Misiones, Argentina). Bagasse pith was removed in two stages. In the former, bagasse was wet-depithed to break its structure in a Bauer disc refiner, using a plate gap of 0.005 inches (0.13 mm), after which the bagasse pith was removed by screening, using a plate with 2 mm wide slits (Wenmber). Finally, depithed bagasse was centrifuged. Acetic acid and sulfuric acid (A.C.S. reagent grade) were used as catalysts.

Bagasse was delignified by an organosolv

ethanol process carried out in a 7 L MK digester (M/K Systems, Inc., Maryland) with liquor circulation, under different conditions. Alkaline extraction was applied as a pre-treatment in the F_8 fractionation (called pre-F_8) using 3.6 g/L NaOH, at 100 ºC, for 1 h. The ratios of liquor/bagasse and ethanol/water remained constant at 14/1 (v/w) and 50% (% v/v), respectively. The time necessary to reach the maximum temperatures was of 30 min. The variables involved were as follows: maximum temperature, time at maximum temperature and catalyst.

The extractives in alcohol-benzene (TAPPI T204), solubility in hot water (TAPPI T207), solubility in 1% sodium hydroxide (TAPPI T212), acetyl groups content (National Renewable Energy Laboratory, Laboratory Analytical Procedure – NREL–LAP002) and ashes at 525 °C (TAPPI T211), were determined on the bagasse samples. Acid-insoluble lignin (TAPPI T222), acid-soluble lignin (TAPPI T250) and the structural carbohydrate content in biomass were determined by HPLC with IR detection (NREL LAP-002), on both bagasse and the fibrous material (Fig. 1).

During fractionation, the residual liquors were sampled at different time values. The dissolved lignin was measured and the residual lignin *(L)* was determined as the lignin content in the fibrous material.

After fractionation, the fibrous materials were washed to remove the residual liquor, their yields being determined as percentages of oven-dry bagasse. The performance of the fractionation process was evaluated by the delignification degree, represented by Kappa number and yield. The Kappa number (TAPPI T236) is the volume (mL) of 0.1 N potassium permanganate solution consumed by 1 g of oven-dry fibrous material under the conditions specified.

The residual liquid contained carbohydrate degradation products, such as hydroxymethyl furfural (HMF) and furfural, and other components, such as organic acids. The organic compounds in the residual liquors were quantified according to the procedures for the determination of sugars, by-products and degradation products present in the liquid samples (NREL LAP-015), as shown in Figure 1.

The compositions of sugars (glucose, xylose, arabinose), organic acids (acetic and formic acids), furfural and HMF were determined by HPLC (Waters Corp. Massachusetts, USA), on an AMINEX-HPX87H (SEE) column, under the following conditions: 4 mM H_2SO_4 as eluent at a flow rate of 0.6 mL/min, at 35 ºC, with Refractive Index and Diode Array as detectors.

The polyoses were converted into monomers by multiplying by the hydrolysis factor (hexoses to hexosanes: 0.900, pentoses to pentosanes: 0.880, acetic acid to acetyl groups: 0.683). The lignin from the residual liquors was quantified by UV (Techcomb 8500 II Spectrophotometer) at 210 nm, on liquor aliquots diluted in ethanol. The absorptivity determined and used in this work was of 78.2 L/g·cm. The value obtained was corrected by subtracting the absorptivity of furfural, obtained by HPLC.

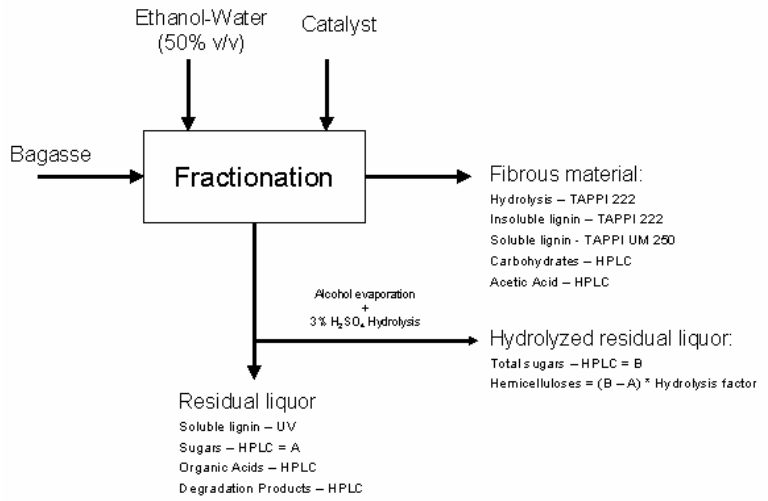

Figure 1: Procedure for the analysis of materials

The results (concentrations of carbohydrates, organic acids, degradation products and lignin) were analyzed by multifactorial analysis of variance (ANOVA), with time, temperature and catalyst as independent variables. The statistical software employed was Statgraphics and the level of significance applied was of 95%.

RESULTS AND DISCUSSION

The chemical composition of the sugar cane bagasse (mass percent on oven-dry bagasse) was the following: glucan – 43.1%; xylan – 23.8%; arabinan – 1.66% and lignin – 21.3% (Table 1).

The conditions of fractionation F_5 were the same as those for F_1, except for the use of sulfuric acid instead of acetic acid. The treatment turned out too strong, producing the degradation of the fibrous material and the darkening of the residual liquor (condensation products). Fractionation F_8 repeated the conditions of F_7, yet pre-treating the fibrous material with sodium hydroxide to favour the access of the liquor. However, the high yield obtained indicates that this pre-treatment was inefficient. The conditions of the F_7 treatment with 0.5 g/L sulfuric acid (160 ºC, 120 min) produced a yield similar to that obtained under stronger conditions, with acetic acid. Table 2 shows the results for the liquors and fibrous materials during final fractionation.

The first-order kinetics was tested with respect to residual lignin, by calculating the reaction rate constants under different fractionation conditions. Assuming that the delignification rate is proportional to the product of the residual lignin by the concentration of chemical reagents in the liquor for a given temperature, the reaction rate can be represented by the equation:

$$-\frac{dL}{dt} = k_0\,[Ethanol]\,L \qquad (1)$$

where:
-dL/dt = delignification rate
k_0 = delignification rate constant
[Ethanol] = ethanol concentration in the liquor
L = lignin content in the fibrous material (% o.d.b.)

Under conditions of constant temperature and liquor composition, equation (1) takes the following form:

$$-\frac{dL}{dt} = k_0'\,L \qquad (2)$$

By integrating equation (2), one obtains:

$$\ln L = -k_0'\,t + \ln L_0 \qquad (3)$$

where L_0 is the initial lignin content of bagasse.

According to this equation, in the plot of the natural logarithm of residual lignin in the fibrous material *vs*. time, the slope of the straight line represents the delignification reaction rate constant, valid for the fractionation conditions applied. The reaction rate constants for short and long delignification time periods are presented in Tables 3 and 4. Figures 2 and 3 show the kinetics of the more efficient fractionations: F_3 (at 180 ºC, catalyzed with acetic acid) and F_7 (at 160 ºC, catalyzed with sulfuric acid).

Table 1
Chemical characterization of bagasse (% o.d.b.)

Alcohol-benzene extractives	2.12
Hot water solubility	2.73
1% sodium hydroxide solubility	34.2
Ash at 525 °C	1.53
Total lignin	21.3
Soluble lignin	1.51
Insoluble lignin	19.8
Total carbohydrates	70.2
Glucans	43.1
Xylans	23.8
Arabinans	1.66
Acetyls	1.66

Table 2
Results of fractionation

Fractio-nation	Catalyst	Maximum temperature (ºC)	Time at max. temperature (min)	Catalyst (g/L)	Initial pH	Final pH	Kappa	Yield (%)
F_1	Acetic acid	175	240	2.0	3.9	3.8	37.8	48.9
F_2	Without	175	240	0.0	5.9	3.9	37.5	48.2
F_3	Acetic acid	180	240	2.0	3.9	3.7	31.1	46.9
F_4	Without	180	240	0.0	5.9	3.8	34.2	46.8
F_5	Sulfuric acid	175	240	2.0	2.1	2.4	–	–
F_6	Sulfuric acid	150	120	0.5	2.4	3.0	58.6	52.3
F_7	Sulfuric acid	160	120	0.5	2.4	3.0	23.5	45.4
F_8*	Sulfuric acid	160	120	1.0	2.8	3.1	–	63.5

* NaOH-pre-treated material (Pre-F_8)

Table 3
Kinetic parameters of the equation for short time periods (F_1 at F_4, F_6 and F_7)

Fractionation	ln L_0	L_0	- k_0	R^2
F_1 (0 to 40 min)	2.665	14.37	1.93×10^{-2}	0.98
F_2 (0 to 40 min)	2.750	15.64	1.95×10^{-2}	1.00
F_3 (0 to 40 min)	2.621	13.75	2.26×10^{-2}	0.98
F_4 (0 to 40 min)	2.695	14.81	2.21×10^{-2}	0.99
F_6 (0 to 40 min)	2.736	15.43	2.23×10^{-2}	0.98
F_7 (0 to 20 min)	2.272	9.694	4.19×10^{-2}	0.85

Table 4
Kinetic parameters of the equation for long time periods (F_1 at F_4, F_6 and F_7)

Fractionation	ln L_0	L_0	- k_0	R^2
F_1 (60 to 240 min)	1.281	3.60	0.603×10^{-3}	0.96
F_2 (60 to 240 min)	1.241	3.46	0.387×10^{-3}	0.89
F_3 (60 to 240 min)	1.112	3.04	0.494×10^{-3}	0.98
F_4 (60 to 240 min)	1.363	3.91	1.44×10^{-3}	0.99
F_6 (60 to 120 min)	1.918	6.81	2.98×10^{-3}	1.00
F_7 (40 to 120 min)	1.346	3.84	3.05×10^{-3}	0.83

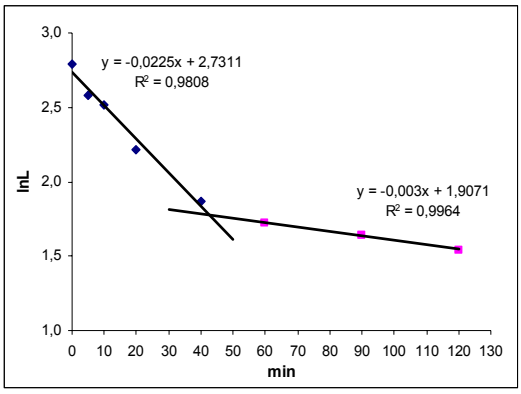

Figure 2: Kinetics of fractionation F_3

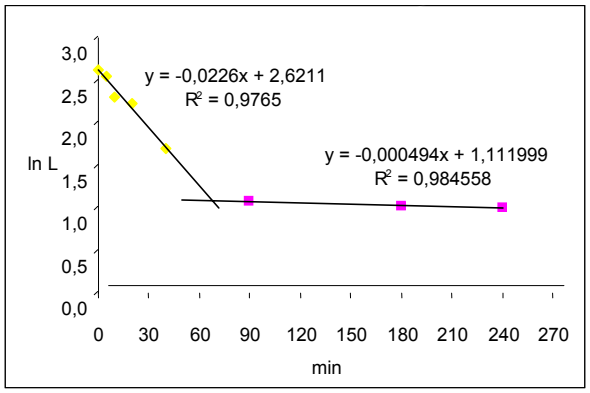

Figure 3: Kinetics of fractionation F_7

The calculated initial lignin L_0 varied in the different fractionations, being 6-8% o.d.b and 10-12% o.d.b. lower than the lignin content of bagasse in the fractio-nations catalyzed with acetic and sulfuric acid, respectively (Tables 3 and 4), as the digester reached the maximum temperature within 30 min, and therefore, different levels of delignification were reached under different conditions.

The fractionations carried out at low temperatures presented inferior initial reaction rates. The highest reaction rate occurred at 160 ºC, with sulfuric acid in the first 20 min, with a change in the slope between 40 and 120 min (Fig. 3). The highest speed in the long time periods corresponded to the reactions catalyzed with sulfuric acid.

The lignin content of the fibrous material and the delignification percentages at different time values (all calculated from the kinetic equations) are shown in Table 5.

The results of delignification at 120 min (Table 5) showed that the temperature had a significant influence on the total amount of extracted lignin (p = 0000). Fractionation F_6 (at 150 ºC) was the most inefficient one. Fractionations F_7 (with sulfuric acid at 160 ºC) and F_3 (with acetic acid at 180 ºC) were the most efficient, presenting similar degrees of delignification (homogeneous groups in the multiple-range test).

The delignification values calculated from the kinetic equations showed that fractionation could be stopped at 90 or at 120 min, with a difference of only 1% in the residual lignin from the fibrous material. The carbohydrates remaining in the fibrous material are characterized in Table 6.

Table 5
Lignin in the fibrous material and delignification percentages

Fractionation	F_1	F_2	F_3	F_4	F_6	F_7
Insoluble lignin (% o.d.p.)	5.73	6.20	5.03	5.70	9.10	4.51
Soluble lignin (% o.d.p.)	0.69	0.61	0.55	0.63	0.49	0.25
Total lignin (% o.d.p.)	6.42	6.81	5.58	6.33	9.59	4.76
Total lignin (% o.d.b.)	3.14	3.28	2.61	2.96	5.01	2.16
Delignification at 90 min (%)*	84.0	84.3	86.3	83.9	75.5	86.3
Delignification at 120 min (%)*	84.3	84.5	86.5	84.6	77.6	87.5
Delignification at 240 min (%)*	85.4	85.2	87.3	87.0	–	–

(% o.d.p.): on oven-dry pulp; (% o.d.b.): on oven-dry bagasse; * calculated from the kinetic equations

Table 6
Remaining carbohydrates in the fibrous materials

Fractionation	Glucan (%o.d.p.)	Glucan (%o.d.b)	Xylan (%o.d.p)	Xylan (%o.d.b.)	Total carbohydrates (%o.d.p.)	Total carbohydrates (%o.d.b.)	Loss *** (%)
F 1	84.73	41.5	10.59	5.18	95.33	46.64	33.6
F 2	86.63	41.7	10.77	5.19	97.40	46.93	33.1
F 3	88.06	41.3	8.76	4.10	96.82	45.37	35.4
F 4	87.17	40.8	9.38	4.39	96.55	45.23	35.6
F 6	63.80	33.4	13.00	6.80	76.80	40.17	42.8
F 7	75.89	34.4	7.88	3.58	83.77	38.03	45.8

(%o.d.p.): on oven-dry pulp; (%o.d.b.): on oven-dry bagasse; *** Loss= (Carbohydrates in bagasse - Carbohydrates in pulp) / Carbohydrates in bagasse)

Table 7
Composition of non-hydrolyzed liquor (% o.d.b.) at different fractionation time periods

Fractionation	Time (min)	Glucose	Xylose	Arabinose	Formic acid	Acetic acid	Furfural
F_1	90	0.14	1.03	0.69	0.29	1.34	0.52
F_1	180	0.10	2.85	0.21	0.67	1.75	1.93
F_1	240	0.14	3.75	0.11	0.77	2.00	3.08
F_2	90	0.14	0.97	0.64	0.20	1.71	0.38
F_2	180	0.08	3.12	0.28	0.79	2.51	1.70
F_2	240	0.11	4.30	0.16	0.57	2.84	2.81
F_3	90	n.d.	1.47	0.38	0.39	1.60	0.99
F_3	180	0.12	3.27	0.07	0.81	1.96	3.06
F_3	240	0.33	3.53	n.d.	0.97	2.36	4.51
F_4	90	0.14	1.05	0.25	0.25	2.14	0.70
F_4	180	0.12	3.07	0.08	0.79	2.82	2.78
F_4	240	0.34	4.11	0.24	0.95	3.18	3.86
F_6	20	0.06	0.99	0.42	0.29	0.25	n.d.
F_6	90	0.14	5.02	0.66	0.62	0.68	n.d.
F_6	120	0.17	6.48	0.69	0.69	0.88	n.d.
F_7	20	0.23	7.27	0.68	0.82	0.66	n.d.
F_7	90	0.86	13.1	0.89	0.96	1.22	0.34
F_7	120	1.36	14.5	1.40	1.14	1.77	0.78

n.d.: not detected; (% o.d.b.): on oven-dry bagasse

A total loss of arabinans and acetyl groups (not detected in pulp hydrolysates) and of 90% xylans (xylan content in bagasse: 25.0%) occurred in fractionations F_1 to F_4. On the contrary, these components remained in the fibrous material after fractionations F_6 and F_7. Fractionation F_7 produced the largest xylan extraction. The sugars, organic acids and furfural contents of the residual liquor at various reaction time values are shown in Table 7.

The generated acetic acid differed significantly under all fractionation conditions ($p = 0.0001$), the highest amount corresponding to fractionation F_4 and the lowest – to F_6. Fractionation F_7 presented the largest amount of formic acid, arabinose, glucose and xylose in the spent liquors, while

fractionations F_3 and F_4 generated the largest amount of furfural. The spent liquors of F_6 and F_7 showed a significant amount of xylose, as compared to all the others (p = 0000).

Acetic and formic acids, xylose and furfural increased progressively in the liquor throughout the fractionation time (p = 0000). Xylose and formic acid contents increased significantly in the liquor when sulfuric acid was present ($p < 0.01$). Arabinose and glucose showed the same trend. The balance of the components in the residual liquor is shown in Table 8.

The conditions of F_5 produced a high degradation of the fibrous material. The alkaline pre-treatment of F_8 caused considerable deacetylation, with a subsequent increase in the acetic acid content present in the liquor and a loss of pentosans. The conditions of F_5 produced a high degradation of the fibrous material. The alkaline pre-treatment of F_8 caused considerable deacetylation, with a subsequent increase in the acetic acid content in the liquor and a loss of pentosans. Fractionation F_7 caused a larger hydrolysis of pentosans and increased the xylose content in the spent liquor. The evolution of most organic components in the residual liquor of fractionations F_6 and F_7 (both with sulfuric acid) is shown in Figure 4.

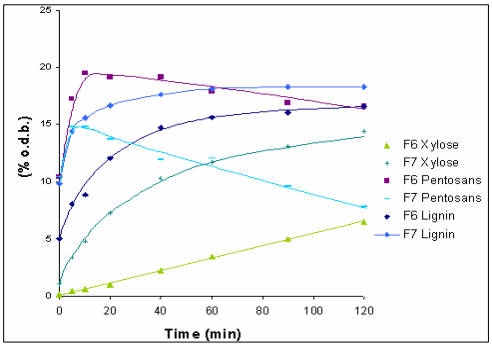

Figure 4: Organic compounds in the residual liquor from fractionations F6 and F7

Table 8
Organic compounds in residual liquors (% o.d.b.)

Fractionation	Glucose	Xylose	Arabinose	Pentosans*	Formic acid	Acetic acid	HMF**	Furfural	Lignin
F_1	0.14	3.75	0.11	7.64	0.77	2.00	n.d.	3.08	18.2
F_2	0.11	4.30	0.16	6.75	0.57	2.84	n.d.	2.81	18.2
F_3	0.33	3.53	n.d.	5.02	0.97	2.36	0.21	4.51	18.6
F_4	0.34	4.11	0.24	3.45	0.95	3.18	n.d.	3.86	18.6
F_5	1.93	1.29	n.d.	0.58	2.58	3.91	3.18	11.5	17.3
F_6	0.17	6.48	0.69	16.6	0.69	0.88	n.d.	n.d.	16.6
F_7	1.36	14.5	1.40	7.79	1.14	1.77	n.d.	0.78	18.3
Pre-F_8	–	–	–	1.49	–	4.53	n.d.	–	–
F_8	0.35	1.60	1.37	6.50	0.85	0.34	n.d.	0.09	10.8

* (Pentoses in hydrolyzed liquor – Pentoses in liquor such as it is) x hydrolysis factor; ** 5-(Hydroxymethyl)furfural; n.d.: not detected; (% o.d.b.): on oven-dry bagasse

CONCLUSIONS

The use of sulfuric acid as a catalyst at 160 ºC and 120 min generates delignification degrees similar to those obtained with acetic acid and without catalyst at 180 ºC and 240 min (> 87%).

Under optimum conditions (fractionation with sulfuric acid at 160 ºC), the delignification values (calculated from the kinetic equations) showed that fractionation can be stopped at 90 min, with an approximately 1% decrease in delignification, as compared to the treatment at 120 min.

In all cases, there was a significant increase in carbohydrates, organic acids and furfural in the liquor with the fractionation time, except for arabinose, which decreased as it was degraded to furfural.

The largest amount of xylose in the liquors (14.5% o.d.b.) was obtained in the fractionation with sulfuric acid, which also produced the largest cellulose degradation.

Fractionation at 160 ºC with sulfuric acid extracted the largest amounts of carbohydrates and lignin, producing the largest hydrolysis of pentosans and a moderate degradation to furfural.

ACKNOWLEDGEMENTS: The authors gratefully acknowledge the financial support of the Productive Innovation Federal Projects (PFIP) 2006, Secretariate for Technology, Science and Productive Innovation of the Ministry of Education, Science and Technology. They also acknowledge the assistance of the technical staff: Sebastián González, María Antonieta Klein, Hugo Ferreira, Christian Sanabria and Daniel Baez.

REFERENCES

[1] E. Frollini and M. J. A. Pimenta, *Anais Assoc. Bras. Quím.*, **46**, 43 (1997).
[2] B. P. Lavarack, G. J. Griffin and D. Rodman, *Catal. Today*, **63**, 257 (2000).
[3] W. Alonso Pippo, P. Garzone and G. Cornacchia, *Waste Manag.*, **27**, 869 (2007).
[4] A. Ashok Pandey, C. R. Soccol, P. Nigam and V. T. Soccol, *Bioresource Technol.*, **74**, 69 (2000).
[5] B. P. Lavarack, G. J. Griffin and D. Rodman, *Biomass Bioenerg.*, **23**, 367 (2002).
[6] J. E. Paiva, I. R. Maldonade and A. R. P. Scamparini, *Rev. Bras. Eng. Agríc. Ambient.*, **13**, 75 (2009).
[7] R. Aguilar, J. A. Ramírez, G. Garrote and M. Vázquez, *J. Food Eng.*, **55**, 309 (2002).
[8] A. Rodríguez-Chong, J. A. Ramírez, G. Garrote and M. Vázquez, *J. Food Eng.*, **61**, 143 (2004).
[9] S. Gamez, J. J. Gonzalez-Cabriales, J. A. Ramirez, G. Garrote and M. Vazquez, *J. Food Eng.*, **74**, 78 (2006).
[10] M. Sasaki, T. Adschiri and K. Arai, *Bioresource Technol.*, **86**, 301 (2003).
[11] J. X. Sun, X. F. Sun, R. C. Sun and Y. Q. Su, *Carbohyd. Polym.*, **56**, 195 (2004).
[12] Z. Pernalete, F. Piña, M. Suárez, A. Ferrer and C. Aiello, *Bioagro*, **20**, 3 (2008).
[13] D. Pasquini, M. T. B. Pimenta, L. H. Ferreira and A. A. S. Curvelo, *J. Supercrit. Fluid.*, **34**, 125 (2005).
[14] D. Pasquini, M. T. B. Pimenta, L. H. Ferreira and A. A. S. Curvelo, *J. Supercrit. Fluid.*, **36**, 31 (2005).
[15] J. X. Sun, R. Sun, X. F. Sun and Y. Su, *Carbohyd. Res.*, **339**, 291 (2004).
[16] S. Aziz and K. Sarkanen, *Tappi J.*, **72**, 169 (1989).
[17] T. J. McDonough, *Tappi J.*, **76**, 186 (1993).
[18] E. Muurinen, *Academic Dissertation*, Faculty of Technology, University of Oulu, Linnanmaa, Acta Universitatis Ouluensis, Technica, 2000, F146.
[19] P. E. Kendall and J. H. Lora, *Tappi J.*, **74**, 113 (1991).
[20] T. N. Kleinert, *Tappi J.*, **58**, 170 (1975).
[21] G. Papatheofanous, E. Billa, D. P. Koullas, B. Monties and E. G. Koukios, *Bioresource Technol.*, **54**, 305 (1995).
[22] D. Sidiras and E. Koukios, *Bioresource Technol.*, **94**, 91 (2004).
[23] K. V. Sarkanen, *Tappi J.*, **73**, 215 (1990).
[24] A. A. S. Curvelo and R. Pereira, *8th International Symposium on Wood and Pulping Chemistry (ISWPC)*, Helsinki (Finland), 1995, vol. 2, p. 473.