Chemical and morphological characterization of sugar cane bagasse

MARCELA FREITAS ANDRADE, JORGE LUIZ COLODETTE, AND HASAN JAMEEL

ABSTRACT: The sugar cane industry in Brazil is expanding, leading to great interest in using the leftover bagasse for other uses, beyond burning it for its energy. A thorough physical and chemical characterization of bagasse, particularly regarding its lignin structure, is relevant for a more rational utilization of the bagasse in the production of printing and writing pulp grades, dissolving pulp, ethanol, and power. The main goals of this study were characterizing the chemical (pith and fibers fractions) and morphologic (fibers fraction) properties of the sugar cane bagasse and the structure of the depithed bagasse lignin by two-dimensional nuclear magnetic resonance spectroscopy. Industrial whole bagasse was separated into two fractions: pith and depithed bagasse. The pith was only characterized chemically. The depithed bagasse was chemically and morphologically characterized. The cellulose, hemicelluloses, and lignin contents of the two materials varied significantly. The lignin composition of the depithed bagasse showed very high contents of phenolic cinnamic acids (PCAs). The depithed bagasse lignin presented fractions with different structural monomer distributions. The morphological analyses of the depithed bagasse indicated a short fiber material, similar to hardwoods.

Application: The thorough physical and chemical characterization of bagasse presented here, particularly regarding its lignin structure, is relevant for a more rational use of the bagasse.

The sugar cane industry in Brazil is expanding, leading to great interest in using the leftover bagasse for other uses, beyond burning it for its energy. A thorough physical and chemical characterization of the bagasse, particularly regarding its lignin structure, is relevant for a more rational use of the bagasse in the production of printing and writing pulp grades, dissolving pulp, ethanol, and power.

Among the many agricultural fibers used for pulp manufacture, sugar cane bagasse is the one with most promise. Bagasse is easily accessible and readily available in many countries. Brazil is the world-leading producer of sugar cane (*Saccharum officinarum*). In 2011 and in 2012, the average productivity of sugar cane in Brazil was 68 tons/ha [1]. About 80 million tons of bagasse is generated per year on 8.4 million ha of land [1]. About 90% of the bagasse is burnt in ethanol and sugar mills to produce energy for steam and electricity [2], with about 10% being wasted. This wasted material can potentially be used for producing high added value pulp.

The sugar cane bagasse is a low cost raw material and has a high carbohydrate content (51%-78%) [3-7]. Because of its low ash content (2%-3%), bagasse offers numerous advantages in comparison to other crop residues, such as rice straw and wheat straw, which have ash contents of about 17% and 11%, respectively, [8]. In addition, bagasse provides longer fibers than straw, low refining energy consumption, and good sheet formation, and contributes to paper smoothness [9]. These are relevant raw material traits for pulp production and other industrial uses. The bagasse pulp is generally comparable to hardwood pulps [10].

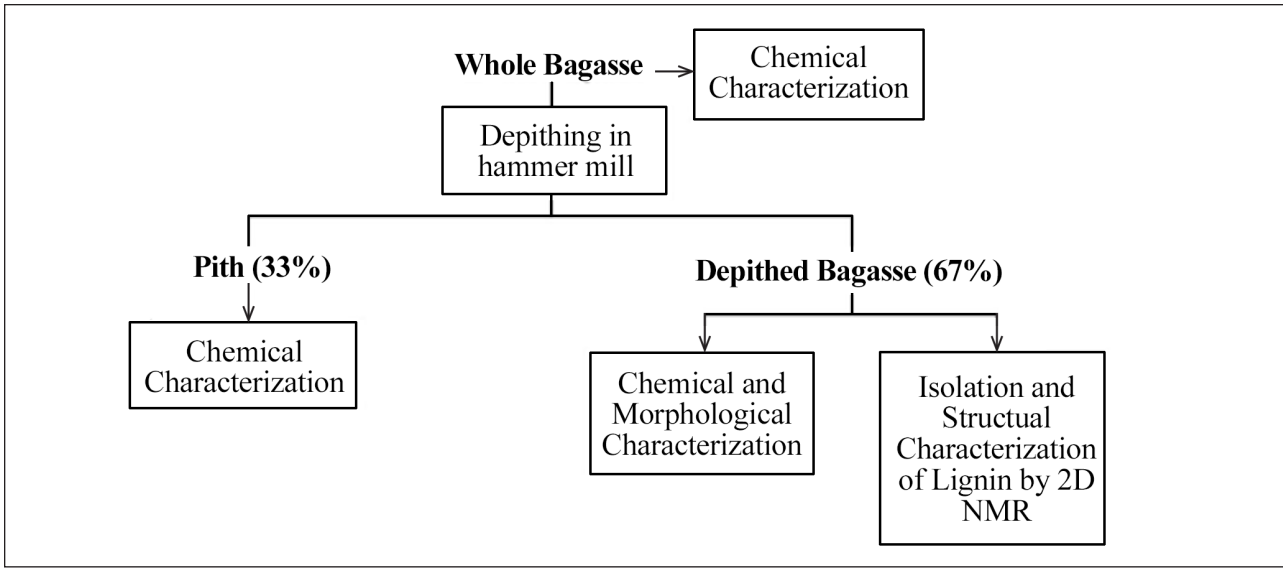
The properties of felting index, flexibility coefficient, wall fraction, ratio between length and thickness, and the Runkel can facilitate an understanding of the characteristics of cellulose pulps as they relate to resistance properties [11]. The fiber variables responsible for determining the physical characteristics and quality of pulp and paper are classified under fiber morphological aspects. Sugar cane bagasse fibers have a length and diameter similar to those of hardwoods fibers [12,13].

Because sugar cane bagasse is a promising raw material, a comprehensive study of chemical composition was performed. The main goal of this study was characterizing the chemical (pith and fibers fractions) and morphologic (fibers fraction) properties of the sugar cane bagasse, as well as the structural properties of the depithed bagasse lignin, by two-dimensional nuclear magnetic resonance (2D NMR) spectroscopy.

EXPERIMENTAL

Working plan

Figure 1 depicts the working plan for the experiment. The two fractions of the sugar cane bagasse (pith and fibers) were separated in a hammer mill. The whole bagasse, pith, and depithed bagasse were chemically characterized. The depithed bagasse fraction (fibers) was also morphologically characterized and the structural characterization of the



1. Working plan for bagasse fractionation.

isolated lignin from the depithed bagasse was accomplished by 2D NMR spectroscopy.

Material

About 150 kg of industrial whole bagasse was provided by a Brazilian pulp mill. About 100 kg of the whole bagasse was separated into two fractions (pith and depithed bagasse) using a hammer mill. The pith fraction represented 33%, while the other 67% remained as the depithed bagasse. The pith, depithed bagasse, and whole bagasse were dried to about 85% dryness in a conditioning room (23.0 ± 1.0°C and 50.0 ± 2.0% moisture) and stored in polyethylene bags for further use. The air-dried samples of pith, depithed bagasse, and whole bagasse were ground in a Wiley mill and sieved, and the fraction that passed through a 40 mesh screen but was retained by a 60 mesh screen was collected.

Methods

The pith and depithed bagasse fractions were analyzed according to standard procedures for moisture content (TAPPI T 264 om-88 "Preparation of wood for chemical analysis"), total extractives content (TAPPI T 264 cm-97 "Preparation of wood for chemical analysis"), acid soluble lignin [14], Klason lignin [15], lignin syringyl:guaiacyl ratio [16], preparation of biomass for sugar analysis (TAPPI T 249 cm-85 "Carbohydrate composition of extractive-free wood and wood pulp by gas-liquid chromatography"), sugars [17], acetyl groups [18], uronic acids [19], silica (TAPPI T 245 cm-98 "Silicates and silica in pulps [wet ash method]"), and ash (TAPPI 211 om-93 "Ash in wood, pulp, paper, and paperboard: combustion at 525°C").

From the depithed bagasse fraction, two different isolation methods were used to obtain lignin fractions: milled nonwood lignin (MWL) and cellulolytic enzyme lignin (CEL96). Crude MWL (MWLc) was isolated according to the classic Björkman method [20]. A planetary ball mill was used in the current experiment in contrast to a vibratory ball mill used in Björkman's protocol.

The nonwood meal obtained after planetary ball milling according to Björkman's protocol was treated with a cellulase (from *Trichoderma viride*, 9.1 U/mg solid) in an acetate buffer solution (pH 4.5) at 45°C for 48 h. The enzyme charge was 500 U/g nonwood substrate.

Heteronuclear single-quantum correlation 2D NMR spectra were acquired using methods described by Balakshin et al. [21]. The spectra were acquired at a sample concentration of about 10% with a Bruker (Billerica, MA, USA) AVANCE 500 MHz spectrometer equipped with a BBI probe with a cryoplatform or a Bruker 5 mm ID CPTCI (^{1}H / ^{13}C / ^{15}N/D) cryo-probe with z-axis gradient. The acquisition parameters used on the 500 MHz spectrometer were 160 transients (scans per block) acquired using 1 K data points in F2 (^{1}H) dimension for an acquisition time of 151 ms and 256 data points in F1 (^{13}C) for an acquisition time of 7.68 ms for a total of 20 h. A coupling constant $^{1}J_{C-H}$ of 147 Hz was used. The 2D NMR data sets were processed with 1 K × 1 K data points using a Q-sine window function in both dimensions.

Morphological analyses were performed from the depithed bagasse fraction, after hydration and mild agitation for complete fiber individualization. Slides were prepared and images were captured with a video microscope equipped with image analysis software. A small amount of colorant (Astra blue) was added for better visualization of the fibers. Approximately 100 whole fibers were measured. The following fiber biometric traits were measured: length, width, lumen diameter, and cell wall thickness. Fiber length was measured using a 70× magnification; other traits were measured using 1000× magnification. The variation coefficient was taken at every 25 measurements. The data obtained from the various measurements were transferred to a computer spreadsheet program (Micro-

soft Excel 2007) to determine statistical parameters (e.g., averages, standard deviations, and variation coefficients).

From the fiber biometry measurements, mathematical inferences were performed to determine the following fiber properties:

Felting index (FI) = relation between fiber length (FL) and fiber width (FW),
(FI) = (FL/FW)*1000 (1)
where 1000 is a conversion factor of μm to mm.

Flexibility coefficient (FC) = relation between lumen diameter (LD) and fiber width (FW),
(FC) = (LD/FW)*100 (2)
where 100 is a conversion factor for percentage.

Wall fraction (WF), relation between wall thickness (WT) and fiber width (FW),
(WF) = (2*WT/FW)*100 (3)
where 100 is a conversion factor for percentage.

Ratio length/thickness (L/T) = relation between fiber length (FL) and wall thickness (WT):
(L/T) = (FL/WT)*1000 (4)
where 1000 is a conversion factor of μm to mm.

Runkel index (RI) = relation between wall thickness (WT) and lumen diameter (LD),
(RI) = (2*WT/LD) (5)

The analysis of coarseness, fiber number/gram, and fines content were carried out in a Galai CIS-100 particle size analyzer.

RESULTS AND DISCUSSION

Chemical characterization of sugar cane bagasse biomass

Three different fractions of sugar cane bagasse biomass were characterized: pith, depithed bagasse, and whole bagasse. The proportions of pith and depithed bagasse after separation in the hammer mill were 33% and 67% on the basis of the whole bagasse dry weight, respectively. The contents of cell wall structural constituents (cellulose, hemicelluloses, and lignin) in the pith, depithed bagasse, and whole bagasse varied significantly (**Table I**), with the pith showing higher lignin and lower xylans and glucans contents than the depithed bagasse. Xylans are the main component of the hemicellulose of pith and depithed bagasses with trace amounts of galactans, mannans, and arabinans.

The content of xylans in the pith (12.4%) was only about half of that in the depithed bagasse (22.1%); the glucan content was 37.8% and 43.4%, respectively, for the pith and the depithed bagasse. The acetyl group content in the pith (0.5%) was much lower than that in the depithed bagasse (2.6%); however, the contents of uronic acids of these materials were similar.

Analyses, %	Whole Bagasse	Pith	Depithed Bagasse
Klason lignin	21.3	23.2	20.2
Acid soluble lignin	1.2	1.0	1.4
Total lignin	22.5	24.2	21.6
Glucans	41.6	37.8	43.4
Xylans	18.9	12.4	22.1
Galactans	0.5	0.6	0.5
Mannans	0.0	0.0	0.0
Arabinans	1.2	0.9	1.4
Uronic acids	1.0	0.9	1.0
Acetyl	1.9	0.5	2.6
Total sugars*	65.0	52.9	71.1
Ash	4.0	9.5	1.3
Silica	2.7	7.5	0.4
Extractives	8.5	13.4	6.1
Grand total, %**	100	100	100

* Includes glucans, xylans, galactans, mannans, arabinans, uronic acids, and acetyl groups.
** Includes total lignin, total sugars, ash, and extractives.

I. Chemical composition of sugar cane bagasse in percentage of biomass dry weight.

Preparation	Preparation Yield, %
DB - MWLc	29.8
DB - CEL96	68.1

II. Yield from crude milled nonwood lignin (MWLc) and CEL96 preparations.

The pith presented much larger amounts of nonstructural constituents (extractives and inorganics) versus the depithed bagasse. Most of the total inorganic material was composed of silica, particularly with the pith bagasse fraction. The whole bagasse presented structural and non-structural chemical composition in between the pith and depithed bagasse ones, reflecting their proportions in the whole bagasse.

Lignin content of this bagasse was similar to values reported elsewhere. Values in the range of 19%-24% were reported by Hurter [22]. Luz et al. [23] have reported values of 43.8%, 28.6%, 23.5%, 1.3%, and 2.8% for cellulose, hemicelluloses, lignin, ash, and other components, respectively. High ash contents on bagasse have been reported by Wolf [5]. The very high content of extractives in the pith fraction also has been reported elsewhere [4,5,24].

Considering that pith has low fiber content and rather inadequate chemical composition to make pulp, this fraction was excluded for the next analyses in this study. The remainder of this study focused on the depithed bagasse fiber frac-

Lignin Sample	Aromatic Rings					Inter-unit Linkages		
	Percentage					Percentage		
	S:G:H	PCAs	FA	p-CA	Ratio FA/p-CA	β-O-4′	β-5′	β-β′
DB-MWLc	54/42/4	60.3	19.2	41.0	0.468	83	10	2
DB-CEL96	49/47/4	52.8	7.0	45.8	0.154	82	9	1

III. Syringyl (S):guaiacyl (G):p-hydroxyphenyl (H) (S:G:H) ratio, content of phenolic cinnamic acids (PCAs), and relative abundances of inter-unit linkages.

Dimension	Statistic	Depithed Bagasse Soda Pulp (kappa 17.5)
Fiber length, mm	Average	1.44
	Standard deviation	0.5
	C.V., %	34.9
Fiber width, µm	Average	21.3
	Standard deviation	6.5
	C.V., %	30.5
Lumen diameter, µm	Average	15.5
	Standard deviation	5.8
	C.V., %	37.8
Wall thickness, µm	Average	2.9
	Standard deviation	1.3
	C.V., %	43.6

IV. Depithed bagasse fiber dimensions in the soda brownstock (kappa 17.5).

tion. The high extractive and inorganic contents are quite a challenge for pulp production.

From the depithed bagasse fraction, two different isolation methods were used to obtain lignin fractions: MWLc and CEL96. Both lignin fractions were analyzed by 2D NMR spectroscopy. **Table II** shows the isolation yield from MWLc and CEL96 preparations. The isolation yield of CEL96 was far higher than that of MWLc. This means that the enzymatic digestion of the carbohydrates increases the accessibility for the dioxane 96% solvent.

Wood lignins mainly contain guaiacyl (G) and syringyl (S) units, whereas the lignins of nonwoody plants contain all three units: *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S). In nonwood materials, *p*-coumaric and ferulic acids are attached to lignin and hemicelluloses via ester and ether bonds forming lignin/phenolics–carbohydrate complexes.

The most abundant lignin linkages are β-O-4 ether bonds followed by β-5 and β-β′ structures. In nonwood materials, where *p*-coumaric acid (*p*-CA) is ester-linked to the γ-carbon of the lignin side-chain, we can find an acylated version of β-O-4′ ethers.

Table III shows the monomeric ratios, phenolic cinnamic acids (PCAs) contents, and the ratios of the different inter-unit linkages. Regarding the S:G:H ratio, the differences in the S and G units were relevant. Regarding PCAs, the estimated content was very high for both lignins (>50%); the ferulic acid (FA) content was higher in MWLc than CEL96; and the *p*-CA content was higher in CEL96 than MWLc preparations. In MWLc and CEL96 preparations, the FA:*p*-CA ratio was always in favor of *p*-CA (stronger for CEL96 than MWLc).

Depithed bagasse lignins of herbaceous crops are known to be structurally different from those of softwood or hardwoods. The PCAs are also attached to lignin via acid-labile ester bonds and serve as bridges between lignin and hemicelluloses [25]. The depithed bagasse lignin presented fractions with different structural monomer distributions. The lignin composition showed very high contents in PCAs.

Morphological characterization of depithed bagasse

Table IV shows the average fiber dimensions for the depithed bagasse brownstock after soda pulping to kappa 17.5. The average fiber length, fiber width, lumen diameter, and wall thickness of the depithed bagasse were similar to those

Relationship	Depithed Bagasse Soda Pulp (kappa 17.5)	Bagasse Biomass [11]	Eucalyptus Hybrids [26]
Felting index	67.6	69.0	48.0 - 59.0
Flexibility coefficient, %	72.8	58.0	28.0 - 47.0
Wall fraction, %	27.2	42.0	52.0 - 73.0
Ratio length/thickness	496.6	329.0	145.0 - 196.0
Runkel index	0.37	0.73	1.10 - 2.67

V. Relationship of morphological properties.

Fiber analysis	Depithed Bagasse Soda Pulp (kappa 17.5)	Eucalyptus Hybrids Kraft Pulp [28]	Eucalyptus Hybrids Kraft Pulp [29]
Coarseness, mg/100 m	4.3	6.4	7.6
Fibers number/gram, millions	25.8	23.7	22.0
Fines content, %	12.0	6.2	14.7

VI. Analysis of depithed bagasse soda pulp fiber suspension carried out in Galai Cis-100 particle size analyzer.

reported by Carvalho [26] and Boechat [27]. In general, the fiber morphology of bagasse is somewhat similar to that of many hardwoods, thus being qualified as a short-fibered material [26,27]. The values shown for the wall thickness were much lower in relation to lumen diameter. We anticipate that pulp fibers produced from this raw material will have high collapsibility and low drainability, in addition to good bonding capacity. Other significant information can be obtained from the fiber morphology data through the correlations among the various fiber dimensions. **Table V** shows the relations calculated based on the average value of each morphological parameter measured in Table IV. These calculated relationships help with understanding the influence of fiber morphology on paper formation and sheet physical properties [11]. Fibers with a high Runkel index and wall fraction, and a low flexibility coefficient are more rigid; these relationships correlate to tear resistance, which is a property dependent on the intrinsic strength of the fiber. However, tensile and burst indexes are properties that depend on fiber bonding [11].

The depithed bagasse soda pulp fibers exhibited a Runkel index of 0.37% and wall fraction of 27.2%. These values are lower than those reported for eucalypt woods, which were 1.10-2.67 and 52%-73%, respectively [11]. The pulp's fiber flexibility coefficient value was higher than that reported for eucalypt woods. Pulps of high flexibility coefficient produce more inter-fiber bonding during beating, which increases tensile and burst strengths. The felting index for depithed bagasse soda pulp was similar to that reported by Carvalho [26], but was higher than that of eucalypt wood fibers [11]. The length to thickness ratio of the depithed bagasse soda pulp was higher (496.6) than that of eucalyptus wood fibers (145-196) [11] and bagasse biomass fibers (329) [26].

Table VI shows the results of fiber coarseness, fiber population per unit mass, and fiber fines content for depithed bagasse soda pulp and eucalyptus hybrids kraft pulps. The coarseness of the depithed bagasse fibers was lower than values reported in the literature for eucalypt kraft pulps. The coarseness is closely related to the fiber wall thickness and increases with increasing wall thickness [29]. The fiber population (fibers/gram) of depithed bagasse pulp was somewhat similar to those observed for eucalypt pulps. The fines content of the depithed bagasse pulp (12.0%) was higher than the values commonly reported in the literature for eucalypt pulps. This is explained by the presence of significant parenchyma cells in the bagasse pulp, in spite of being depithed. Pulps containing higher fines content and high number of fibers per gram of pulp, coupled with low fiber coarseness and thin-walled fibers, will likely exhibit high resistance to drainage at the wet end of the paper machine.

CONCLUSIONS

- The contents of cell wall structural constituents in the three bagasse materials varied significantly, with the pith showing significantly higher lignin, and lower xylans and glucans contents than the depithed bagasses.
- The pith presented much larger amounts of extractive and inorganic contents in relation to depithed bagasse.
- The depithed bagasse lignin composition by 2D NMR analysis showed very high contents of PCAs.
- The morphological analyses of the depithed bagasse (fiber fraction) indicated a short fiber material, similar to hardwoods. **TJ**

LITERATURE CITED

1. Companhia Nacional de Abastecimento (CONAB), "Acompanhamento de safra brasileira: cana-de-açúcar, terceiro levantamento – December 2011." Available [Online] http://www.conab.gov.br/OlalaCMS/uploads/arquivos/11_12_08_11_00_54_08.pdf>[06June2014].
2. Maués, J.A., *Engenharia* 583: 88(1997).
3. Caraschi, J.C., Campana Filho, S.P., and Curvelo, A.A.S., *Polim.: Cienc. Tecnol.* 6(3): 24(1996).
4. Canilha, L., Carvalho, W., Rocha, G.J.M., et al., "Caracterização do bagaço de cana-de-açúcar in natura, extraído com etanol ou ciclohexano/etanol," Associação Brasileira de Química - Seção Regional do Rio Grande do Norte, Natal, Brasil, 2007. Available [Online] http://www.abq.org.br/cbq/2007/trabalhos/11/11-570-713.htm>[06June2014].
5. Wolf, L.D., "Pré-tratamento organossolve do bagaço da cana-de-açúcar para produção de etanol e obtenção de xilooligômeros," M.S. dissertation, Universidade Federal de São Carlos, São Carlos, Brasil, 2011.
6. De Ramos e Paula, L.E., Trugilho, P.F., Napoli, A., et al., *Cerne* 17(2): 237(2011).
7. Marabezi, K., "Estudo sistemático das reações envolvidas na determinação dos teores de lignina e holocelulose em amostras de bagaço e palha da cana-de-açúcar," M.S. dissertation, Instituto de Química de São Carlos da Universidade de São Paulo, São Carlos, Brasil, 2009.
8. Pandey, A., Soccol, C.R., Nigam, P., et al., *Bioresour. Technol.* 74(1): 69(2000).
9. Rajesh, K.S. and Rao Mohan, N.R., *IPPTA J.* 10(3): 151(1998).
10. Casey, J.P., Ed., *Pulp and Paper: Chemistry and Chemical Technology*, 3rd edn., vol. 2, John Wiley & Sons Inc., New York, 1980.
11. Foelkel, C.E.B. and Barrichelo, L.E.G., *8th ABCP Annu. Congr., Proc.*, Brazilian Technical Association of Pulp and Paper, São Paulo, 1975, pp. 40-53.
12. Vena, P.F., "Integration of xylan extraction prior to kraft and soda AQ pulping from South African grown *Eucalyptus grandis*, giant bamboo and sugarcane bagasse to produce paper pulps, value added biopolymers and fermentable sugars," Ph.D. dissertation, Stellenbosch University, Stellenbosch, South Africa, 2013.
13. Belayachi, L. and Delmas, M., *Biomass Bioenergy* 8(6): 411(1995).
14. Goldschimid, O., in *Lignins: Ocurrence, Formation, Structure and Reactions* (K.V. Sarkanem and C.H. Ludwig, Eds.), Wiley-Interscience, New York, 1971, pp. 241-266.
15. Gomide, J.L., and Demuner, B.J., *O Pap.* 47(8): 36(1986).
16. Lin, S.Y. and Dence, C.W., *Methods in Lignin Chemistry*, Springer-Verlag, Berlin, 1992, p. 578.
17. Wallis, A.F.A., Wearne, R.H., and Wright, P.J., *TAPPI J.* 49(4): 262(1996).
18. Solar, R., Kacik, F., and Melcer, I., *Nord. Pulp Pap. Res. J.* 2(4): 139(1987).
19. Scott, R.W., *Anal. Chem.* 51(7): 936(1979).
20. Björkman, A., *Sven. Papperstidn.* 59(13): 477(1956).
21. Balakshin, M.Y., Capanema, E.A., Gracz, H., et al., *Planta* 233(6): 1097(2011).
22. Hurter, A.M., *TAPPI Pulping Conf.*, TAPPI PRESS, Atlanta, GA, USA, Book 1, 1988, pp. 139–160.

ABOUT THE AUTHORS

We chose this topic because the sugar cane industry in Brazil is expanding and there has been great interest in the utilization of the leftover bagasse for other uses, in addition to burning it for energy. Bagasse is easily accessible and readily available in many countries. Given this, a complete chemical and morphological characterization of sugarcane bagasse becomes essential.

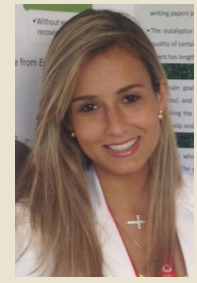
A thorough physical and chemical characterization of the bagasse, particularly regarding its lignin structure, is relevant for a more rational utilization of the bagasse in the production of printing and writing pulp grades, dissolving pulp, ethanol, and power.

The most difficult aspect of this research was to thoroughly elucidate the lignin structure of bagasse, because the available information about lignins of herbaceous crops is scattered and the available reviews generally address wood lignins. To address this, our complementary study was performed.

The most interesting surprise was that the depithed bagasse lignin composition by two-dimensional nuclear magnetic resonance spectroscopy analysis showed very high contents of phenolic cinnamic acids.

Mills can use the information about the complete chemical and morphological characterization to better understand the composition of the bagasse for special cellulosic pulp production.

The next steps will be to produce special cellulose pulps from sugar cane bagasse, such as dissolving pulp production, and to perform a complementary study about lignin structure and characterization.



Andrade

Colodette

Jameel

Andrade is a Ph.D. candidate and Colodette is professor, Pulp and Paper Laboratory, Department of Forestry Engineering, Federal University of Viçosa, Minas Gerais, Brazil. Jameel is professor, Department of Wood and Paper Science, North Carolina State University, Raleigh, NC, USA. Email Andrade at andrade-marcela@hotmail.com.

23. Luz, S.M., Gonçalves, A.R., Ferrão, P.M.C., et al., "Water Absorption Studies of Vegetable Fibers Reinforced Polypropylene Composites," *Proc. Int. Symp. Nat. Polym. Compos., 6th; XI Int. Macromol. Colloq.*, 2007, Vol. 1, p. 73.
24. Pitarelo, A.P., "Avaliação da susceptibilidade do bagaço e da palha de cana de açúcar à bioconversão via pré-tratamento a vapor e hidrólise enzimática," Ph.D. dissertation, Universidade Federal do Paraná, Curitiba, Brasil, 2007.
25. Buranov, A.U. and Mazza, G., *J. Chem. Eng.* 28(3): 237(2008).
26. Carvalho, D.M., "Caracterização físico-química e polpação etanol/soda do bagaço e da palha de cana-de-açúcar," Ph.D. dissertation, Universidade Federal de Viçosa, Brasil, 2012.
27. Boechat, J.V.B., "Produção de papel tissue a partir de bagaço de cana," M.S. dissertation, Universidade Federal de Viçosa, Viçosa, Brasil, 2010.
28. Bassa, A., Bassa, A.G.M.C., Sacon, V.M., et al., "Seleção e caracterização de clones de eucalipto considerando parâmetros silviculturais, tecnológicos e de produto final," *II Int. Colloq. Eucalyptus Pulp*, Universidade Federal de Viçosa, Viçosa, Brasil, 2005.
29. Ferreira, C., Junior, M.F., Oliveira, R.C., et al., "Critérios de seleção de clones para maximizar rendimento e qualidade da cellulose," *I Int. Colloq. Eucalyptus Pulp*, Universidade Federal de Viçosa, Viçosa, Brasil, Brasil, 2003.