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Sugarcane biomass for biorefineries: Comparative composition of carbohydrate and non-carbohydrate components of bagasse and straw

Danielle Szczerbowski[a,c], Ana Paula Pitarelo[b,c], Arion Zandoná Filho[c], Luiz Pereira Ramos[c,d,*]

[a] Novozymes Latin America, 683—Professor Francisco Ribeiro St., 83606-770 Araucária, PR, Brazil
[b] Centro de Tecnologia Canavieira (CTC), Fazenda Santo Antônio, 13400-907 Piracicaba, SP, Brazil
[c] Research Center in Applied Chemistry (CEPESQ), Department of Chemistry, Federal University of Paraná, PO Box 19081, 81531-980 Curitiba, PR, Brazil
[d] INCT Energy & Environment (INCT E&A), Department of Chemistry, Federal University of Paraná, Curitiba, PR, Brazil

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ABSTRACT

Two fractions of sugarcane, namely bagasse and straw (or trash), were characterized in relation to their chemical composition. Bagasse presented values of glucans, hemicelluloses, lignin and ash of 37.74, 27.23, 20.57 and 6.53%, respectively, while straw had 33.77, 27.38, 21.28 and 6.23% of these same components. Ash content was relatively high in both cane biomass fractions. Bagasse showed higher levels of contaminating oxides while straw had a higher content of alkaline and alkaline-earth oxides. A comparison between the polysaccharide chemical compositions of these lignocellulosic materials suggests that similar amounts of fermentable sugars are expected to arise from their optimal pretreatment and enzymatic hydrolysis. Details about the chemical properties of cane biomass holocellulose, hemicelluloses A and B and α-cellulose are provided, and these may offer a good opportunity for designing more efficient enzyme cocktails for substrate saccharification.

1. Introduction

Sugarcane is an energy crop that is largely grown on flat lands in countries like Brazil, China, India, Thailand and Australia (Soccol et al., 2010), and its harvesting and processing wastes, namely straw (dried and green leaves and tops, sometimes referred to as cane trash) and bagasse (pressed and water-washed culms), are examples of lignocellulosic biomasses that can be used to produce fuels and chemicals (Saad et al., 2008). In Brazil, the production of sugarcane in 2011 reached 625 million tons (Chandel, Silva, Carvalho, & Singh, 2012). Since the handling and processing of fresh sugarcane produce 14 and 12.5% of straw and bagasse (dry basis), it is estimated that nearly 82.4 and 73.6 million tons of these residues were produced during this same period, respectively. Such lignocellulosic materials are potential sources for ethanol production due to their relatively low cost, availability, favorable logistics and suitable chemical composition (Soccol et al., 2010). Table 1 shows that the chemical composition of cane bagasse has been extensively studied while cane straw is still emerging as a promising bioenergy feedstock.

In general, ethanol can be produced from cane polysaccharides such as glucans (cellulose and β-glucans), hemicelluloses (xyloglucans and heteroxylans) and pectins after hydrolysis and fermentation. However, some monosaccharides (mostly pentoses) are more difficult to ferment to fuel ethanol than hexoses such as glucose. Hence, along with agronomic issues, the proper characterization of a given plant biomass is critical for the optimization of its use for cellulosic ethanol because this would provide valuable information about properties, storage and handling criteria, theoretical yields and potential environmental problems that are related to large scale industrial operations (Vassilev, Baxter, Andersen, & Vassileva, 2010). For such purpose, the objectives of this work were to develop a detailed characterization of the chemical composition of the two main lignocellulosic fractions of sugarcane (bagasse and straw) and compare their potential as a suitable source of fermentable sugars for bioprocesses such as those involved in the cellulosic ethanol production chain.

* Corresponding author. Tel.: +55 41 33613175; fax: +55 41 33613186.
E-mail addresses: dszc@novozymes.com (D. Szczerbowski), anapitarelo@gmail.com (A.P. Pitarelo), a.zandona@globo.com (A. Zandoná Filho), luiz.ramos@ufpr.br, lramos@quimica.ufpr.br (L.P. Ramos).

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Table 1
Compositional analysis of sugarcane bagasse and straw.

Reference	Component (wt%)					
	Glucan	Hemicellulose	Lignin[a]	Ash	Extractives[b]	Total
Bagasse						
Hoareau, Trindade, Siegmund, Castellan, & Frollini (2004)	55.2	16.8	25.3	1.1	–	98.4
Carrasco et al. (2010)	43.4	28.3	22.8	1.9	1.6	98.0
Silva et al. (2010)	38.8 ± 0.7	26.0 ±0.4	32.4[d]	2.8 ± 0.9	-	100.0
Krishna, Prasanthi, Chowdary, & Ayyanna, 1998	40.1 ± 0.2	31.1 ± 0.1	19.1 ± 0.5[c]	1.0 ± 0.2	9.2 ± 0.1	100.5
Maeda et al. (2011)	34.1 ± 1.2	29.6 ± 1.4	19.4 ± 0.4	7.9 ± 1.1	–	91.0
Monavari, Galbe, & Zacchi (2011)	40.9	32.2	22.9	3.9	–	99.9
Rocha et al. (2011)	45.5 ± 1.1	27.0 ± 0.8	21.1 ± 0.9	2.2 ± 0.1	4.6 ± 0.3	100.4
Rocha, Gonçalves, Oliveira, Olivares, & Rossell (2012)	43.1 ± 1.4	25.2 ± 1.9	22.9 ± 1.1	2.8 ± 1.4	4.3 ± 1.6	98.3
Velmurugan and Muthukumar (2012)	35.6	26.6	17.1	–	–	79.3
Yoon et al. (2012)	41.0	30.1	21.2	0.7	7.0	100.0
Straw						
Saad et al. (2008)	39.4 ± 0.3	26.2 ± 0.1	21.5 ± 0.3	-	–	87.1
Silva et al. (2010)	33.6 ± 0.9	28.9 ± 0.6	31.8[d]	5.7 ± 0.1	–	100.0
Krishna, Prasanthi, Chowdary, & Ayyanna, 1998	35.3 ± 0.6	26.4 ± 0.6	19.6 ± 0.7[c]	7.8 ± 2.1	7.5 ± 0.6	96.6
Moutta et al. (2012)	40.8 ± 0.9	30.8 ± 1.0	25.8 ± 0.1	2.6 ± 0.1	–	100.0
Costa et al. (2013)	33.5 ± 0.2	27.1 ± 0.3	25.8 ± 0.5	2.5 ± 0.2	–	88.9
Oliveira et al. (2013)	39.8 ± 0.3	28.6 ± 0.2	22.5 ± 0.1	2.4 ± 0.3	6.2 ± 0.3	99.5

[a] Total lignin content (acid-soluble plus acid-insoluble lignin).
[b] Total extractives content.
[c] Reported exclusively as acid-insoluble lignin.
[d] Reported as lignin and others.

2. Material and methods

2.1. Material

The two types of sugarcane (*Saccharum officinarum*) biomass (straw and bagasse) were obtained from the São Martinho Mill (Pradópolis, SP, Brazil) with the technical help of CTC (Cane Technological Center, Piracicaba, SP, Brazil). The variety SP79-1049 was mechanically harvested in the beginning of the harvest season (April 29, 2009) in green conditions after 4 years of growth. Both sugarcane culms and straw were collected in the field at the same time. Bagasse was obtained directly from the cane crusher after pressing and water washing to extract the majority of the sugarcane juice. Both sugarcane biomass were packed and stored in vacuum sealed plastic bags at 4 °C.

2.2. Methods

The average composition analysis of both sugarcane biomass fractions was always obtained from three independent replicates. Total extractives, ash and protein contents were determined directly on unextracted samples, whereas measurements of both cane polysaccharide and lignin components were performed in extractive-free materials (see below for details).

Ash content was carried out according to the NREL/TP-510-42622 method (Sluiter et al., 2008a). Bagasse was analyzed as received while straw was washed with water at room temperature to remove the excess of soil and dust. The chemical composition of cane biomass ashes was determined by X-ray fluorescence in a Philips Analytical XRF apparatus, model PW 2400/00, provided with a Sample Changer model 2510. XRF analyses were carried out using a wafer that was obtained by melting 0.9 g of ashes with lithium tetraborate and ammonium nitrate at 900 °C for 20 min.

Protein determination was performed using the micro-Kjeldahl method according to AOAC 991.20 standard procedure (Horwitz & Latimer, 2005). Extractives were determined quantitatively in a Soxhlet apparatus using the following sequence of extraction solvents: ethyl ether (EE), dichloromethane (DCM), ethanol:toluene 1:2 vol/vol (ET) and ethanol 95% (E95), followed by a hot-water extraction under reflux for 1 h. Besides that, the total extractive content was also determined by solvent extraction with E95, following the NREL/TP-510-42619 method (Sluiter et al., 2008b).

Different fractions of cane biomass extractives were analyzed by Fourier-transform infrared spectrometry (FTIR) using a Bomen BM-100 spectrometer (Hartmann & Braun, Quebec, Canada) in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}, 64 scans and triangular apodization. FTIR spectra were acquired from potassium bromide (KBr) pellets containing 1% of the ground sample.

Total lignin content was determined in accordance to the NREL/TP-510-42618 method (Sluiter et al., 2008c). However, in this case, the extractive-free materials were those derived from extraction with a sequence of solvents with increased polarity. HPLC analyses of neutral sugars were carried out in acid hydrolysates using an Agilent 1200 Infinity Chromatograph with an Aminex HPX-87P column (Bio-Rad, Hercules, USA) and detection by differential refractometry. Acetic acid and dehydration by-products (furfural and hydroxymethylfurfural) were analyzed in the same HPLC system using an Aminex HPX-87H column (Bio-Rad) and detection by differential refractometry and ultraviolet spectrophotometry at 280 nm, respectively. All analytes measured by HPLC were converted to their anhydro form to compile the full composition analysis of both cane biomass fractions (Sluiter et al., 2008c).

Holocellulose, hemicelluloses A and B and α-cellulose were prepared by treatment of the biomass with sodium chlorite under acidic conditions. The experimental procedure was an adaptation made by Guerra, Mendonça, & Ferraz, 2003 from the classical method described by Browning (1967). Analyses of both holocellulose and α-cellulose were performed by acid hydrolysis using the NREL/TP-510-42618 method (Sluiter et al., 2008c). On the other hand, hemicelluloses A and B were analyzed by HPLC after acid hydrolysis with 1 mol L^{-1} H_2SO_4 for 2 h at 105 °C.

The molecular mass (MM) distribution of cane biomass polysaccharides was obtained by gel permeation chromatography (GPC) of their tricarbanyl derivatives (Ramos, Zandoná Filho, Deschamps, & Saddler, 1999). GPC was carried out in a Waters HPLC Model 1515 using a series of four TSK gel columns (6000HXL, 4000HXL, 3000HXL and 1000HXL; 7.8 × 300 mm each) with tetrahydrofuran (THF) as mobile phase. The number (MM_N) and mass (MM_M) average MM of per-carbanylated cellulose were calculated by universal

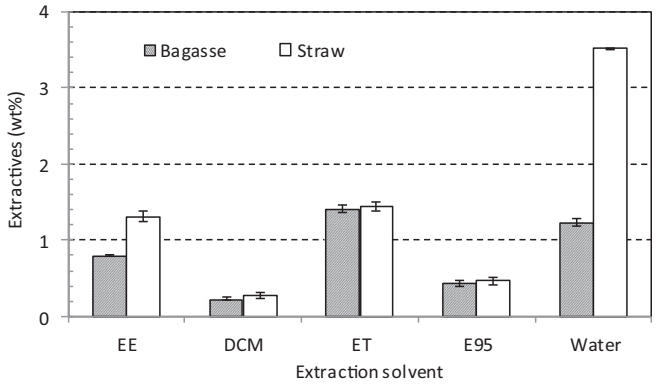

Fig. 1. Extractive contents derived from cane biomass in different solvents. **EE**: ethy ether; **DCM**: dichloromethane; **ET**: ethanol:toluene 1:2 vol/vol; **E95**: ethanol 95%.

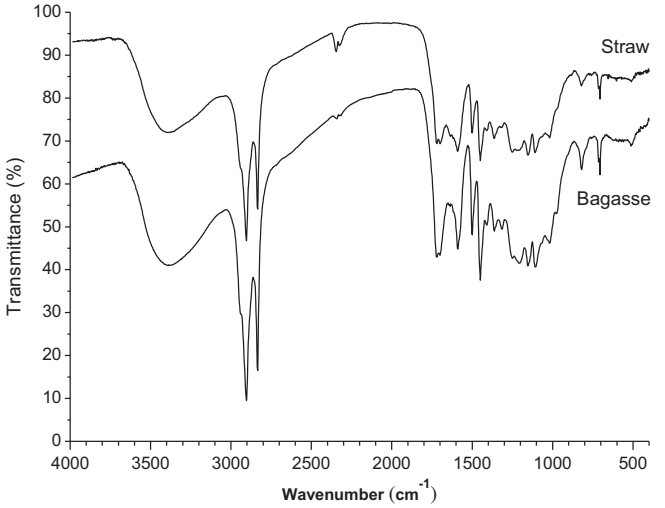

Fig. 2. FTIR analyses of E95 extractives that were obtained from cane bagasse and cane straw.

calibration and the degree of polymerization (DP) was determined by dividing MM by 519. Sample polydispersity was the ratio between MM_M and MM_N.

3. Results and discussion

3.1. Compositional analysis of cane biomass organic components

Cane bagasse and cane straw were shown to have similar ash contents of 6.53 and 6.23 wt%, respectively. Vassilev et al. (2010) reported an ash content of 2.1 wt% for cane bagasse and an average of 8.6 wt% for different samples of biomass straws, while Silva, Inoue, Endo, Yano, & Bon (2010) reported 2.8 wt% for cane bagasse and 5.7 wt% for cane straw. In this work, a higher amount of ash was observed in cane bagasse and this might have been related to the conditions used for harvesting. Variable amounts of ash are usually associated with inorganic soil contaminations, along with mechanical contaminations arisen from wearing of industrial crushers such as iron and titanium oxides. Regarding straw, lower amounts of ash were reported by other authors (Table 1) and this was probably due to contamination with soil and dust as well. If the straw is washed before ash determination, most of the silica present in the surface of the leaves is lost, resulting in lower ash contents. Thus, any comparative analysis based on data available in the literature requires caution because the observed differences may be due to differences in sample preparation and handling.

Cane bagasse and cane straw had ash contents lower than those observed in other biomass types such as rice and wheat straw (17.5 and 11.0%, respectively) (Barcelos, Maeda, Betancur, & Pereira Jr., 2013). Hence, both cane biomass fractions have a relatively low buffering capacity, offering advantages over other materials for pretreatment methods based on acid catalysis.

As stated earlier, both cane biomass fractions were extracted before characterization with a sequence of five solvents with increased polarity, with water being used in the last washing stage. This method was used to ensure the complete removal of non-structural compounds that are not directly linked to the cell wall, since these can interfere in the quantification of carbohydrates and lignin. The amount of extractives obtained for each solvent was quantified gravimetrically and the results are shown in Fig. 1. Ethyl ether (EE) was able to extract twice as much material from cane straw compared to cane bagasse and this in probably due to the presence of waxes and other non-polar compounds in its composition. Straw was also richer in water-extractable compounds and this is due to the presence of secondary metabolites and energy storage compounds that are critical for photosynthesis and plant growth.

The preliminary characterization of these multiple cane biomass extractives was carried out by FTIR (data not shown). In general, the extractives obtained by treating cane bagasse with EE in a Soxhlet apparatus had a FTIR spectrum typical of aliphatic organic compounds of low polarity. This conclusion arose from the relative intensity of both axial and angular deformations of C—H in CH_3 e CH_2 and the absence of other bands that could be assigned to aromatic and/or oxygenated compounds. The breathing vibration of the aromatic ring was initially observed in the FTIR spectrum of the DCM extract and became relatively predominant in the ET extract, together with other bands that are characteristic of oxygenated aromatic compounds such as phenolic acids. The characteristic vibrations of O—H in hydroxyl groups were also evident in the FTIR spectra of both DCM and ET extracts and these bands became predominant in the last two families of extractives, particularly those obtained in water, whose profile was typical of water-soluble carbohydrates such as sucrose and pectin. Although relatively different in their intensities, the FTIR assignments were typically the same in the spectra of both cane straw and cane bagasse extractive components. In this case, the similarity among the EE, DCM and ET extracts suggest that a single extraction procedure with DCM would suffice for these cane biomass fraction.

Sluiter et al. (2008b) proposed a method to remove extractives of grasses and energy crops using only water and E95. When both cane biomasses were submitted to a single extraction with E95, the resulting amount of extractable materials was $2.79 \pm 0.01\%$ for bagasse and $4.89 \pm 0.09\%$ for straw. Therefore, a single extraction with E95 was almost as efficient for cane bagasse as the sequence of extraction solvents, while its use led to an even higher percentage of total extractable compounds for straw. No clear differences were observed between the FTIR spectra of E95 extractives from both bagasse and straw and this indicative for the presence of the same functional groups but not necessarily the same organic compounds in their chemical composition (Fig. 2). However, further analyses of these extractives are still required to reveal their chemical nature using techniques such as liquid chromatography and mass spectrometry.

Both carbohydrates and lignin contents were determined in extractive-free materials but the results in Table 2 are expressed in relation to the dry mass of the original samples. Bagasse and straw had similar hemicellulose (mostly xylans) and ash contents. However, the former had a lower amount of extractives and a glucan content four points percent higher than that of straw. By contrast,

Table 2
Chemical composition of cane biomass.

Component (wt%)	Bagasse	Straw
Anhydroglucose (glucans)	36.74 ± 0.26	32.76 ± 0.51
Anhydroxylose (hemicelluloses)	20.30 ± 0.07	20.47 ± 0.74
Anhydrogalactose (hemicelluloses)	0.65 ± 0.05	0.97 ± 0.28
Anhydroarabinose (hemicelluloses)	1.80 ± 0.37	2.57 ± 0.64
Unidentified anhydrohexose (glucans)	1.00 ± 0.06	1.01 ± 0.03
Unidentified anhydropentose (hemicelluloses)	0.22 ± 0.03	0.11 ± 0.01
Acetyl groups (hemicelluloses)	4.26 ± 0.08	3.26 ± 0.07
Acid-soluble lignin	0.23 ± 0.04	0.71 ± 0.03
Acid-insoluble lignin	20.34 ± 0.40	20.57 ± 0.19
Ash	6.53 ± 0.20	6.23 ± 0.20
Protein	1.13 ± 0.16	3.72 ± 0.11
Extractives in organic solvents	2.85 ± 0.03	3.50 ± 0.06
Extractives in hot water	1.22 ± 0.05	3.52 ± 0.01
Total	97.27	99.40
Total glucan content	37.74	33.77
Total hemicellulose content	27.23	27.38
Total lignin content	20.57	21.28

Table 3
X-ray fluorescence (XRF) analyses of cane biomass inorganic compounds (ashes); values were expressed in relation to the original dry mass.

Oxide (wt%)			Corresponding element (g kg^{-1})		
Type	Bagasse	Straw	Type	Bagasse	Straw
CaO	0.11 ± 0.01	0.77 ± 0.01	Ca	0.79 ± 0.01	5.49 ± 0.01
MgO	0.08 ± 0.01	0.24 ± 0.01	Mg	0.48 ± 0.01	1.44 ± 0.01
SiO_2	1.79 ± 0.01	2.68 ± 0.01	Si	8.35 ± 0.02	12.51 ± 0.06
I_2O_3	0.98 ± 0.01	0.35 ± 0.01	I	5.17 ± 0.01	1.83 ± 0.01
Fe_2O_3	1.54 ± 0.01	0.30 ± 0.02	Fe	10.74 ± 0.09	2.11 ± 0.02
Na_2O	0.01 ± 0.01	0.01 ± 0.01	Na	0.04 ± 0.01	0.07 ± 0.02
K_2O	0.17 ± 0.01	0.33 ± 0.01	K	1.37 ± 0.01	2.70 ± 0.01
SrO	nd[a]	0.01 ± 0.01	Sr	nd	0.04 ± 0.01
TiO_2	0.45 ± 0.01	0.10 ± 0.01	Ti	2.72 ± 0.01	0.62 ± 0.01
MnO	0.08 ± 0.01	0.02 ± 0.01	Mn	0.11 ± 0.02	0.15 ± 0.01
P_2O_5	0.11 ± 0.01	0.11 ± 0.01	P	0.48 ± 0.01	0.46 ± 0.01
Loss[b]	0.02 ± 0.01	0.03 ± 0.01			
Total ash	5.25 ± 0.01	4.93 ± 0.02			

[a] nd, Not detected.
[b] Loss inherent to heating (flame loss).

the protein content of straw was three times higher than that of bagasse.

The compositional analysis of both cane biomass fractions differs considerably from one study to another (Table 1). However, not all data found in the literature are reported as the average of independent replicates with their corresponding standard deviations. Also, the chemical composition of cane biomass and other lignocellulosic materials are often reported as normalized data (Silva et al., 2010; Yoon, Ang, Ngoh, & Chua, 2012) and this is not suitable for calculating recoveries and mass balances because, in this case, the relative contribution of each component is clearly overestimated.

In general, the composition analysis of lignocellulosic materials may vary quantitatively within the same plant species according to its age, storage time, type of plant tissue and both cultivation and harvesting conditions (Fernandes, Bos, Zeeman, Sanders, & van Lier, 2009; Liu, Ye, Womac, & Sokhansanj, 2010). However, a great deal of this data scattering is also due to differences in the methods used for characterization, including (a) the use of extractives-free samples for lignin analysis, (b) the extent of extractives removal during treatment with different solvents, (c) the proper quantification of minor components such as pectic materials and proteins, (d) the use of HPLC columns with different resolutions among monosaccharides, and (e) the application of suitable hydrolysis factors to account for sugar losses due to dehydration. Along with agronomic and edafoclimatic drivers, these are probably the main reasons why there is such a wide range of differences in the reported chemical composition of both cane bagasse and cane straw.

Regarding lignin, a straight comparison among different studies available in the literature is even more complicated due to differences in the nature of the reported data, including the mandatory use of extractive-free materials (Sluiter et al., 2008c) and the subtraction of the amount of ash found in acid-insoluble lignin (Gouveia, Nascimento, & Souto-Maior, 2013). This source of error leads to an overestimation of the total lignin content of cane biomass since both bagasse and straw acid-insoluble lignin had an ash content of 2.67 ± 0.05% and 2.75 ± 0.02% in relation to its dry mass, respectively.

A small percentage of unidentified anhydrosugars is observed in Table 2 and these correspond to dehydration byproducts that are formed during acid hydrolysis. Since it is impossible to precise where these dehydration components came from, furfural and hydromethylfurfural (HMF) were expressed as anhydrosugars and added to the total hemicellulose and glucan contents of cane biomass, respectively. Also, minor components such as 4-*O*-methyl-glucuronic acid were not expressed in the proposed compositional analysis due to the lack of a suitable chromatographic standard for its quantification. For this reason, cumulative values such as those reported in Table 2 shall not reach 100% unless the experimental data are normalized.

3.2. Compositional analysis of cane biomass inorganic components

Ash analysis by X-ray diffraction revealed that SiO_2 was the predominant oxide in the inorganic matter of both cane biomass fractions, being particularly sharp for straw in which it contributes with 51% of the total ash content (Table 3). Besides that, straw had a relatively higher content of basic oxides (CaO, MgO and K_2O) compared to bagasse, suggesting a higher buffering capacity for pretreatment technologies that are based on acid hydrolysis, while bagasse showed a higher content of contaminating oxides that are probably due to soil impurities (Al_2O_3) and wearing of crushing facilities that are used for extracting the juice from cane culms (Fe_2O_3 and TiO_2).

Leal et al. (2013) showed considerably lower amounts of these contaminating oxides in both bagasse and straw, with bagasse having four times less Al_2O_3 and more than six times less Fe_2O_3 than the samples involved in this study. Regarding basic oxides, Leal et al. (2013) detected half of the CaO content in cane straw while K_2O was present in concentrations five times higher than those obtained in this work. On the other hand, Vassilev et al. (2010) reported lower amounts of contaminating oxides and similar levels of basic oxides in cane bagasse, while silica was found in higher amounts, representing 46% of the total ash content compared to the 34% that was obtained in the present work. In general, the amount of inorganic components in plant biomass may vary due to several factors including climate, cultivation conditions, soil type, location, growth stage, harvesting and storage conditions (Vassilev et al., 2010).

3.3. Extraction and characterization of cane biomass polysaccharide fractions

Holocellulose content of cane bagasse was slightly higher than that of cane straw (Table 4), with 45.06 wt% of it corresponding to α-cellulose in relation to the bagasse dry matter. By contrast, the cane straw holocellulose contained a greater amount of both hemicelluloses A and B and the ratio between these two hemicellulose fractions was different than that of cane bagasse. Guimarães, Frollini, Silva, Wypych, & Satyanarayana (2009) found 71.39 ± 0.60 wt% of holocellulose and 23.33 ± 0.02 wt% of lignin in cane bagasse. Such results were lower than those obtained in this work because these authors did not use extractives-free materials in their analytical procedure. On the other hand, Caraschi, Campana

Table 4
Holocellulose, hemicelluloses and α-cellulose content of bagasse and straw.

Fraction	Straw (wt%)	Bagasse (wt%)
Holocellulose	77.71 ± 0.29	79.71 ± 1.82
Fractions derived from holocellulose		
Hemicellulose A	29.89 ± 1.87	26.74 ± 0.39
Hemicellulose B	10.25 ± 1.11	6.14 ± 0.54
α-Cellulose	37.53 ± 1.15	45.06 ± 0.95
Total	77.67	77.94

Filho, & Curvelo (1996) reported holocellulose and α-cellulose contents of 76.5 and 51.0 wt% in cane bagasse, respectively, and these are very similar to the values described in Table 4. However, their corresponding value for the total cane bagasse hemicellulose content (hemicelluloses A and B) was approximately 6% points percent higher than the corresponding value obtained in this work.

Table 5 shows the chemical composition of both cane biomass holocellulose and α-cellulose fractions. Sodium chlorite is reported as a selective oxidative agent for delignification of different types of wood and non-woody biomass (Kumar, Fran, Hubbell, Ragauskas, & Wyman, 2013). However, in both cases (bagasse and straw), nearly 1.2–1.4 wt% of acid-insoluble lignin remained attached to the delignified materials after chlorite bleaching (Table 5). This acid-insoluble lignin was probably highly condensed because alkali washing was not able to remove it from the fibrous material. The even higher total lignin contents in cane biomass α-celluloses were a result of hemicellulose removal by alkali washing. It is also worth noticing that a residual amount of hemicelluloses also survived the extraction procedure, leaving both α-cellulose fractions with as much as 4-5 wt% of hemicelluloses (primarily xylans) in their chemical composition.

The molar ratios between monosaccharide constituents (Glc:Xyl:Ara:Gal) were 3:56:11:1 and 1:28:3:1 for bagasse and straw hemicellulose A, respectively. These results show that both cane biomass hemicelluloses A were primarily formed by arabinoxylans with different Xyl:Ara ratios and relatively low hexose (Glc and Gal) content. Xyloglucans did not seem to be present in these fractions in large amount but the glucose content in cane bagasse hemicellulose A was three times higher than that of cane straw. Similarly, both hemicelluloses B had xylose as their main monosaccharide constituents but their Glc:Xyl:Ara:Gal ratios were considerably different, being 8:33:8:1 for cane bagasse and 5:25:4:1 for cane straw. Cane straw hemicelluloses A and B had similar Glc:Xyl:Ara:Gal molar ratios except for the slightly higher glucose content of the latter. The same was also observed in cane bagasse hemicellulose B but the higher Glc:Xyl ratio in this fraction strongly suggests the presence of a greater amount of xyloglucans, compared to the corresponding hemicellulose A fraction.

Other components may be present in cane hemicelluloses A and B with acid sugars, such as the 4-*O*-methyl-glucuronic acid, being the most important "unknown" in the HPLC analysis of cane biomass acid hydrolysates. Hence, the molar ratios reported above are slightly offset since this hemicellulose component may correspond to as much as 4 wt% of the cane biomass dry mass (Spiridon & Popa, 2005).

3.4. Degree of polymerization (DP) of cane biomass polysaccharide fractions

The molecular mass (MM) distribution of cane biomass polysaccharides are shown in Table 6. The FTIR analysis of all percarbanylated cellulosic derivatives revealed the almost absence of the axial deformation of associated O-H groups at 3300–3400 cm^{-1}. Hence, most if not all of this chemical groups were derivatized as a result of carbanylation (data not shown), rendering the product completely soluble in THF.

The DP of cane biomass polysaccharides were slightly higher in cane straw compared to that of cane bagasse (Table 6), not only in the holocellulose but also in their corresponding hemicellulose and α-cellulose fractions. Hemicellulose DP was also measured using the same analytical technique and the GPC distribution of hemicellulose tricarbanyl derivatives was chromatographically identical to the shoulder found in the GPC distribution of the corresponding holocellulose analysis. In absolute numbers, both hemicelluloses A found in cane biomass had almost the same DP_M value, whereas the DP_M of cane bagasse hemicellulose B was nearly half of that found in the hemicellulose B of cane straw. Table 6 also indicated that, regardless the DP value of cane biomass holocellulose, treatment of native cane biomass with sodium chlorite resulted in α-cellulose fractions with the DP_M in the range of 510–580 AnGlc units. This data revealed to be in the same order of magnitude as the average leveling-off DP (LODP) of microcrystalline cellulose whose accumulation was a result of cellulose depolymerization during chlorite bleaching. However, this consequence is not usually expected from chlorite bleaching since this method is supposed to be selective for delignification, therefore maintaining unaltered the chain length of structural plant polysaccharides (Kumar et al., 2013; Kumar, Mago, Balan, & Wyman, 2009). Our data has shown that this is not the case for agroindustrial residues such as cane bagasse and cane straw.

A similar reduction in cellulose DP_M was also observed by Kumar et al. (2009) in studies involving Whatman #1 filter paper. Treatment of filter paper fibers with sodium chlorite led to a 73% decrease in cellulose DP_M in relation to the untreated sample (1500 to 410 units of AnGlc, respectively). These authors postulated that the observed reduction in DP was due to acid hydrolysis and/or oxidative cleavage of glycosidic bonds in cellulose chains. On the other hand, treatment with sodium chlorite showed a negative effect of only 15% in the DP_M of microcrystalline cellulose (Avicel PH-101) because its DP_M was already in the range of 330 AnGlc units. These results can be explained by the general theory of cellulose acid hydrolysis, which is known to occur in two steps, one very fast that involves less-organized regions of the cellulose

Table 5
Chemical composition of cane biomass holocellulose and α-cellulose.

Component	Holocellulose (wt%)		α-Cellulose (wt%)	
	Straw	Bagasse	Straw	Bagasse
Anhydroglucose	45.73 ± 0.15	48.41 ± 0.74	89.17 ± 0.24	88.22 ± 0.35
Anhydroxylose	28.25 ± 0.17	27.10 ± 1.42	3.59 ± 0.13	2.06 ± 0.40
Anhydroarabinose	3.92 ± 0.83	2.88 ± 0.98	0.67 ± 0.19	0.68 ± 0.01
Anhydrogalactose	1.43 ± 0.37	1.25 ± 0.41	0.67 ± 0.13	1.03 ± 0.19
Acetyl groups	4.04 ± 0.02	4.82 ± 0.07	nd[a]	nd
Acid insoluble lignin	4.15 ± 0.05	4.06 ± 0.37	1.18 ± 0.37	1.26 ± 0.09
Acid soluble lignin	0.23 ± 0.02	0.30 ± 0.01	0.62 ± 0.03	0.56 ± 0.04
Total	87.75	88.82	95.90	93.81

[a] nd, Not detected.

Table 6
Degree of polymerization of the polysaccharide fractions derived from cane biomass, as determined by GPC of tricarbanyl derivatives.

Fraction	MM_M[a]	DP_M[b]	MM_N[c]	DP_N[d]	PD[e]
Cane bagasse polysaccharides					
Holocellulose	621,115 ± 26,149	1196	375,319 ± 19,441	723	1.65
Error (%)	4.21		5.18		
Hemicellulose A	187,505 ± 19,257	361	134,170 ± 13,739	259	1.40
Error (%)	10.27		10.24		
Hemicellulose B	110,348 ± 1,280	213	102,430 ± 3,677	197	1.08
Error (%)	1.16		3.59		
α-Cellulose	301,110 ± 6,383	580	221,610 ± 28,455	427	1.36
Error (%)	2.12		12.84		
Cane straw polysaccharides					
Holocellulose	662,791 ± 3,049	1277	300,711 ± 0,872	579	2.20
Error (%)	0.46		0.29		
Hemicellulose A	204,883 ± 4,897	395	188,765 ± 18,914	364	1.09
Error (%)	2.39		10.02		
Hemicellulose B	211,058 ± 4,728	407	192,540 ± 5,199	371	1.10
Error (%)	2.24		2.70		
α-Cellulose	264,104 ± 25,618	509	170,457 ± 37,126	328	1.55
Error (%)	9.70		21.78		

[a] MM_M, mass-average molecular mass.
[b] DP_M, mass-average degree of polymerization.
[c] MM_N, number-average molecular mass.
[d] DP_N, number-average degree of polymerization.
[e] PD, polydispersity (PD = MM_M/MM_N).

structure (amorphous regions) and causes a subtle decrease in cellulose DP, and a rate limiting step that involves the peeling of cellulose chains from the surface of the crystalline regions. In this way, the cellulose DP decreases rapidly to a limit, with a concomitant increase in cellulose crystallinity due to the easiness with which these short chains organize themselves in structures of higher molecular order (Hubbell & Ragauskas, 2010).

The polydispersity (PD) of cane straw holocellulose was higher than that of the corresponding cane bagasse holocellulose, indicating that chlorite bleaching of cane straw led to a greater accumulation of low molecular mass oligomers. Also, compared to the original cane biomass holocelluloses, higher PD values were observed in both α-cellulose fractions that were derived from bagasse and straw, indicating that the alkaline treatment for hemicellulose removal reduced the amplitude of the DP distribution in both cases. On the other hand, the PD value for cane bagasse hemicellulose A was higher than that of the corresponding hemicellulose B and this was probably due to its relatively higher DP_M range.

4. Conclusion

The proper chemical characterization of energy crops is a critical step toward the implementation of a commercial process involving bioenergy and/or biorefineries. However, this step is not always treated in high priority as many research groups are used to reporting incomplete and/or misleading chemical compositions that are not adequate to achieve closed mass balances in biomass processing technologies. In this work, a detailed investigation was carried out to provide the compositional analysis of two cane biomass fractions, namely cane biomass and cane straw. In general, both fractions differed very little in their chemical composition, suggesting that relatively similar amounts of fermentable sugars would be released from their optimal pretreatment and enzymatic hydrolysis.

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References

Barcelos, C. A., Maeda, R. N., Betancur, G. J. V., & Pereira, N., Jr. (2013). The essentialness of delignification on enzymatic hydrolysis of sugar cane bagasse cellulignin for second generation ethanol production. *Waste Biomass Valorization*, *4*, 341–346.

Browning, B. L. (1967). Holocellulose preparations. In *Methods of wood chemistry, vol. II*. New York, NY: Wiley-Interscience Publishers.

Caraschi, J. C., Campana Filho, S. P., & Curvelo, A. A. S. (1996). Preparação e caracterização de polpas para dissolução obtidas a partir de bagaço de cana-de-açúcar. *Polímeros: Ciência e Tecnologia*, *3*, 24–29.

Carrasco, C., Baudel, H. M., Sendelius, J., Modig, T., Roslander, C., Galbe, M., Hahn-Hängerdal, B., Zacchi, G., & Lidén, G. (2010). SO_2-catalyzed steam pretreatment and fermentation of enzymatically hydrolyzed sugarcane bagasse. *Enzyme and Microbial Technology*, *46*, 64–73.

Chandel, A. K., da Silva, S. S., Carvalho, W., & Singh, O. V. (2012). Sugarcane bagasse and leaves: Foreseeable biomass of biofuel and bio-products. *Journal of Chemical Technology and Biotechnology*, *87*, 11–20.

Costa, S. M., Mazzola, P. G., Silva, J. C. A. R., Pahl, R., Pessoa, A., Jr., & Costa, S. A. (2013). Use of sugar cane straw as a source of cellulose for textile fiber production. *Industrial Crops and Products*, *42*, 189–194.

Fernandes, T. V., Bos, G. J. K., Zeeman, G., Sanders, J. P. M., & van Lier, J. B. (2009). Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass. *Bioresource Technology*, *100*, 2575–2579.

Guerra, A., Mendonça, R., & Ferraz, A. (2003). Molecular weight distribution of wood components extracted from *Pinus taeda* biotreated by *Ceriporiopsis subvermispora*. *Enzyme and Microbial Technology*, *33*, 12–18.

Gouveia, E. R., Nascimento, R. T., & Souto-Maior, A. M. (2013). Validação de metodologia para caracterização química de bagaço de cana-de-açúcar. *Química Nova*, *32*, 1500–1503.

Guimarães, J. L., Frollini, E., Silva, C. G., Wypych, F., & Satyanarayana, K. G. (2009). Characterization of banana, sugarcane bagasse and sponge gourd fibers of Brazil. *Industrial Crops and Products*, *30*, 404–415.

Hoareau, W., Trindade, W. G., Siegmund, B., Castellan, A., & Frollini, E. (2004). Sugar cane bagasse and curaua lignins oxidized by chlorine dioxide and reacted with furfuryl alcohol: characterization and stability. *Polymer Degradation and Stability*, *86*, 567–576.

Horwitz, W., & Latimer, G. W. (2005). *Official Methods of Analysis of AOAC International* (18th ed.). Gaithersburg, MD: AOAC International (Chapter 33)

Hubbell, C. A., & Ragauskas, A. J. (2010). Effect of acid-chlorite delignification on cellulose degree of polymerization. *Bioresource Technology*, *101*, 7410–7415.

Krishna, S. H., Prasanthi, K., Chowdary, G. V., & Ayyanna, C. (1998). Simultaneous saccharification and fermentation of pretreated sugar cane leaves to ethanol. *Process Biochemistry*, *33*, 825–830.

Kumar, R., Fran, H., Hubbell, C. A., Ragauskas, A. J., & Wyman, C. E. (2013). Comparison of laboratory delignification methods, their selectivity, and impacts on physicochemical characteristics of cellulosic biomass. *Bioresource Technology*, *130*, 372–381.

Kumar, R., Mago, G., Balan, V., & Wyman, C. E. (2009). Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresource Technology*, *100*, 3948–3962.

Leal, M. R. L. V., Galdos, M. V., Scarpare, F. V., Seabra, J. E. A., Walter, A., & Oliveira, C. O. F. (2013). Sugarcane straw availability, quality, recovery and energy use: A literature review. *Biomass and Bioenergy*, *53*, 11–19.

Liu, L., Ye, X. P., Womac, A. R., & Sokhansanj, S. (2010). Variability of biomass chemical composition and rapid analysis using FT-NIR techniques. *Carbohydrate Polymers*, *81*, 820–829.

Maeda, R. N., Serpa, V. I., Rocha, V. A. L., Mesquita, R. A. A., Santa Anna, L. M. M., Castro, A. M., Driemeier, C. E., Pereira, N., Jr., & Polikarpov, I. (2011). Enzymatic hydrolysis of pretreated sugar cane bagasse using *Penicillium funiculosum* and *Trichoderma harzianum* cellulases. *Process Biochemistry*, *46*, 1196–1201.

Monavari, S., Galbe, M., & Zacchi, G. (2011). Influence of impregnation with lactic acid on sugar yields from steam pretreatment of sugarcane bagasse and spruce for bioethanol production. *Biomass and Bioenergy*, *35*, 3115–3122.

Moutta, R. O., Chandel, A. K., Rodrigues, R. C. L. B., Silva, M. B., Rocha, G. J. M., & Silva, S. S. (2012). Statistical optimization of sugarcane leaves hydrolysis into simple sugars by dilute sulfuric acid catalyzed process. *Sugar Tech*, *14*, 53–60.

Oliveira, F. M. V., Pinheiro, I. O., Souto-Maior, A. M., Martin, C., Gonçalves, A. R., & Rocha, G. J. M. (2013). Industrial-scale steam explosion pretreatment of sugarcane straw for enzymatic hydrolysis of cellulose for production of second generation ethanol and value-added products. *Bioresource Technology*, *130*, 168–173.

Ramos, L. P., Zandoná Filho, A., Deschamps, F. C., & Saddler, J. N. (1999). The effect of *Trichoderma* cellulases on the fine structure of a bleached softwood kraft pulp. *Enzyme and Microbial Technology*, *24*, 371–380.

Rocha, G. M. J., Gonçalves, A. R., Oliveira, B. R., Olivares, E. G., & Rossell, C. E. V. (2012). Steam explosion pretreatment reproduction and alkaline delignification reactions performed on a pilot scale with sugarcane bagasse for bioethanol production. *Industrial Crops and Products*, *35*, 274–279.

Rocha, G. J. M., Martin, C., Soares, I. B., Maior, A. M. S., Baudel, H. M., & Abreu, C. A. M. (2011). Dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production. *Biomass and Bioenergy*, *35*, 663–670.

Saad, M. B. W., Oliveira, L. R. M., Cândido, R. G., Quintana, G., Rocha, G. J. M., & Gonçalves, A. R. (2008). Preliminary studies on fungal treatment of sugarcane straw for organosolv pulping. *Enzyme and Microbial Technology*, *43*, 220–225.

Silva, A. S., Inoue, H., Endo, T., Yano, S., & Bon, E. P. S. (2010). Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. *Bioresource Technology*, *101*, 7402–7409.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2008a). *Determination of ash in biomass. NREL/TP-510-42622* Golden, CO: National Renewable Energy Laboratory.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2008b). *Determination of extractives in biomass. NREL/TP-510-42619* Golden, CO: National Renewable Energy Laboratory.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). *Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618* Golden, CO: National Renewable Energy Laboratory.

Soccol, C. R., Vandenberghe, L. P. S., Medeiros, A. B. P., Karp, S. G., Buckeridge, M., Ramos, L. P., Pitarelo, A. P., Ferreira-Leitão, V., Gottschalk, L. M. F., Ferrara, M. A., Bon, E. P. S., Moraes, L. M. P., Araújo, J. A., & Torres, F. A. G. (2010). Bioethanol from lignocelluloses: Status and perspectives in Brazil. *Bioresource Technology*, *101*, 4820–4825.

Spiridon, I., & Popa, V. I. (2005). Hemicelluloses: Structure and properties. In S. Dumitriu (Ed.), *Polysaccharides: Structural diversity and functional versatility* (pp. 475–489). New York, NY: Marcel Dekker.

Vassilev, S. V., Baxter, D., Andersen, L. K., & Vassileva, C. G. (2010). An overview of the chemical composition of biomass. *Fuel*, *89*, 913–933.

Velmurugan, R., & Muthukumar, K. (2012). Sono-assisted enzymatic saccharification of sugarcane bagasse for bioethanol production. *Biochemical Engineering Journal*, *63*, 1–9.

Yoon, L. W., Ang, T. N., Ngoh, G. C., & Chua, A. S. M. (2012). Regression analysis on ionic liquid pretreatment of sugarcane bagasse and assessment of structural changes. *Biomass and Bioenergy*, *36*, 160–169.