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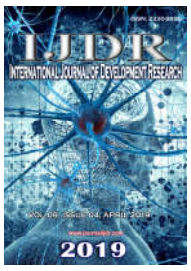

RESEARCH ARTICLE OPEN ACCESS

CHARACTERIZATION OF CELL WALL POLYSACCHARIDES AND CELLULOSIC ETHANOL POTENTIAL IN GENOTYPES OF SORGHUM BIOMASS

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ABSTRACT

In this study, the physicochemical characterization of the cell wall polysaccharides of different genotypes of sorghum bicolor, (*Sorghum bicolor* (L.) Moench) were obtained using the raw biomass and after the chemical pretreatments, with the objective of this culture being a potential source for the production of cellulosic ethanol. Among the evaluated materials two hybrids brown midrib (*bmr*) sorghum biomass were tested compared to three conventional sorghum biomass hybrids. The lignocellulosic composition of the raw biomass of each genotype, and after acid and alkaline pretreatments was determined for future investigations of improved ethanol yields. The lignocellulosic composition of the biomass of the *bmr* hybrid 2015B002 and 2015B003 stood out, presenting significantly lower lignin contents (4.63%) than the conventional hybrids (7.15%).The results showed that the acid treatment followed by the alkaline treatment showed the best performance after the enzymatic hydrolysis, supported by the Scanning Electron Microscopy (SEM), Fourier Transform Infrared Analysis (FTIR), Thermal analysis (TG) and crystallinity index analyzes that demonstrated the desired structural modification. Cellulosic ethanol production for the evaluated hybrids ranged from 6,612 to 11,838 liters per hectare per cycle of 180 days.

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INTRODUCTION

The lignocellulosic composition of the biomass and the deconstruction capacity of the plant cell wall are extremely important to indicate a culture as a potential biomass supply chain for bioenergy market. This physicochemical composition can be differentiated between species, cultural management and even in different parts of the same plant (ZHANG *et al*., 2014; VERBANČIČ *et al*., 2018). The cell wall consists mainly of cellulose microfibrils embedded in a polysaccharide matrix, in addition to proteins and phenolic substances (SANDHU *et al*., 2009, DOMÍNGUEZ *et al*., 2017). Due to its specificity and high recalcitrance (JOHNSON, 2016), unit operations are necessary to efficiently and economically break down these polymers.

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In this context, lignocellulosic biomass has been considered an alternative in the expansion of ethanol production, since it is the raw material used in cellulosic biofuel production technologies. Unlike the first generation technologies, the raw materials used in the production of cellulosic ethanol are complex, consisting of a fibrous material, which cannot be directly digested by the traditional yeasts (MONOCHIO *et al*., 2017). However, cellulosic ethanol technology is possible through enzymatic hydrolysis processes of biomass (RAELE *et al*., 2014; REIS *et al*., 2016), thus releasing fermentable sugar monomers. As a major constituent of the cell wall, cellulose is considered the most abundant polymer on earth and structurally microfibrils are coated by hemicelluloses and lignin (KANNAM *et al*., 2017). Because of the presence of intermolecular hydrogen bonds and a strong tendency to form intramolecular hydrogen bonds, cellulose is characterized by high stiffness, highly insoluble and resistant to most organic

solvents (TEIXEIRA; SIQUEIRA; BATISTA, 2016). In addition, cellulose microfibrils may exhibit different degrees of crystallinity and polymerization, which may be determinant for many future applications, such as the reduction of recalcitrance of biomass to hydrolysis in the production of cellulosic biofuels (CARROLL & SOMERVILLE, 2009). The hemicelluloses are characterized by being a heteropolysaccharide, composed of polymers of pentoses and hexoses (ANWAR; GULFRAZ; IRSHAD, 2014). Its branched configuration makes the hemicelluloses structure irregular, conferring absence of crystallinity and its hydrophilic nature, increasing the flexibility of the aggregate and being able to decrease the recalcitrance of the polymer (ANWAR; GULFRAZ; IRSHAD, 2014; FARHAT *et al*., 2017). Unlike polysaccharides, lignin is the third most important polymer in plant cell wall composition, and represents a set of amorphous, high molecular weight polymers with phenolic and highly recalcitrant nature (SHAHZADI *et al*., 2014). Among the biomass components, lignin acts negatively on enzymatic hydrolysis by three main factors: i) competitive adsorption, ii) chemical inhibition and iii) steric hindrance (KE & CHEN, 2013). In the biomass processing the physical, chemical and or enzymatic pretreatments are summarized in breaking the lignocellulosic structure increasing the access of the enzymes to the polysaccharides that will later be fragmented into fermentable monomers (LIU *et al*, 2017). However, the pretreatment steps with polysaccharide hydrolysis also lead to the formation of sugar-degrading products, namely inhibitors such as furfural and 5-hydroxymethylfurfural (HMF) (NEUREITER *et al*., 2002), as well as phenolic aldehydes and weak organic acids (BALAN, 2014). The degradation products not only reduce the yield of sugar monomers but also act as inhibitors of fermentation.

At this moment in which the vegetal biomass is highlighted as an alternative source of energy, sorghum biomass (*Sorghum bicolor* (L.) Moench) appears as a promising plant for the production of cellulosic ethanol. Sorghum is a C4 plant belonging to the Poaceae family, and sorghum emerges as an alternative for the biomass supply chains for bioenergy and biorefining (ZEGADA-LIZARAZU & MONTI, 2012). This type of sorghum has a large height, can reach 6 meters (EMBRAPA, 2014), is a fully mechanizable crop (from planting to harvest), established by seeds, and has well-known agricultural production systems (PARRELLA, 2010). Sorghum genotypes have a large variation in relation to the cell wall content, mainly in relation to lignin (GURAGAINA *et al*., 2014). Alleles called brown midrib (*bmr*), which confer the phenotype of brown midrib to the plant, are responsible for the low lignin content (PEDERSEN *et al*., 2005; SABALLOS *et al*., 2008; SATTLER *et al*., 2014). In sorghum, *bmr* mutant plants were obtained through chemical induction with diethyl sulfate (PORTER *et al*., 1978). Several studies have demonstrated that genes responsible for the *bmr* mutation act by reducing the activity of enzymes involved in the lignin biosynthesis process (Halpin *et al*., 1998; Sattler *et al*., 2009). To determine the potential use of lignocellulosic material in the production of cellulosic ethanol, it is necessary to obtain detailed knowledge about the structure and composition of the plant cell wall that make up the species of interest. The feasibility of using any lignocellulosic raw material as a biological resource depends on the saccharification potential of the structural carbohydrates. In turn, this depends on the accessibility and susceptibility of the cell wall polysaccharides to the hydrolytic enzymes.

The objective of the present study was to evaluate the cellulosic ethanol production from different contrasting genotypes of sorghum biomass, conventional and *bmr* genotypes after pretreatments, enzymatic hydrolysis and fermentation.

MATERIAL AND METHODS

Plant Material: Five high biomass sorghum hybrids were evaluated, all sensitive to the photoperiod and developed by the genetic breeding program of EMBRAPA. Five experimental hybrids (201556B001, 201556B002, 201556B003, CMSXS7027 and CMSXS7016), and a commercial hybrid (BRS 716) were used, the first two experimental hybrid being *brown midrib*.

Experimental Environments: High biomass sorghum planting was conducted in two distinct regions of the State of Minas Gerais, Brazil, in the agricultural years 2015/2016 and 2016/2017. One of the regions was the city of SeteLagoas, at the experimental area of Embrapa Maize and Sorghum (-19 ° 28 'S and 44 ° 15' W), at 732m altitude. Alto Jequitinhonha Valley was the other region, with trials conducted at the Rio Manso Experimental Farm, of the Federal University of the Jequitinhonha and Mucuri Valleys (18°04'S and 43°28'W), located in Couto de Magalhães de Minas city, at 733 m altitude. The climate of the two regions, according to the classification of Köppen, is Aw type, with dry season from May to October and humid from November to April. Soil preparation of the experimental area at each site was done by plowing one to two days before sowing. Weed control was performed using Atrazine. The other cultural treatments related to the control of pests and diseases were carried out following the recommendations for the culture in the region. In both environments, a 5x3 randomized block design (RBD) was used, consisting of five plots with two rows of five meters each, spaced 0.7 m apart. Three replicates were used for each plot. The material was manually sown along the line, and at 21 days after emergence, thinning was performed for 40 plants per row, providing a stand with approximately 140,000 plants per hectare (8 plants per linear meter). Planting was applied at a dose of 400 kg ha^{-1} of NPK (08-28-16) and cover fertilization with 80 kg ha^{-1} of N, when the plants were with 4-6 leaves final.

Lignocellulosic composition: The contents of acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin detergent acid (LDA) were determined according to methodologies proposed by Robertson & Van Soest (1981). The cellulose content was calculated from the difference between the content of FDA and LDA; and the hemicellulose content was calculated from the difference between the FDN and the FDA content.

Pretreatments: The conditions used in the acid pretreatment steps for 75.0 g of sample were 20% (w/v) ratio, 4% sulfuric acid concentration and autoclave time of 30 minutes, (121 ºC). For alkaline pretreatment, 7.0% NaOH solution and temperature at 85 °C were used under a reaction time of 12 hours for each 28.0 g of sample previously treated with acid. Enzymatic hydrolysis (Enzymatic hydrolysis) 150 µL commercial cellulase (Celluclaste-Novozymes ® -Bagsværd, Denmark) per gram of plant biomass, 10% solid-liquid ratio, 50 °C, 50 $mmolL^{-1}$ pH 5.0 bicarbonate buffer under stirring (150 rpm) for 72 hours.

Table 1. Mean values of cellulose, hemicellulose, lignin (%) of the raw sorghum biomass, after acid pretreatment, and after acid followed by alkali pretreatment, cultivated in Sete Lagoas and Couto de Magalhães de Minas in the 2015/2016 and 2016 / 2017

	Raw			After acid pretreatment			After ac+alk pretreatment		
Hybrids	Cel	Hemicel	Lig	Cel	Hemicel	Lig	Cel	Hemicel	Lig
201556(B)002	34.98 [a]	26.80 [a]	4.63 [b]	69.97 [a]	3.06 [a]	16.72 [c]	97.02 [a]	2.97 [a]	0.2 [b]
201556(B)003	34.65 [a]	27.86 [a]	4.80 [b]	67.41 [b]	2.68 [a]	17.97 [c]	94.99 [a]	3.52 [a]	1.15 [b]
CMSXS7927	36.82 [a]	27.50 [a]	7.15 [a]	66.35 [c]	4.12 [a]	21.71 [b]	91.14 [b]	2.91 [a]	5.33 [a]
CMSXS7016	35.19 [a]	26.32 [a]	6.47 [a]	65.37 [c]	3.90 [a]	22.02 [b]	91.45 [b]	2.68 [a]	4.55 [a]
BRS716	35.05 [a]	25.26 [a]	6.81 [a]	63.55 [c]	3.00 [a]	24.20 [a]	89.13 [b]	4.23 [a]	6.46 [a]
Sete Lagoas	38.46 [a]	27.08 [a]	7.10 [a]	65.41 [b]	3.61 [a]	19.92 [a]	93.71 [a]	3.66 [a]	2.82 [a]
Couto de Magalhães de Minas	35.76 [a]	27.01 [a]	5.46 [b]	67.91 [a]	2.72 [b]	19.99 [a]	93.06 [a]	2.82 [a]	3.07 [a]

Means followed by the same lowercase letter in the columns do not differ by the Scott Knott test, at a 5% probability level for each location. Cel = cellulose; Hemicel = hemicelulose; Lig = lignin; after acid pretreatment (H_2SO_4 4%, 121 ºC, 30 min); after ac+alktrat= acid followed alkaline pretreatment (NaOH 7.0%, 85 °C, 12 h)

Fermentation: The microorganism used for the fermentation was *Saccharomyces cerevisiae*, yeast commercially available at 2% concentration. The fermentation was carried out in bottles with screw cap with capacity of 50 mL at 30 °C in static condition up to 24 h. Two distinct experiments of fermentation were carried out, the first was used the biomass pretreated with acid followed by alkaline solution, and the second was used the biomass treated with acid alone as described in the pretreatments item.

Infrared Spectroscopy – FTIR: Mid Fourier Transform Infrared (FTIR) spectra are often used to investigate the structure of constitutions and chemical changes in lignocellulosic biomass. The FTIR spectra were obtained using a spectrophotometer (Perkin Elmer) equipped with a diffuse reflectance accessory. All spectra were recorded in transmittance mode in the range of 550-4000 cm^{-1} wave numbers with a detection resolution of 4 cm^{-1} and 32 scans per sample. Unscrambler X® (version 10.3, CAMO Software Inc., Woodbridge NJ USA) software was used to determine peak positions and intensities.

Scanning electron microscopy – SEM analysis: The morphology of the sample particles, before and after the acid pretreatment and acid followed by basic, was observed by scanning electron microscopy (TESCAN model VEGA3 LMH, Czech Republic) and was operated at 30 kV with 5000x * average magnification (* in the images there is the magnification and voltage for each photo). Samples were attached to a double-sided tape on a stub (sample port) with a diameter of 1 cm and a height of 1 cm. This system described above was gold plated using a metallizer (model Q150RS QUORUM Quorum Technologies Ltda., UK).

X-Ray Diffraction: The determination of the crystallinity of the cellulose was carried out in the equipment XDR-7000 X-ray diffractometer from SHIMADZU (Tokyo, Japan). The configuration adopted for the analysis was the slotted monochromator (divergence: 0.5; convergence: S1 and 0.3), operated at 40 kV with a current of 20 mA. The velocity adopted was $1^{o}min^{-1}$, using a Cu-Kα radiation with a wavelength of 0.15418 nm (1.541838Âμ). The crystallinity index (CI) of the fibers was calculated according to Equation 1:

$$IC = ((I200 - I_{am}) / I200) \times 100 \quad \text{Equation 1}$$

Where: I200 = intensity corresponding to the peak of the crystalline material; Iam = intensity of the band referring to the amorphous material. The CI of Cellulose Avicel PH-101(Sigma) was measured for comparison with sorghum biomass.

Thermal Analysis: Thermogravimetry / Derivative Thermogravimetry - (TG / DTG): Thermal analysis was assessed by TG and DTG analysis techniques. The thermogravimetry was performed in an instrument model TGA-Q50 Instruments, in the temperature range of 20 °C to 800 °C, under nitrogen atmosphere, under a flow of 100 $mLmin^{-1}$ and a heating rate of 10 $°Cmin^{-1}$. The samples used for the thermogravimetric analysis were 4 to 6 mg of biomass in the form of powder. The mass losses associated with certain temperature ranges are considered as indicative of the amount of different chemical components present in the biomass. The TG and DTG of Cellulose Avicel PH-101(Sigma-Aldrich (Darmstadt, Germany) was measured for comparison with sorghum biomass.

Statistical analysis: We performed the analysis of variance for each environment, to verify the existence of equal residual variances by the relation between the largest and the smallest mean square of the residue of the environments in all the characteristics (GOMES &GARCIA, 2002). Then, the combined analysis was made based on the following statistical model: Yijk = m + (B / L) / Ajkm + Gi + Aj + Lk + GAij + GLik + ALjk + GALijk + Eijk, where Yijk = observation of genotype i in year y at site k; m = general average; Gi = effect of the i-th genotype; Aj = effect of j-th year; Lk = effect of local k-th; GAij = effect of the i-th genotype interaction in the jth year; GLik = effect of the i-th genotype interaction at the kth site; ALjk = effect of j-th year interaction at the kth site; GALijk = effect of the i-th genotype in the jth year within the kth site; Eijkm = mean experimental random error associated with the observation Yijlm. All experiments were carried out in triplicate, and analyzes of variance and grouping of means by the Schott-Knott test using the 5% level of significance ($p<0.05$) were done by the statistical software GENES (CRUZ, 2013).

RESULTS AND DISCUSSION

Lignocellulosic composition: The lignocellulosic composition of raw sorghum biomass and after the pretreatments by dilute acid and alkali are presented in Table 1. Efficient and economical cellulose production is achieved by extracting the maximum amount of cellulose fibers from the biomass through delignification. Therefore the pretreatments are fundamental for the removal of hemicellulose and lignin, decrease of the crystallinity of the biomass and increase of the surface area of the materials, thus facilitating the subsequent enzymatic hydrolysis. Most of the hemicellulosic fraction was removed with acid, as shown by the percent reduction of about 25.26 to 27.86% to 2.68 to 4.23%, in Table 1.

The relative percentage of lignin in the samples increased slightly with acid pretreatment due to the removal of other components (mainly hemicelluloses), and then progressively decreased with pretreatment using NaOH. It was verified that the percentage of cellulose increased continuously after each pretreatment with acid or alkaline pretreatments obtaining significant difference, compared to raw biomass. The amount of cellulose ranged from an initial content of 35% to about 90%, under pretreatment using 7% NaOH. It was also observed that acid pretreatment followed by alkaline was efficient in the removal of lignin, especially for *brown midribs* hybrids 2015(B)002 and 2015(B)003. In the vast majority of pretreatment processes there is no total or efficient removal of lignin. (TALEBNIA *et al*., 2010), so in some cases another delignification process is required. Therefore, it was verified that the *brown midrib* hybrids (*bmr*) presented a significant difference in the lignin removal, resulting in values close to zero.

Ethanol yields by genotype (Saccharification and Fermentation): The results (Table 2) presented that the acid followed by alkaline pretreatment showed the best yield after enzymatic hydrolysis than using only acid as a pretreatment, which demonstrated the desired structural modification by Scanning Electron Microscopy (SEM) and Infrared Analysis by Fourier (FTIR).

enzymatic hydrolysis. This result shows the advantage of the hybrids*bmr*. It is also worth noting that when tested for the property g Ethanol per g Cellulose, the hybrid *bmr* once again demonstrates an advantage over the conventional ones, since it is shows that small amounts of biomass of this hybrid are needed to obtain the same amount of ethanol. This result shows that changes in the structure and chemical composition of mutant sorghum are more favored than in conventional hybrids, which naturally present higher lignin contents. It was verified that in the city of SeteLagoas the hybrids 201556 (B)002 and BRS 716 presented values of ethanol per hectare significantly higher than the others, obtaining values of 11,567.86 Lha^{-1} and 10,587.97 Lha^{-1}, respectively. In the city of Couto de Magalhães de Minas the hybrid BRS 716 presented the best ethanol productivity, reaching 11,838.46 Lha^{-1}. It is worth noting that when the g ethanol per g cellulose is evaluated, the *bmr*hybrid once again shows an advantage over the conventional ones, since it is perceived that smaller amounts of biomass of this hybrid are needed to obtain the same quantity of ethanol. The ethanol values for the sorghum hybrids evaluated here (Table 2) were higher than those already registered for sugarcane, the main raw material used to produce first generation ethanol, with yield values of 4,508 Lha^{-1} to 7,167 Lha^{-1} (MENDES *et al*., 2017). This potential advantage of sorghum is maintained even if the amount of ethanol that can be obtained from sugarcane bagasse is

Table 2. Average values of hydrolysis yield and ethanol (L ha^{-1}) of sorghum biomass after acid and acid followed by alkalipretreatment

Hybrids	(g EtOH g Cel^{-1})		Hydrolysisyield (%) SSFác.		Hydrolysis ac+alk (%) yield SSF		L EtOH ha^{-1}(SSF ac)		L EtOH ha^{-1} (SSF ac+alk)	
	Sete Lagoas	Couto M. Minas	Sete Lagoas	Couto M. Minas	Sete Lagoas	Couto M. Minas	Sete Lagoas	Couto M. Minas	Sete Lagoas	Couto M. Minas
201556(B)002	0.42 ^{Aa}	0.33 ^{Ba}	33.16 ^{Ba}	39.52 ^{Aa}	74.66 ^{Aa}	72.15 ^{Aa}	3821.21 ^{Aa}	4220.28 ^{Aa}	11567.86 ^{Aa}	9232.21 ^{Bb}
201556(B)003	0.26 ^{Ab}	0.31 ^{Aa}	25.41 ^{Bb}	39.86 ^{Aa}	77.51 ^{Aa}	76.43 ^{Aa}	3715.52 ^{Aa}	4196.27 ^{Aa}	6612.57^{Ab}	8080.50 ^{Ab}
CMSXS7027	0.23 ^{Bc}	0.29 ^{Aa}	15.77 ^{Bc}	24.89 ^{Ab}	62.68 ^{Ab}	67.19 ^{Aa}	2806.64 ^{Bb}	3577.77 ^{Ab}	6671.33 ^{Bb}	8459.30 ^{Ab}
CMSXS7016	0.20 ^{Bc}	0.26 ^{Aa}	18.23 ^{Bc}	26.89 ^{Ab}	62.60 ^{Ab}	63.11 ^{Aa}	3788.22 ^{Ba}	4520.18 ^{Aa}	7256.25 ^{Bb}	9316.34 ^{Ab}
BRS716	0.28 ^{Ab}	0.31 ^{Aa}	13.12 ^{Bc}	20.52 ^{Ac}	63.39 ^{Ab}	71.78 ^{Aa}	3976.62 ^{Aa}	4401.47 ^{Aa}	10587.97 ^{Aa}	11838.46 ^{Aa}

EtOH = Ethanol; Cel = cellulose; Lha^{-1} = liters per hectare; SSF = saccharification and separate fermentation; Averages followed by the same lowercase letter in the columns and uppercase in the lines do not differ from each other by the Scott Knott test, at a 5% probability level for each location.

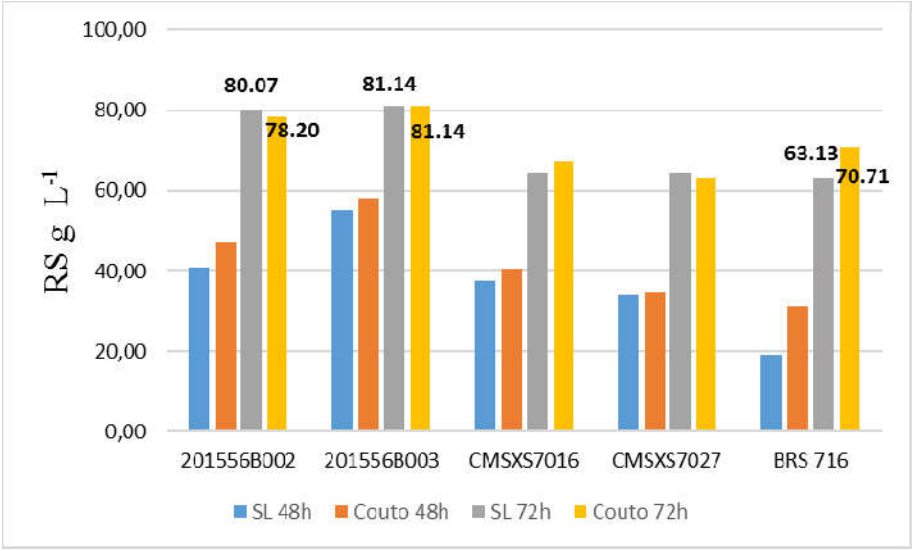

Figure 1. Reducing sugar released after saccharification and fermentation process

It is noted that the *bmr* hybrids (201556B002 and 201556B003) resulted in higher hydrolysis yields in the two saccharification conditions. The efficiency of pretreated samples to produce fermentable sugars, Figure 1, was evaluated by measuring the total amount of reducing sugar released from the samples after 48 hours and 72 hours of

considered using the 2nd generation technology, approximately 3,000 Lha^{-1} (BENJAMIN *et al*., 2014). The ethanol production obtained in this study also stands up an advantage when compared to maize, the main raw material used in the United States of America. Duvernay *et al*. (2013), reported productivity between 2,800 and 3,800 Lha^{-1} of corn ethanol,

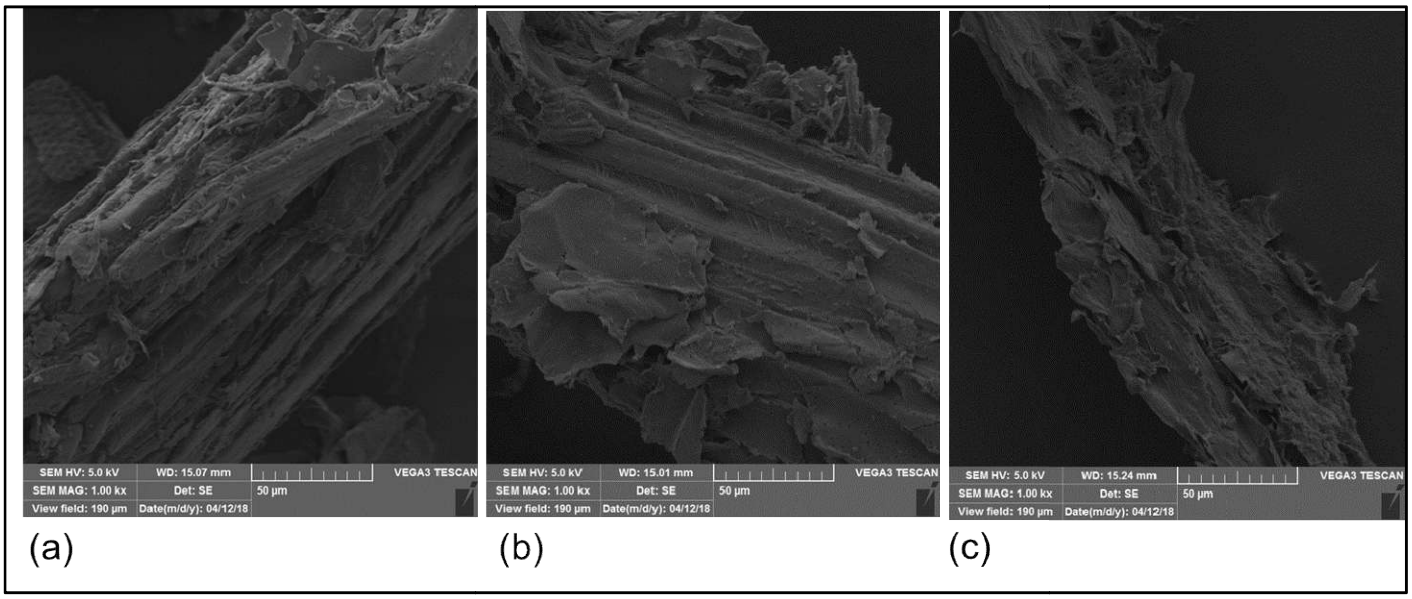

Figure 2. SEM 1000x hybrid *bmr*201556B002 (a) *raw*; (b) after acid pretreatment (H_2SO_4 4%, 121 ºC, 30 min); (c) after acid followed by alkali pretreatment (H_2SO_4 4%, 121 ºC, 30 min and NaOH 7.0%, 85 °C, 12 h)

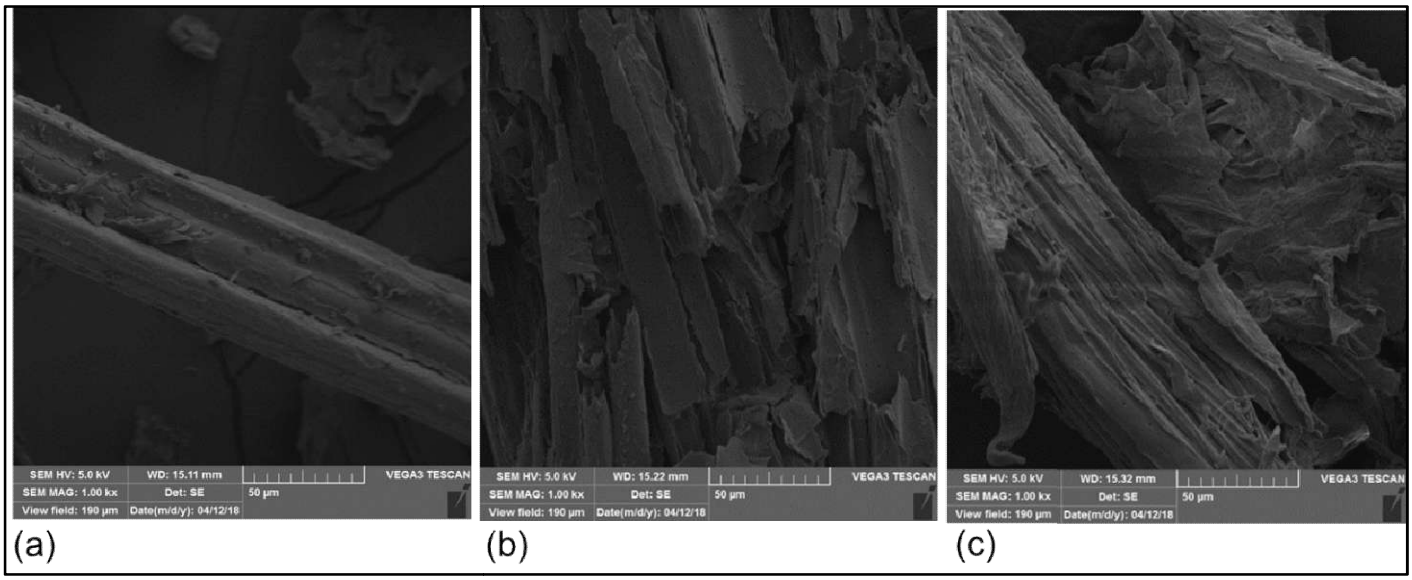

Figure 3. SEM 1000x conventional hybrid BRS 716 (a) *raw*; (b) after acid pretreatment (H_2SO_4 4%, 121 ºC, 30 min); (c) after acid followed by alkali pretreatment (H_2SO_4 4%, 121 ºC, 30 min and NaOH 7.0%, 85 °C, 12 h)

that is, values lower than the biomass sorghum hybrid with the lowest productivity in this study (20155(B)003 = 6,612.57 Lha^{-1}), after the separate fermentation of the pretreated material with acid and alkaline solution. The ethanol yields obtained from biomass sorghum show high and satisfactory results for the potential of this crop for the supply of biofuels, besides making possible the supply of raw material in a maximum period of six months, being therefore much more efficient than sugarcane, which has a cycle of twelve to eighteen months.

SEM analysis

Scanning Electron Microscopy (SEM) was performed to evaluate morphological structural modifications of sorghum biomass. SEM images with magnification of 1000 × explained the changes that occurred during pretreatments (Figures 2 and 3). In the case of raw, untreated biomass (Figures 2 (a) and 3 (a)), the structure observed was rigid, highly conserved, compact and non-porous.

It was verified that the pretreated samples were disrupted, which may be due to the breakage of the cellulose-hemicelluloses-lignin network, leading to the removal of hemicellulosic fractions (Figures 2 (b) and 3 (b)) and lignin (Figures 2 (c) and 3 (c)). During acid pretreatment, intermolecular hydrogen bonds within the cellulose may be partially interrupted and thus result in porous structure with high surface area (LINDMAN *et al*., 2017). This is beneficial for the enzymes to penetrate, adsorb and easily hydrolyze the biomass during the hydrolysis. Identical observations have been reported previously for sugarcane tops pretreated with acid (SINDHU *et al*., 2011).

FTIR Analysis: Fourier Transform Mid-Infrared (MIR-FTIR) spectroscopy is used to investigate constituent structure and chemical changes in lignocellulosic biomass have advantages over conventional chemical methods that consume time, reagents and may result in the degradation of natural polymers (XU *et al*., 2013). The FTIR spectra for the raw sorghum and pretreated were different, explaining the structural changes in

the cellulose that occurred during the pretreatments. Different authors reported transmittance profiles of lignocellulosic materials, serving as a basis for the analysis of the attributions to the bands observed in the FTIR analysis of the genotypes of sorghum biomass. The FTIR spectra of biomass sorghum show several absorption bands that can be attributed to the main structural components: cellulose, hemicellulose and lignin. The 4000-1800 cm^{-1} region of the absorbance spectra shows few bands, which are attributed to the O-H group (around 3340 cm^{-1}) and the C-H group (2927 cm^{-1}). The region between 1800 and 900 cm^{-1} presents more complex bands, due to several modes of vibration of the carbohydrates and lignin (GILBERT *et al*., 1993; PANDEY, 1999).

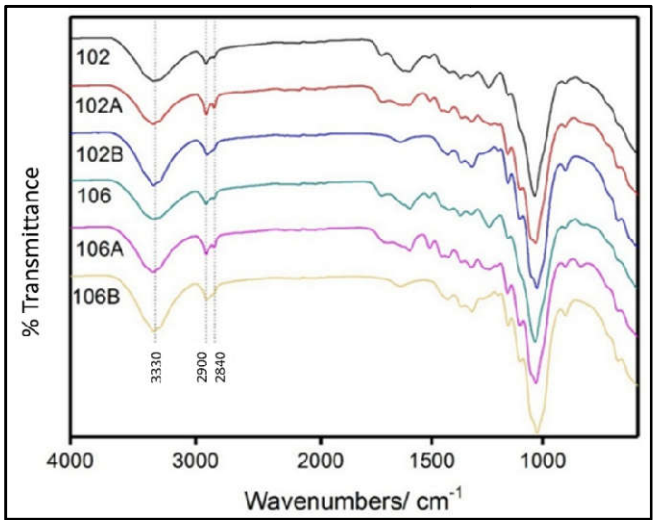

Figure 4. FTIR spectra of raw, acid pretreatment (H_2SO_4 4%, 121 ºC, 30 min), (A) and acid followed by alkaline pretreatment (H_2SO_4 4%, 121 ºC, 30 min and NaOH 7.0%, 85 °C, 12 h), (B) sorghum biomass. 102 = Hybrid 201556B002; 106 = Hybrid BRS 716

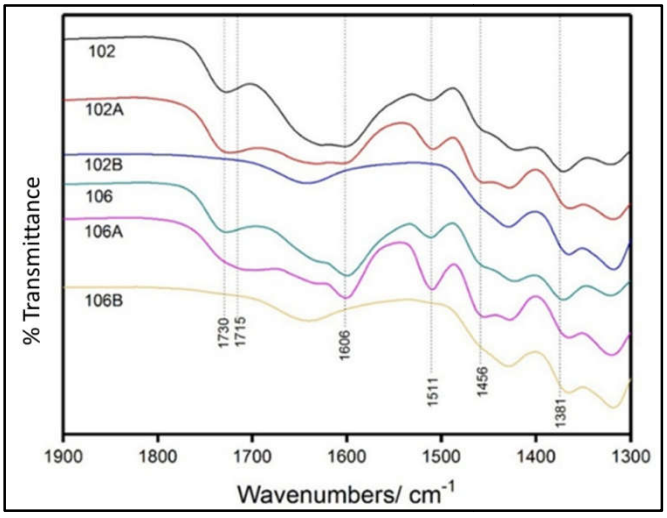

Figure 5. FTIR spectra of raw, acid pretreatment (H_2SO_4 4%, 121 ºC, 30 min), (A) and acid followed by alkaline pretreatment (H_2SO_4 4%, 121 ºC, 30 min and NaOH 7.0%, 85 °C, 12 h), (B) sorghum biomass. 102 = Hybrid 201556B002; 106 = Hybrid BRS 716

Although all sorghum biomass spectra show similar characteristics, small changes were observed between the spectra. For example, there were no peaks at 1714-1642 cm^{-1} for raw biomass. The hemicellulose band appeared at 1730 cm^{-1} (Figure 5) for all raw samples, and gently for the acid treatment of the *bmr* hybrid, indicating the removal of hemicellulose (GUO *et al*., 2008; KUMAR *et al*., 2009, PANDEY, 1999, SUN &TOMKINSON, 2004). According to Liu *et al*. (2005), this peak has been related to saturated alkyl esters from hemicellulose.

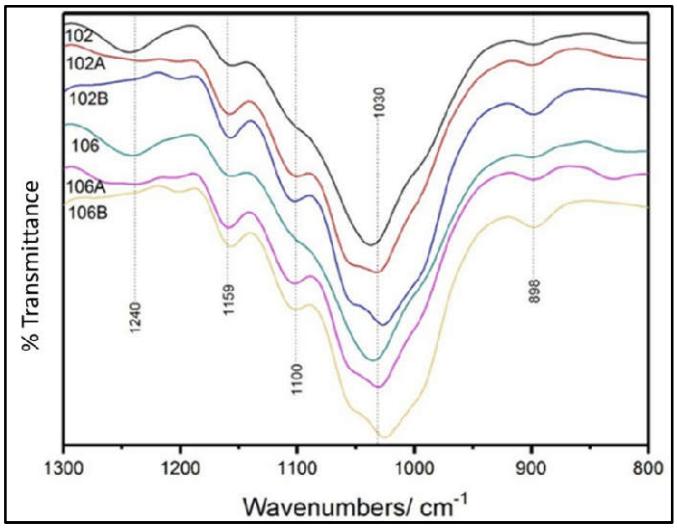

Figure 6. FTIR spectra of raw, acid pretreatment (H_2SO_4 4%, 121 ºC, 30 min), (A) and acid followed by alkaline pretreatment (H_2SO_4 4%, 121 ºC, 30 min and NaOH 7.0%, 85 °C, 12 h), (B) sorghum biomass. 102 = Hybrid 201556B002; 106 = Hybrid BRS 716

It was also observed that in the hemicellulose band was detected after pretreatments (Figure 5), indicating that the hemicellulose was greatly hydrolyzed. The analysis of the chemical composition of the sorghum biomass (Table 1) shows with FTIR observations that the hemicellulose content of the biomass decreases significantly after the pretreatments. Lignin-related bands in the FTIR spectra may be observed around 1268, 1329, 1610 and 1715 cm^{-1} (KUMAR *et al*., 2009; PANDEY, 1999; SUN *et al*., 1998). The band at 1511 cm^{-1}, attributed to the C = C bond of lignin was observed for all untreated biomasses (Figure 5), and was of great intensity for the biomass after acid treatment. After acid treatment the percentage of lignin increases due to the removal of hemicellulose. This band was not observed after the alkaline pretreatment, indicating the efficient removal of this polymer as can be seen in Table 1. Detection of an absorption band at 1715 cm^{-1} (Figures 4 and5), due to the stretching of C=O of the side chains of the phenyl ester group of the lignin structure, in pretreated solid residues showed that some hemicelluloses were not cleaved after pretreatment with dilute acid. This result confirms the previous result for analyzing the chemical composition of biomass samples, in which the diluted acid pretreatment can remove most of the hemicellulose from the biomass, while most of the cellulose and lignin remains in the solid residues. Biomass sorghum has two main rings components of lignin: guaiacyl and syringyl rings. The band at 1508-1511 cm^{-1} is associated with the guaiacyl ring in lignin (CORREDOR *et al*., 2009; Pandey, 1999; SUN *et al*., 1998). This band was observed (Figure5) in all untreated biomass samples, and after basic pretreatment was not observed. The range around 1456 cm^{-1} is due to the absorption of syringyl rings in lignin (CORREDOR *et al*., 2009; GASTALDI *et al*., 1998; PANDEY, 1999). This band was observed in all untreated samples (Figure 5). Cellulose-related bands in the FTIR spectra were observed at around 898, 1320 (Figure 6), 1423, 2920 and 3340 cm^{-1} (Figure 5) (GASTALDI *et al*.,

1998; GILBERT *et al*., 1993; Kumar *et al*., 2009, PANDEY, 1999, SUN *et al*., 1998).

X-Ray Diffraction: The different pretreatments applied in sorghum biomass provided a variation in the crystallinity index (CI) in relation to commercial cellulose, which presented a CI of 79.9%. The crystallinity and amorphous peaks intensities were obtained through the graphs of Figures 7, 8 and 9. The crystallinity index corresponds to the ratio of crystalline (I200) to amorphous (I_{am}) (LENGOWSKI *et al*., 2013). Table 3 shows an increase in the CI in the treated samples, according with that found by MIRANDA *et al*., (2015), which detected increasing CI proportional to the concentration of alkaline solution used to treatment of piaçava fibers. The increase in crystallinity index was also observed by PHITSUWAN, SAKKA & RATANAKHANOKCHAI (2016) when applying, among other alkalines pretreatments. In the alkaline pretreatment of elephantgrass (*Pennisetum purpureum*), in which there was an increase of 46.0% of the raw material to 49.8% (pretreated with 2% NaOH). The pretreatments with H_2SO_4 and NaOH used in sorghum biomass demonstrated efficiency in the removal of the amorphous phase (hemicellulose and lignin), thus increasing the crystalline domains.

Table 3. Crystallinity index of sorghum biomass after different pretreatments

Sample	IC
Cellulose	79.9
201556B002	67.2
201556B002A	67.2
201556B002B	78.5
201556B003	46.7
201556B003A	64.6
201556B003B	76.5
CMSXS7027	49.6
CMSXS7027A	64.5
CMSXS7027B	74.8
CMSXS7016	48.7
CMSXS7016A	64.6
CMSXS7016B	76.7
BRS 716	43.9
BRS 716A	64.7
BRS 716B	77.0

A = sample after acid pretreatment (H_2SO_4 4%, 121 ºC, 30 min); B = sample after acid followed by alkaline pretreatment (H_2SO_4 4%, 121 ºC, 30 min and NaOH 7.0%, 85 °C, 12 h)

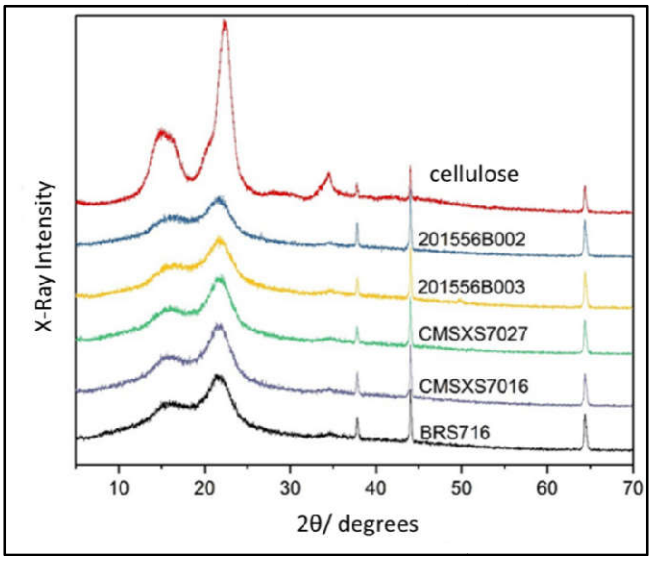

Figure 7. Diffractogram of the raw sorghum biomass

An increase in the significant CI is observed after performing the acid followed by alkaline pretreatment (Figure 9). This can be justified by the change in crystallinity, associated first to the removal of hemicellulose by NaOH and subsequently by amorphous lignin by H_2SO_4, and consequently by the increase in the crystalline cellulose content, and not necessarily to the structural change of the cellulose (KIM; LEE; KIM, 2015).

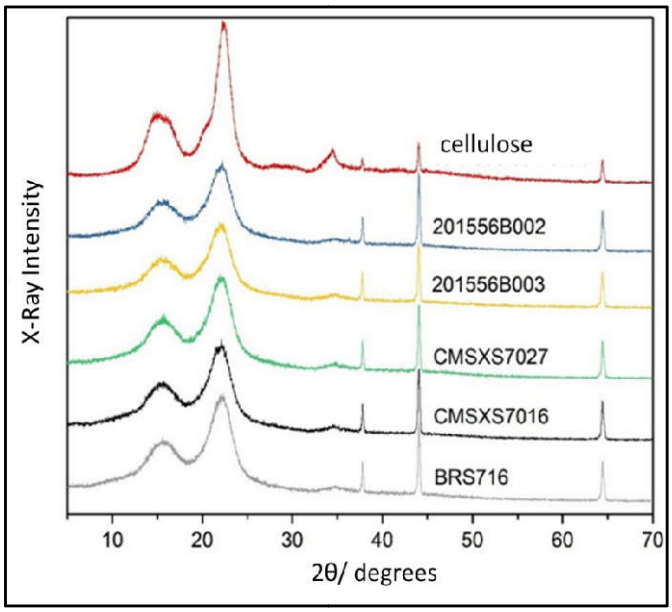

Figure 8. Sorghum biomass diffraction after acid pretreatment (H_2SO_4 4%, 121 ºC, 30min)

There are several reports in the literature that the reduction of cellulose crystallinity, or low crystallinity, resulted in a higher rate of bioconversion for lignocellulosic biomasses (GOSHADROU *et al*., 2011; OSTOVAREH *et al*., 2015). However, this does not always happen, because, several studies have shown that a higher digestibility is obtained with samples presenting a higher crystallinity. In cases where higher crystallinity resulted in higher digestibility, other factors, for example, accessible surface area, porosity, lignin content, hemicellulose and particle size, were the most influential. As an example, in the pretreatment of corn straw, the amorphous parts are removed, resulting in a highly crystalline residue, which is capable of enzymatic hydrolysis, since it has a highly porous structure (KIM and HOLTZAPPLE, 2006). Therefore, crystallinity is an important feature of lignocellulosic biomass for digestibility, but not the only effective factor in all cases (KARIMI *et al*., 2013; SHAFIEI *et al*., 2015).

Thermal Analysis: Thermogravimetry / Derivative Thermogravimetry - (TG / DTG)

The thermal behavior of lignocellulosic biomasses using thermogravimetry (TG) and derived thermogravimetry (DTG) are important because they report the presence of chemical constituents, such as hemicellulose, cellulose and lignin (PARTHASARATHY *et al*., 2013). Figures 10, 11 and 12show the TG and DTG curves for raw sorghum biomass and after pretreatments. It is observed that the curves present a typical behavior of thermal degradation for lignocellulosic biomasses, with three well defined stages: release of humidity in the range of 50 to 100 ºC, decomposition of carbohydrates (hemicellulose and cellulose) in the range of 250 to 400 ºC, (Figure 10). Figure 12shows the presence of lignin at the end

of the process and the formation of coal and ash (RAMBO *et al*., 2015). The degradation of the hemicellulose is only evident in Figure 10, since the acid treatment takes place the removal of this polysaccharide, which can be confirmed by the absence of degradation in the bands before 290 ºC in Figures 11 and 12, besides confirming the results already discussed from the composition and XRD analysis.

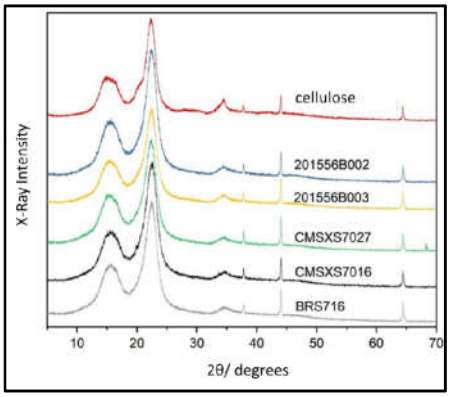

Figure 9. Diffractogram of sorghum biomass after acid followed by alkaline pretreatment (H_2SO_4 4%, 121 ºC, 30 min and NaOH 7.0%, 85 °C, 12 h)

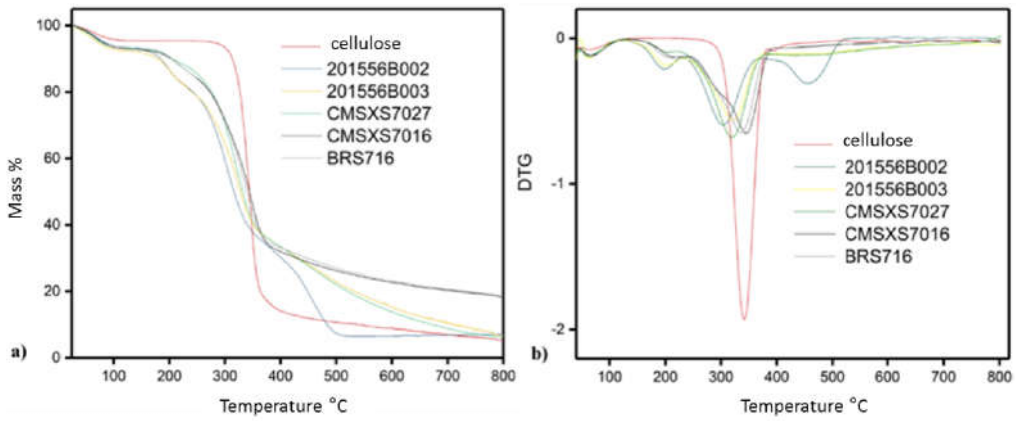

Figure 10. TG (a) and DTG (b) curves of the raw sorghum biomass

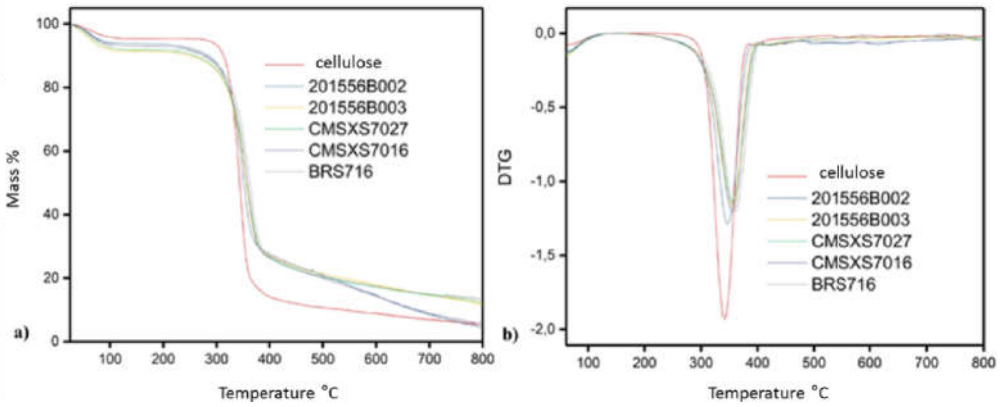

Figure 11. TG (a) and DTG (b) curves of sorghum biomass after acid pretreatment (H_2SO_4 4%, 121 ºC, 30 min)

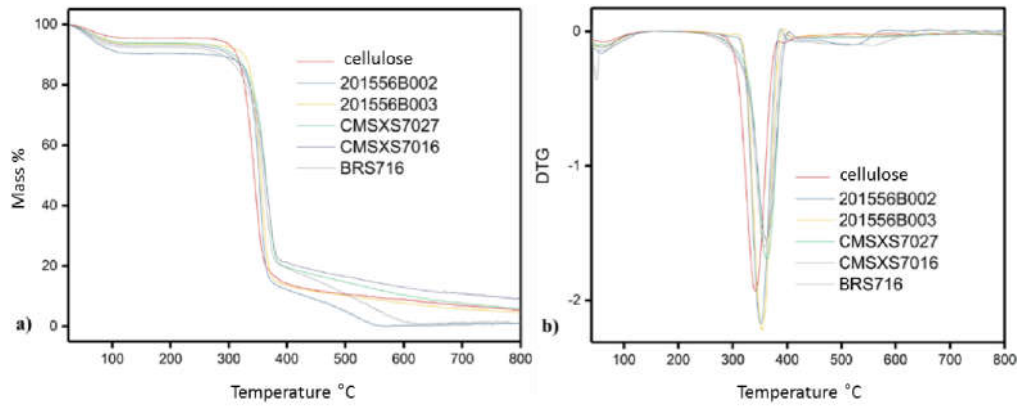

Figure 12. TG (a) and DTG (b) graphs of sorghum biomass after acid followed by alkaline pretreatment (H_2SO_4 4%, 121 ºC, 30 min and NaOH 7.0%, 85 °C, 12 h)

According to the literature, the lignin degradation event starts at approximately 200 °C and ends at 600 °C. (MARTIN *et al*., 2010; BREBU &VASILE, 2010). It is observed in Figure 11, therefore, that the DTG curve is broader in the base, indicating the decomposition of the lignin, a fact that does not occur in Figure 12. The Figure 12represents the acid followed by alkaline treatment of sorghum biomasses. Therefore, only cellulose predominated in the analyzed material, as demonstrated by the TG and DTG curves profile.

Conclusions

The microstructure and lignocellulosic composition of sorghum biomass influenced the bioconversion rate and final yield of ethanol. The best hydrolysis yields were obtained from the pretreated materials, although they presented higher crystallinity index (IC) than *the raw biomass material*. The accessibility of cellulose should be affected by crystallinity, but it is also likely to be affected by several other parameters, such as lignin and hemicellulose content and composition, porosity and particle size. Biomass sorghum *bmr* 201556B002 presented better hydrolysis yield for the two types of saccharification used when compared to conventional genotypes. The yield of ethanol using this mutant hybrid reached 11,838.46 L ha^{-1}, demonstrating that lower amounts of biomass of this material are required to obtain the same amount of ethanol when using other hybrids.

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