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Effect of acid concentration and pulp properties on hydrolysis reactions of mercerized sisal

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ABSTRACT

The influence of sulfuric acid concentration (H_2SO_4 5–25%, 100 °C), crystallinity and fibers size on the hydrolysis reaction of sisal pulps were investigated, with the goal of evaluating both the liquor composition, as an important step in the production of bioethanol, and the residual non-hydrolyzed pulp, to determine its potential application as materials. Aliquots were withdrawn from the reaction media, and the liquor composition was analyzed by HPLC. The residual non-hydrolyzed pulps were characterized by SEM, their average molar mass and crystallinity index, and their size distribution was determined using a fiber analyzer. Sulfuric acid 25% led to the highest glucose content (approximately 10 g L^{-1}), and this acid concentration was chosen to evaluate the influence of both the fiber size and crystallinity of the starting pulp on hydrolysis. The results showed that fibers with higher length and lower crystallinity favored glucose production in approximately 12%, with respect to the highly crystalline shorter fibers.

1. Introduction

Energy consumption has increased over the last century as the world population has grown and more countries have become industrialized. Crude oil has been the primary resource for meeting this increased energy demand. Unlike fossil fuels, ethanol produced through the fermentation of sugars is a renewable energy source (Jamal, Alam, Salleh, & Nadzir, 2005) and can be used as either a partial or complete gasoline replacement. Biological feedstocks, such as sugarcane bagasse (a residue of the sugar and alcohol industries), containing appreciable amounts of sugars or other materials that can be converted into sugars can be used as raw materials for the production of so-called *second generation* or *new generation* ethanol (Balat, Balat, & Öz, 2008; Binod et al., 2010; Chen, Pen, Yu, & Hwang, 2011; He & Zhang, 2011; Kataria & Ghosh, 2011; Lee, Chen, Chang, & Yang, 2012; Sarkar, Ghosh, Bannerjee, & Aikat, 2012).

Sisal can be highlighted within the search for raw materials containing polysaccharides that can be converted to glucose. Brazil is the largest producer and exporter both of sisal fibers and of the manufactured products made from sisal in the world; however, the amount of value added to sisal is still low (besides fibers, sisal is primarily exported as twines, pads, rugs and carpets, Sindifibras, 2012). Sisal fibers are lignocellulosic and have a high cellulose content (approximately 70%, according to Megiatto, Hoareau, Gardrat, Frollini, & Castellan, 2007), which makes them a potential alternative to sugarcane bagasse and other sources for the production of second generation ethanol as well as both micro and nano scaled materials for high value-added items.

The production of bioethanol from lignocellulosic biomasses is very challenging because of the heterogeneous nature of this feedstock. Pretreatment using physical, chemical and physico-chemical methods (Chen et al., 2011; Eggeman & Elander, 2005; Galbe & Zacchi, 2007; Lacerda, de Paula, Zambon, & Frollini, 2012; Rocha, Gonçalves, Oliveira, Olivares, & Rossell, 2012; Sanchez & Cardona, 2008) are frequently used to disrupt the cellulose–hemicelluloses–lignin complex and make the cellulose and hemicellulose more accessible for conversion into sugars via hydrolysis, which allows the subsequent fermentation steps to occur (Porzio, Prussi, & Chiaramonti, 2011). The performance of physical pretreatments, such as biomass comminution (e.g., milling), is usually poor while the costs are high (Yang & Wyman, 2008). However, because the process consists of a reaction in heterogeneous media, the size and crystallinity of the suspended fibers are factors that deserve attention. Higher surface areas and lower crystallinity increase the accessibility of the solution that contains protons to the polysaccharides chains, in the case of acid hydrolysis.

Among the different chemical pretreatments, acid pretreatment is known to separate pentoses and hexoses, whereas alkali pretreatment (mercerization) is known to separate lignin from lignocellulosic biomass (Binod et al., 2012). The mercerization process, which was used as a pretreatment for the initial pulp because it had been proven efficient in a previous study (Lacerda et al., 2012), alters the fine structure and morphology of the fiber as well as the conformation of the cellulose chains (changing them

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from cellulose I to II). During this process, the material swells, the polysaccharide chains are rearranged, and the crystalline portion is usually diminished. These changes result in higher adsorption of reagents because mercerization increases the specific surface area of the fiber, which makes the chains more easily accessible (Ass, Belgacem, & Frollini, 2006).

In the present study, which is part of a broader one using both sisal lignocellulosic fibers and pulp (Almeida, Frollini, Castellan, & Coma, 2010; de Paula, Lacerda, & Frollini, 2008; Lacerda et al., 2012; Megiatto, Silva, Ramires, & Frollini, 2009; Megiatto, Ramires, & Frollini, 2010; Ramires, Megiatto, Gardrat, Castellan, & Frollini, 2010), the influence of sulfuric acid concentration, crystallinity and fibers size on the hydrolysis reaction of sisal pulps was investigated.

The stages of the acid hydrolysis of sisal pulp were reported, with the goal of evaluating the liquor composition (sugars and their decomposition products) as an important step in the production of bioethanol. The physicochemical and morphological characteristics of the non-hydrolyzed celluloses were also evaluated during this reaction to determine its potential application as materials. It should be noted that variations in the average length and thickness of the non-hydrolyzed cellulose were monitored from the beginning to the end of the reaction using a fiber analyzer. Such analysis is routinely used in the pulp and paper industries, and in the present study it has been used for monitoring the hydrolysis of pulps. The reactions described here were performed at 100 °C under different sulfuric acid concentrations (5–25% (v/v)) with various average fibers sizes and crystallinity for the initial pulp.

Sulfuric acid was chosen for this initial broad exploration to make the results comparable to those obtained from studies currently under consideration, which use oxalic acid (produced from renewable sources) (Lacerda, Zambon, & Frollini, 2011) and enzymes as catalysts.

2. Materials and methods

2.1. Materials

The sisal pulp used for all stages of this study was kindly provided by the company Lwarcel (Lençóis Paulistas, São Paulo, Brazil). In this company the lignocellulosic fiber was delignified through the *kraft* process (*elementary chlorine-free* bleaching sequence) to produce a pulp with an average length and thickness of 2.9 mm and 18.4 μm, respectively, and 86% ISO Brightness according to the supplier.

The Klason lignin was determined according to TAPPI T222 om-02 for the pulp received from the company. No lignin was detected. All pulp samples were milled by passing them through cutting knives using a vertical mill with feed hopper (MARCONI MA-048, 30 mesh stainless steel), and dried in a vacuum oven at 60 °C for 4 h.

The pulp was mercerized in a sodium hydroxide solution (NaOH pellets, Qhemis) (ASTM1695-07). Sulfuric acid (H_2SO_4, Qhemis) was used for the hydrolysis reaction. A 2% cupriethylenediamine (CED) solution (Qeel) was used for the viscometric analysis. Glucose, xylose, arabinose, formic acid, acetic acid, furfural and hydroxymethylfurfural (Sigma–Aldrich) were used as standards for high-performance liquid chromatography (HPLC).

2.2. Methods

2.2.1. Initial and residual non-hydrolyzed pulp characterization

2.2.1.1. Hemicellulose and α-cellulose content. The cellulose sample was added to a 17.5% NaOH solution (Sun, Lawther, & Banks, 1995), and the α-cellulose content was calculated from the final mass. The difference between the total mass and the α-cellulose mass found in the pulp sample corresponded to the hemicellulose content (Browning, 1967).

2.2.1.2. Scanning electron microscopy (SEM). LEO-440 equipment was used for the SEM analysis with a tungsten filament to generate electrons, as previously described (Ramires et al., 2010). The samples were dried in a vacuum oven for 4 h and covered with gold.

2.2.1.3. Average molar mass (MM_{vis}) by viscometry. Viscometry was used to determine the average molar mass of both the starting and non-hydrolyzed cellulose using aliquots withdrawn from the reaction medium at different times during the reaction. Cellulose was solubilized in a copper (II)-ethylenediamine:water solution (1:1 (v/v)), and the flow time in a glass capillary viscometer (Ubbelohde, f = 0.63 mm, AVS-350 Schott-Geräte) was measured.

The average molar mass of the samples was determined as previously described (Morgado, Frollini, Castellan, Rosa, & Coma, 2011; TAPPI T230 om-2008). All measurements were performed in triplicate.

2.2.1.4. Crystallinity index (CI) by X-ray diffraction. The initial and non-hydrolyzed cellulose obtained from five pulp samples withdrawn at t = 10, 30, 120, 240 and 360 min, which had been previously dried in a vacuum oven (60 °C, 4 h), was analyzed by X-ray diffraction to determine the CI using a universal X-ray diffractometer (URD-6 model, CARL ZEISS JENA, 40 kV/20 mA, λ(Cukα) = 1.5406 Å). As described elsewhere (Ass et al., 2006), the CI was calculated according to equation $CI = 1 - (I_1/I_2)$, where I_1 is the minimum intensity, which is proportional to the amorphous fraction of the cellulose ($18^\circ \leq 2\theta \leq 19^\circ$ for cellulose I and $13^\circ \leq 2\theta \leq 15^\circ$ for cellulose II), and I_2 is the maximum intensity, which corresponds to the signal for the crystalline fraction ($22^\circ \leq 2\theta \leq 23^\circ$ for cellulose I and $18^\circ \leq 2\theta \leq 22^\circ$ for cellulose II) (Buschle-Diller & Zeronian, 1992).

2.2.1.5. Average thickness and length of the fibers. The average thickness and length of the fibers were obtained using MorFi Compact (Techpap) equipment, which obtains results by measuring approximately 5000 fibers suspended in water. The MorFi automatic fiber-analysis system also provides the *fiber-occurrence density* (Millions-per-Gram), which enables the construction of a density map, ie a three-dimensional graph having the following characteristics: the values of average length in the x axis, the values of average thickness in the y axis, and the values of the fiber-occurrence density in the z axis.

2.2.2. Mercerization (ASTM1695-07)

The mercerization process was performed using 20 g of pulp suspended in 1 L of a 20 wt% NaOH solution for 3 h at 50 °C with stirring. The pulp was filtered, washed with deionized water until the initial pH of the washing water was reached, and dried first at room temperature and then in a vacuum oven at 60 °C for 4 h.

2.2.3. Pulp fractionation

The previously mercerized pulp was fractionated in a Retsch AS 200 vibratory sieve shaker (Embrapa Cattle-Southeast, São Carlos-SP, Brazil) with 106-μm, 75-μm, 45-μm and 25-μm pores.

2.2.4. Acid hydrolysis

For the hydrolysis reactions, 30 g of dried, milled and mercerized pulp were added to 900 mL solutions of between 5 and 25% (v/v) H_2SO_4 at 100 °C. All reactions were performed over a 6-h period. An H_2SO_4 concentration of 25% was chosen to perform the reactions in the next step with differing fibers size.

During the course of the reaction, aliquots were withdrawn and separated via simple filtration. The liquor was diluted for further analysis of the sugar and related decomposition products by high-performance liquid chromatography (HPLC) using a Shimadzu instrument. Glucose, xylose, arabinose, formic acid and acetic acid were analyzed using a refractive index detector (RID-6A), Aminex HPX 87H column (300 × 7.8 mm BIO-RAD), 0.005 mol L^{-1} H_2SO_4 as eluent, 0.6 mL min^{-1} flow rate and 45 °C temperature. Furfural and hydroxymethylfurfural were analyzed using a 274 nm UV detector (model SPD-10AV), Bondapak TM C18 column (Waters); acetonitrile:water (1:8 (v/v)) containing 1% (v/v) acetic acid as eluent, 0.8 mL min^{-1} flow rate and 25 °C temperature.

The non-hydrolyzed cellulose samples taken from the reaction medium (as previously described) were washed thoroughly, stored in Eppendorf tubes and dried in a vacuum oven at 60 °C for 4 h before analyzing the MM_{vis}, CI, SEM and average fiber thickness and length, as described for the starting pulp (Section 2.2.1).

3. Results and discussion

The codes used throughout the text correspond to the following: MSP is the mercerized sisal pulp (pulp that was not fractionated) and FS1, FS2, FS3 and FS4 are the fractionated samples.

3.1. Initial pulp characterization

For all samples (MSP, FS1–FS4), the α-cellulose and hemicellulose contents were 98 ± 1% and 2 ± 1%, respectively. The pulp used as the raw material in this study has a slightly higher crystallinity index (CI) compared to the pulp used previously; however, the observed variation in crystallinity during the reaction followed the same behavior as described before (Lacerda et al., 2012). The main results of initial pulp characterization are summarized in Table 1.

There were no substantial changes in the MM_{vis} values found for the fractionated pulps (FS1 = 107,000 ± 300, FS2 = 112,000 ± 200, FS3 = 98,000 ± 350, FS4 = 100,000 ± 300 g mol^{-1}) and the unfractionated pulp (MSP = 99,900 ± 450 g mol^{-1}). SEM images of the fractionated pulps (figures not shown) indicated that the sieves efficiently separated fibers of different sizes. The average length and thickness calculated by the fiber analyzer were 238 and 20 μm for MSP, 383 and 21 μm for FS1, 313 and 20 μm for FS2, 249 and 20 μm for FS3 and 130 and 19 μm for FS4, respectively. The average lengths of the fractionated pulps of F1 and F2 (383 and 313 μm, respectively) are longer than that of the unfractionated pulp (238 μm), what indicates that the longer fibers are present in lower content when compared to the shorter ones, since the length of the unfractionated pulp corresponds to an average of all fibers lengths present in the pulp. As observed, the fiber thicknesses are approximately the same, and the fiber lengths decrease from F1 to F4. Fractionation may represent an important factor for the reactions in heterogeneous media, and can be considered a physical pretreatment that may enhance the hydrolysis rate.

The alkaline treatment led to the formation of cellulose II, as indicated by the characteristic peaks in the diffractograms with 2θ between 18° and 22° (figures not shown). The smaller sisal pulps exhibited higher CI (MSP = 69%, FS1 = 65%, FS2 = 72%, FS3 = 73%, FS4 = 75%), which can decrease the hydrolysis rate. Indeed, the CI values were inversely related to the average size of the fibers; the shorter the fiber is, the larger its CI value, i.e., the fibers that passed through successively smaller pores showed higher crystallinity than those that were retained. This finding may be a consequence of the pulping process applied to the lignocellulosic sisal fiber (Section 2.1). The average fiber length, as well as the crystallinity of pulps depend on the pulping process. During pulping, for instance, the pulps may suffer localized attack at stress points, and can be swelled by the liquor. The effects of swelling and peeling in kraft pulps can affect the crystallinity and average molecular weight of these pulps (Fernandez & Young, 1996). Pulp also consists of fines, in addition to fibers. Fines correspond to very short fibers, and can be obtained from fibrils. The crystallinity of these fibrils and their accessibility to swelling depend on both, the biological origin of the cellulosic material and its pre-treatment (Ferreira, Matos, & Figueiredo, 1999; Subramanian, Kononov, Kang, Paltakari, & Paulapuro, 2008). All these factors combined lead to a mixture of fibers and fines having different dimensions and properties, such as crystallinity. The conditions of the kraft pulping process, that generated the pulp used in the present study, may be responsible for the correlation found between fiber length and crystallinity.

3.2. Acid hydrolysis

In the first step of the acid hydrolysis experiments, reactions were performed using the MSP with 5, 10, 15, 20 and 25% (v/v) sulfuric acid solutions at 100 °C, conditions that were chosen based on a previous study (Lacerda et al., 2012). In the second step, the optimal concentration, i.e., the one that led to the best glucose yields, was used for the reaction of varying sisal pulp fibers sizes and crystallinities (25% H_2SO_4 (v/v)), as discussed later.

3.2.1. Reactions performed with varying acid concentrations

3.2.1.1. Characterization of the residual non-hydrolyzed pulp. Scanning electron microscopy (SEM). Fig. 1 shows the starting pulp used for the hydrolysis (a) and the non-hydrolyzed cellulose found after the reacting for different time intervals and acid concentrations (b–f).

It can be observed from the gradual reduction in the fiber sizes (Fig. 1b–e) that hydrolysis of the cellulose chains occurred progressively at all of the sulfuric acid concentrations used. Fig. 1f shows that, for the reaction with 25% sulfuric acid, the fiber bundles agglomerated after isolation, which differs from the residual cellulose from the reactions with less concentrated acid solution. This structure is approximately 500 μm but is composed of smaller units approximately 10 μm in size (Fig. 1f).

The average molar mass (MM_{vis}) via viscometry and crystallinity index (CI) via X-ray diffraction. To investigate the influence of the acid concentration on the residual non-hydrolyzed pulp, the MM_{vis} and CI values of the samples were determined. Fig. 1g and h shows the values of MM_{vis} and CI for the residual non-hydrolyzed pulp from the hydrolysis reactions performed at 100 °C with 5, 10, 15, 20 and 25% (v/v) sulfuric acid. The MM_{vis} and CI values of the starting pulp (MSP) were 99900 g mol^{-1} and 69%, respectively, as previously mentioned.

These results show that, for all acid concentrations used and even after only a short reaction time (10 min), the MM_{vis} values decreased relative to its initial value (99900 g mol^{-1}, Fig. 1g) at least 80%. Still, there is a tendency for this value to remain constant after an initial drop, and the 25% sulfuric acid led to the smallest MM_{vis} value (approximately 7000 g mol^{-1}, Fig. 1g). X-ray diffraction analyses were obtained (diffractograms not shown) from five pulp samples obtained at different reaction times for each of the reactions performed to evaluate the influence of the hydrolysis process on the CI of the non-hydrolyzed cellulose. In all cases, there was a significant initial increase in the CI value. This finding indicates that the non-crystalline pulp fraction, which is more easily accessed, was hydrolyzed in the earlier stages of the hydrolysis, whereas the crystalline fraction, which is more difficult to access, did not react, leading to an increase in the CI (Fig. 1h). It was noticed that as higher is the acid concentration as quickly the non-hydrolyzed cellulose achieves the maximum CI (approximately 87% with 20% H_2SO_4). For the reaction with 25% H_2SO_4, there are indications that the crystalline domain began to hydrolyze

Table 1
Values of the crystallinity index and average length and thickness of the pulps used, and the respective maximum concentration of glucose (Gluc) and xylose (Xyl) obtained at certain hydrolysis times (t).

Sample	CI (%)	Average length (μm)	Average thickness (μm)	$[Gluc]_{max}$ (t) ($g\,L^{-1}$) (min)	$[Xyl]_{max}$ (t) ($g\,L^{-1}$) (min)
FS1	65	383	21	11.2 (360)	1.3 (10)
FS2	72	313	20	10.8 (360)	1.2 (30)
FS3	73	249	20	10.1 (330)	1.0 (20)
FS4	75	130	19	10.2 (360)	0.8 (20)

at approximately 30 min, which caused a decrease in the CI value after the initial increase and indicated that the hydrolysis of chains in the crystalline domain started earlier at this concentration. Still, the CI of the non-hydrolyzed cellulose is larger than that of the initial pulp for all H_2SO_4 concentrations. For concentrations of 5, 10, 15 and 20%, the CI values increase approximately 12% after hydrolysis, and then remain approximately constant.

Average fiber thickness and length (fiber analyzer – MorFi). Fig. 2 shows the density maps obtained from the morphological analysis of both the initial pulp (MSP) and non-hydrolyzed cellulose taken from the 5 and 25% H_2SO_4 reaction media after 10 and 360 min hydrolysis using MorFi compact equipment. Reactions using the other acid concentrations (10, 15, 20%, figures not shown) behaved similarly to the reaction performed using 25% sulfuric acid (Fig. 2c and d). For the reaction performed using 5% sulfuric acid (Fig. 2b and c) the changes were only evident after 210 min. When higher concentrations were used, all of the fibers withdrawn from the reaction media (residual non-hydrolyzed pulp) varied severely in length and thickness even after only short times in the acid solution.

The average length and thickness values, calculated using the fiber analyzer, were 238 and 20 μm (Fig. 2a), 218 and 20 μm (Fig. 2b) 149 and 20 μm (Fig. 2c), 147 and 19 μm (Fig. 2d), 111 and 18 μm (Fig. 2e), respectively. Fig. 2 shows that the distribution of the average fibers length tends to shorter lengths (between 46 and 129 μm) after hydrolysis even for short reaction times in the acid solution. Such behavior is not evident for the evolution of fiber thickness over time, which indicates that acid hydrolysis occurs preferentially at the ends of the fibers (average thickness in regions between 11 and 23 μm). Comparing Fig. 2b and c with Fig. 2d and e show that both concentrations result in an increase in the density from the thinner and shorter fibers; however, this increase is more significant when using 25% H_2SO_4 (Fig. 2d and e) than when using 5% H_2SO_4 (Fig. 2b and c). Still, though less apparent, the fraction of greater density is located in a broader thickness region for the density map of the initial pulp (Fig. 2a) than that of

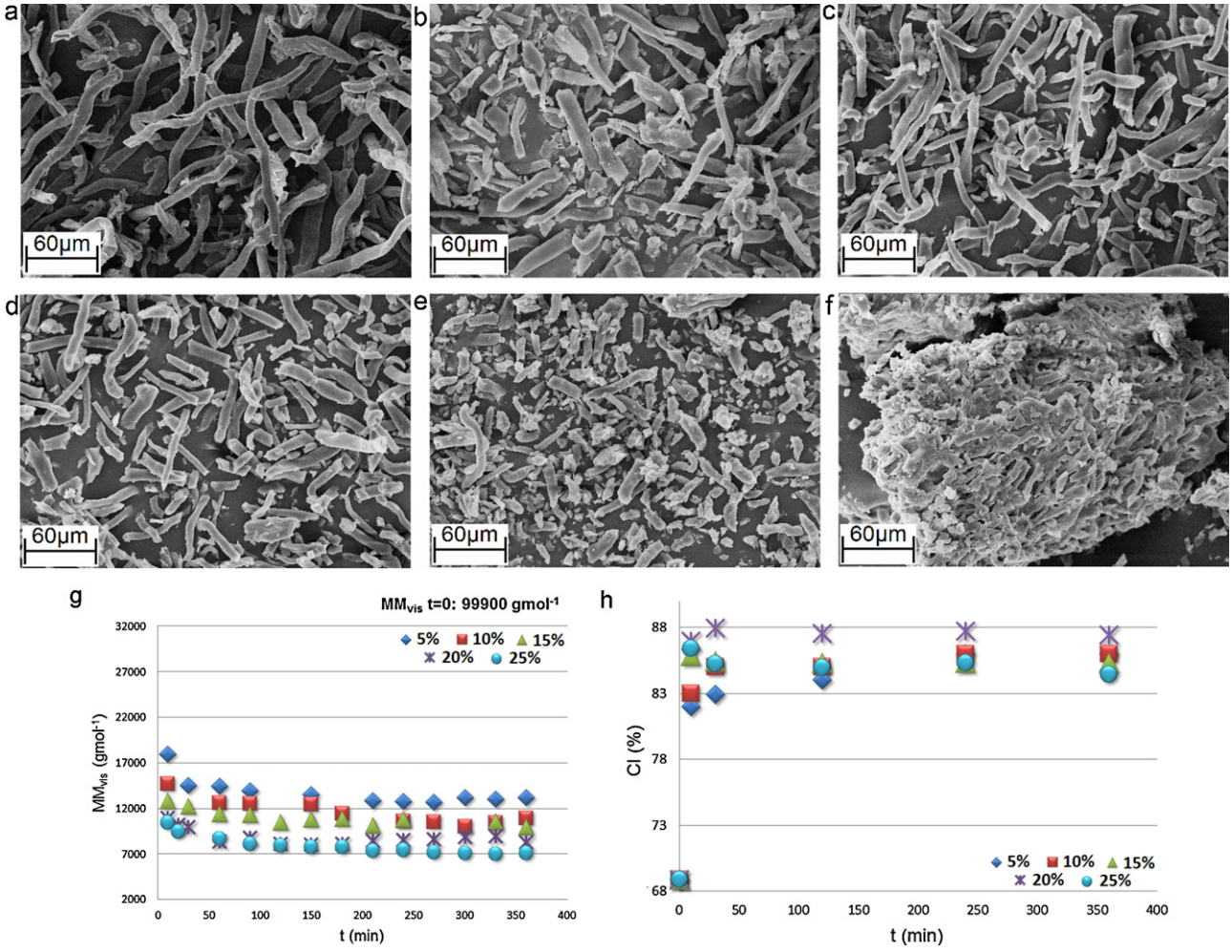

Fig. 1. SEM images for the starting pulp MSP (a), and the non-hydrolyzed pulp, from the reactions with H_2SO_4 5% (b), 10% (c), 15% (d), 20% (e) and 25% (f), after 240 min of reaction. MM_{vis} (g) and crystallinity index (h) as a function of hydrolysis time.

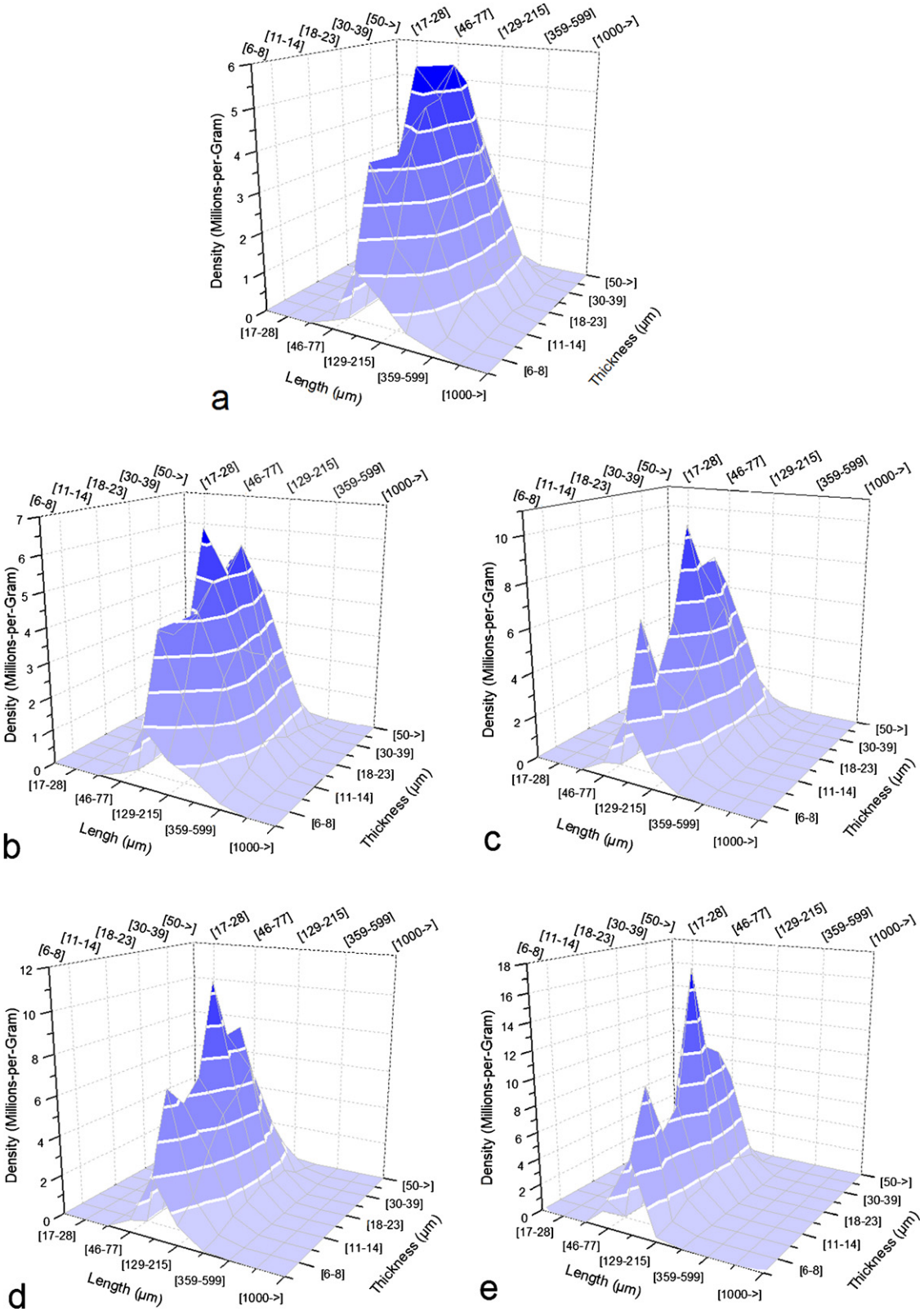

Fig. 2. Fiber-occurrence density maps of the initial pulp (a), and non-hydrolyzed cellulose withdrawn from the reaction performed with 5% sulfuric acid after 10 min (b) and 360 min (c), and 25% sulfuric acid after 10 min (d) and 360 min (e).

the non-hydrolyzed residual pulps (Fig. 2b–e). Fig. 2c–e shows a "valley" dividing the two different thickness regions, which can be explained by the consumption of the fractions between 6–8 and 11–14 μm.

3.2.1.2. Characterization of the products of the hydrolysis reactions. All analyses were made in duplicate, with an average error from 0.4% to 0.8%. As described in Section 2.2.4, the sugars and their respective decomposition products analyzed in the liquor were

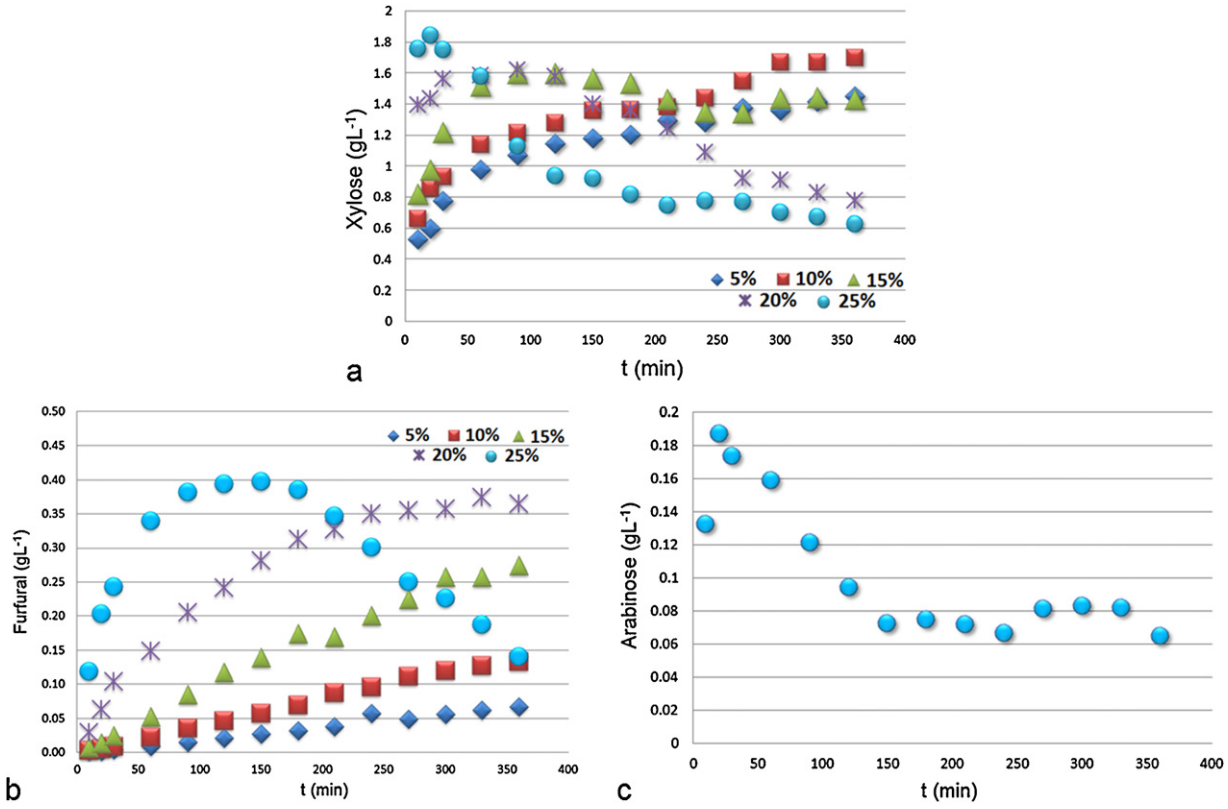

Fig. 3. Concentrations of xylose (a), furfural (b) found in liquor for the reactions with varying sulfuric acid concentration (5–25% H_2SO_4 (v/v)), and arabinose (c), from reaction with 25% H_2SO_4.

glucose, xylose, arabinose, formic acid, acetic acid, hydroxymethylfurfural (HMF) and furfural. Both glucose and xylose, which are products of the direct hydrolysis of cellulose and hemicellulose, and HMF and furfural, which are the primary decomposition products of glucose and xylose, were found in all reaction mixtures. The presence of glucose under all hydrolysis conditions indicates the cleavage of the 1,4-β-glycosidic linkages in the cellulosic chains, and the presence of HMF indicates that all of the reaction conditions led to the partial decomposition of glucose via dehydration. Formic acid, a decomposition product of HMF (Belgacem & Gandini, 2008), was detected only for reactions with 15, 20 and 25% H_2SO_4. Arabinose, produced from the hydrolysis of hemicellulose, was only found in the reaction with 25% H_2SO_4, and acetic acid was not found in any of the reactions considered here.

Fig. 3 refers to the products found in the liquor that directly results from hemicellulose hydrolysis, i.e., xylose (Fig. 3a), its major decomposition product (furfural, Fig. 3b). Arabinose was only detected for the reaction performed using 25% H_2SO_4 (Fig. 3c). Concerning the other reactions, if arabinose has been produced, its concentration was below the detection limit of the chromatographic apparatus used.

For the reactions with 5 and 10% sulfuric acid, the xylose concentration (Fig. 3a) increased for approximately 300 min and then remained constant. With 15 and 20% sulfuric acid, the increase occurred until approximately 90 min, which was followed by decomposition. When 25% sulfuric acid solution was used, the xylose concentration reached a maximum after 20 min and then decreased until the end of the reaction. These results indicate that, at 100 °C, xylose is relatively stable in H_2SO_4 solutions with concentrations between 5 and 15% but is highly unstable at 20%. Fig. 3b shows that, at 100 °C, increasing the acid concentration from 5% to 25% led to a considerable increase in furfural production. It can observed from the profile curves that in 25% sulfuric acid the furfural concentration reached a maximum value after 150 min and then the concentration began to decline over a 6-h reaction time. This pattern is probably related to the formation of polyfurfurals (Belgacem & Gandini, 2008), which is favored at 100 °C (Fig. 3b). Indeed, precipitates were observed in the brown liquors from this reaction, indicating the occurrence of this reaction. Finally, Fig. 3c shows that even small concentrations of arabinose were unstable with concentrated acid solutions, and a maximum concentration was reached (approximately 0.2 g L^{-1}) before it sharply decreased until 150 min mark where it remained roughly constant (approximately 0.07 g L^{-1}) until the end of reaction.

Fig. 4 corresponds to the products in the liquor that came directly from cellulose hydrolysis, i.e., glucose (Fig. 4a) and its decomposition products (HMF and formic acid, Fig. 4b and c) (Belgacem & Gandini, 2008).

It should be noted that hemicellulose may also have glucose in their composition; however, in sisal pulp, hemicellulose contains only approximately 2.2% glucose (Megiatto et al., 2007). As an approximation, glucose will therefore be considered as originating only from cellulose because the starting pulp contained only 2% hemicellulose, as mentioned in Section 3.1.

As expected, the production of glucose (Fig. 4a) was directly related to the acid concentration, and the maximum value obtained was approximately 10 g L^{-1} after a 350-min reaction time using 25% sulfuric acid. However, after this time interval, the decomposition of glucose to HMF intensified, which slightly decreased the glucose concentration (Fig. 4a). The presence of HMF in all analyzed samples indicated that all of the reaction conditions led to the partial decomposition of glucose via a dehydration reaction. It was found that increasing the acid concentration favored the dehydration of glucose and increased HMF production (Fig. 4b). Practically no HMF formed for the reaction with 5% H_2SO_4, with only a small fraction observed after a 120-min reaction time (0.005 g L^{-1}). In

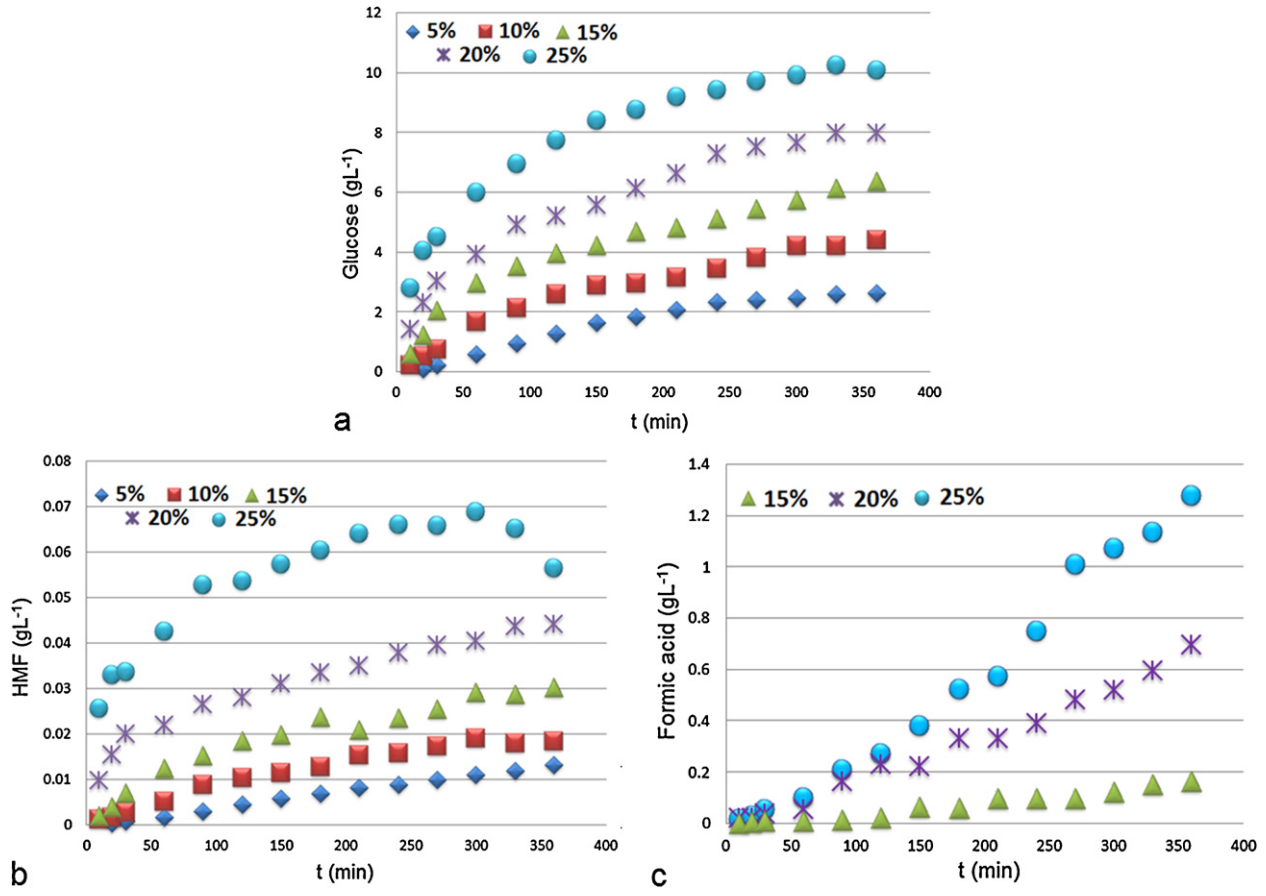

Fig. 4. Concentrations of glucose (a), HMF (b) and formic acid (c) found in liquor for the reactions with varying sulfuric acid concentration.

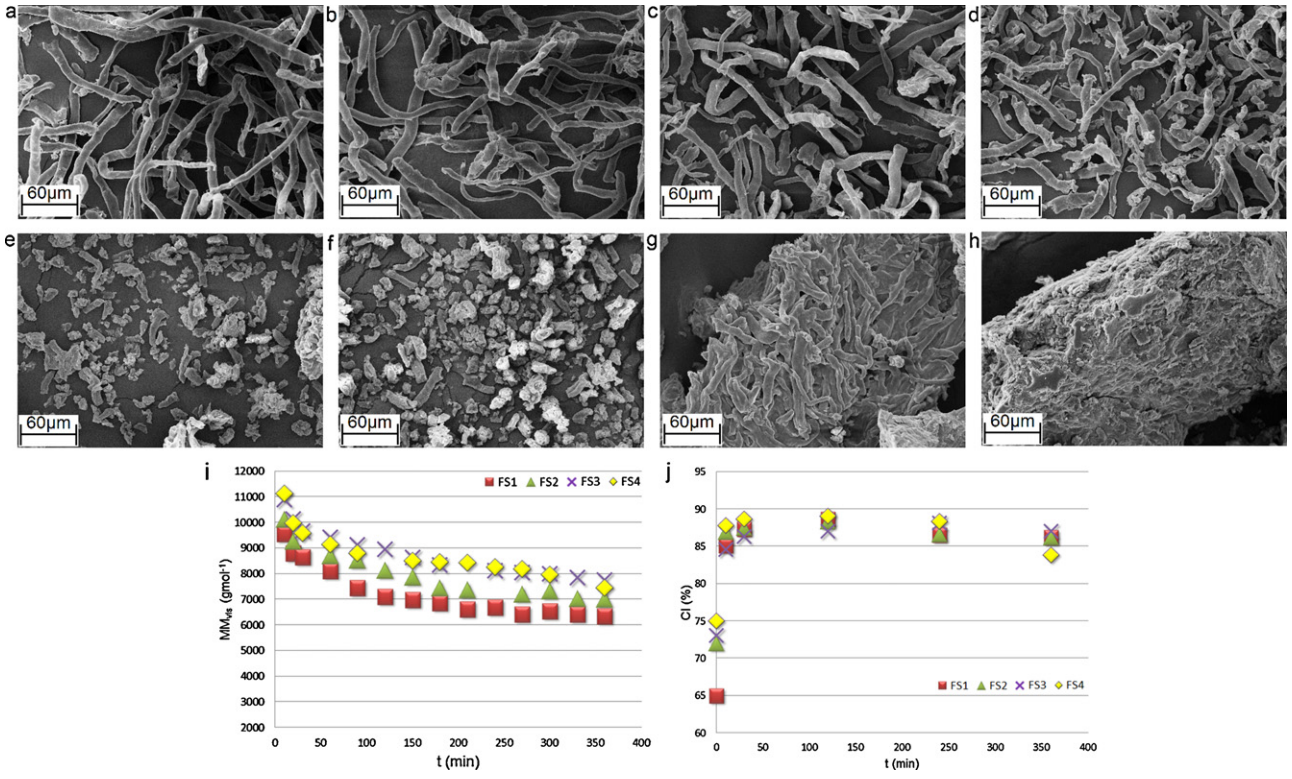

Fig. 5. SEM images for the non-hydrolyzed pulp, from the reactions with FS1 (a), FS2 (b), FS3 (c) and FS4 (d), after 240 min of reaction. MM_{vis} (i) and crystallinity index (j) as a function of reaction length.

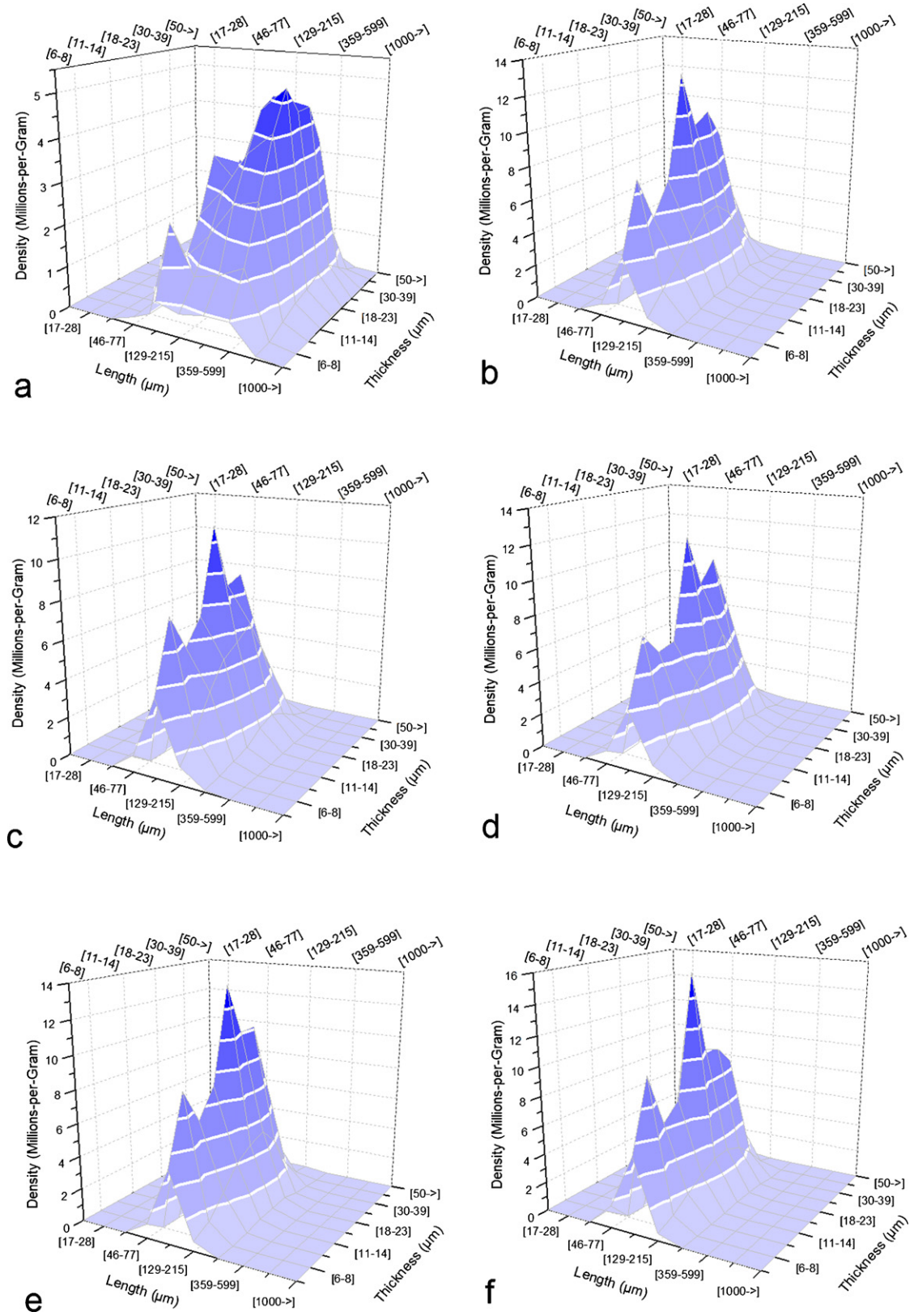

Fig. 6. Fiber-occurrence density maps of the initial pulps FS1 (a) and FS4 (b), and the non-hydrolyzed cellulose withdrawn from the reaction performed with FS1 after 180 min (c) and 360 min (e), and with FS4 after 180 min (d) and 360 min (f).

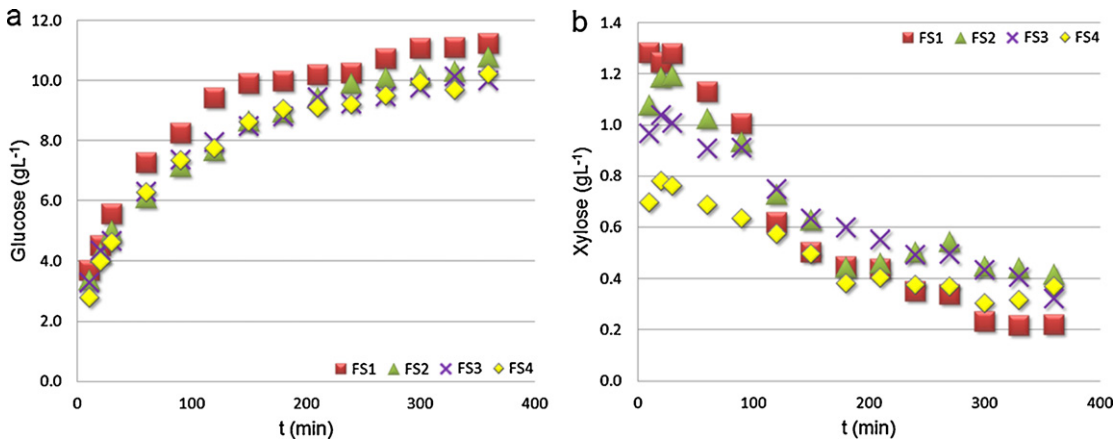

Fig. 7. Concentrations of glucose (a) and xylose (b) as function of time for the reactions with varying particles size.

contrast, the HMF concentration reached approximately 0.055 g L^{-1} with 25% H_2SO_4 over the same time interval. Formic acid (Fig. 4c) is a decomposition product of HMF and was only found for sulfuric acid concentrations between 15 and 25%. With the exception of 15% sulfuric acid, there is a considerable difference between the formic acid and HMF concentrations (Fig. 4b and c) because of the low stability of HMF under the reaction conditions, which leads to the formation of formic and levulinic acids (levulinic acid was not evaluated in the present study). In a previous study (de Paula, Lacerda, Zambon, & Frollini, 2012), a 30% (v/v) sulfuric acid solution was used as the catalyst, and a maximum glucose concentration of approximately 13 g L^{-1} was obtained, which is higher than that found using 25% H_2SO_4. However, the glucose content began to diminish after 240 min when the 30% acid was used and reached a minimum value of 10 g L^{-1} at t = 360 min, which is similar to that obtained using 25% H_2SO_4.

3.2.2. *Reactions performed with varying fibers size and crystallinity of the starting pulp*

3.2.2.1. Characterization of the residual non-hydrolyzed pulp. Scanning electron microscopy (SEM). Fig. 5 shows the non-hydrolyzed cellulose from the reactions starting with pulps of different average sizes.

The SEM images show that the fiber bundles in FS1 and FS2 (Fig. 5e and f) were hydrolyzed after 4 h to smaller and more separate portions relative to the initial pulp, MSP (Fig. 5a and b). However, the fiber bundles in the non-hydrolyzed cellulose from the reactions of FS3 and FS4 (Fig. 5c and d) tended to agglomerate under the same conditions and formed a large, tightly packed material similar to that shown in Fig. 1f for the whole pulp (not fractionated, MSP) under identical conditions. This indicates that using fractions with longer fibers as the starting material (FS1 and FS2) is preferential to smaller ones (FS3 and FS4) when the objective is to isolate the non-hydrolyzed fibers for subsequently application as biomaterials because agglomeration is typically undesirable in this case.

Average molar mass (MM_{vis}) via *viscometry and crystallinity index (CI)* via *X-ray diffraction (figures not shown).* Fig. 5i and j shows that, similarly to Fig. 1, the MM_{vis} values for all fibers sizes initially decreased considerably relative to their starting values (MSP = 99,900 g mol^{-1}, FS1 = 107,000 g mol^{-1}, FS2 = 112,000 g mol^{-1}, FS3 = 98,000 g mol^{-1}, FS4 = 100,000 g mol^{-1}) and stayed relatively constant after this drop. The X-ray diffraction analyses showed an initial increase in the value of CI for all of the used fibers sizes. The CI of all samples decreased after this initial increase with FS4 (shorter and less thick than the others) yielding a more pronounced decrease after a 360 min reaction time, which indicated that the crystalline regions were more easily accessed under these conditions.

Average fiber thickness and length (*Fiber analyzer – MorFi*). Fig. 6 shows the density maps obtained from the morphological analysis, which used MorFi Compact equipment, at t = 0, 180 and 360 min for the hydrolysis of FS1 and FS4. The average length and thickness values calculated by the fiber analyzer were 383 and 21 μm (Fig. 6a), 130 and 20 μm (Fig. 6b), 146 and 18 μm (Fig. 6c), 124 and 20 μm (Fig. 6d), 125 and 18 μm (Fig. 6e) and 116 and 18 μm (Fig. 6f), respectively.

The differences between the density maps promoted from the fractionation of the pulp (Fig. 6a and b) are that the higher density regions in FS4 are located at smaller length (46–215 μm) and thickness intervals (11–23 μm), when compared to FS1 (359–599 μm length, 18–50 μm thickness). The density maps a, c and e (Fig. 6), where map a corresponds to the initial pulp FS1 (t = 0), and maps c and e correspond to the residual non-hydrolyzed pulps after 180 and 360 min, respectively, indicate that the hydrolysis reaction was able to produce smaller fibers (higher density in regions of 46–215 μm length and 11–23 thickness). The same phenomenom occured (not as pronounced as for FS1) when FS4 was evaluated (Fig. 6b, d and f), and it can be observed that, after a 360-min reaction, the population of fibers between 46–77 and 129–215 μm in length increased, while that of fibers with 30- to 39-μm thickness decreased, which indicates an increase in the average fiber thickness. Analogous behavor is observed when FS2 and FS3 were used as the raw material, i.e., longer periods of exposure to the acid solution led to more fibers in regions of smaller thicknesses and length (figures not shown). This change in the density maps was more pronunced when using FS2 than for FS3.

3.2.2.2. Characterization of the products of the hydrolysis reactions. Only the graphs showing the evolution of glucose and xylose as a function of time will be provided for the reactions performed with varying fiber sizes (Fig. 7) because they are the ones where the influence of fibers size on the hydrolysis reaction was most evident. Arabinose, formic acid, furfural and hydroxymethylfurfural were detected in all analyzed samples with maximum contents of 0.18 g L^{-1} (120 min), 1.3 g L^{-1} (360 min), 0.42 g L^{-1} (150 min) and 0.04 g L^{-1} (360 min), respectively, which were all from FS1. This result highlights the ease of hydrolyzing this material, which is probably because of its smaller crystalline regions.

With regard to glucose production as a function of the reaction time (Fig. 7a, Table 1), FS1 provided the highest glucose concentration in the liquor during the reaction with values of up to 22% more glucose at t = 120 min than MSP (i.e., the unfractionated pulp). The graph of xylose concentration (Fig. 7b) clearly indicates a

relationship between the fiber size and xylose production that primarily occurs at the beginning of the reaction; when FS1 was used, the xylose content was up to 82% higher than for FS4 at $t = 10$ min. The results for both glucose and xylose content are closely related to the crystallinity of the initial pulp. The pulp FS1 has a CI of 65% while for pulp FS4 it is 75%. This difference can be decisive when a reaction is carried out in a heterogeneous media, as is the case during cellulose hydrolysis.

4. Conclusions

The influence of sulfuric acid concentration, crystallinity and fibers size on the hydrolysis reaction of sisal pulps was reported. With the aid of a fiber analyzer, it was possible to measure the average length and thickness of the fibers during the hydrolysis reactions. This is important when the primary objective is the isolation of these fibers for biomaterial applications because, under more drastic conditions, they tend to agglomerate, as was demonstrated. Additionally, the liquor obtained from these reactions was also analyzed and primarily contained glucose. Comparing the results described here (H_2SO_4 5–25%) to those found elsewhere (H_2SO_4 30%), the same amount of glucose was obtained using less drastic conditions taking into account that higher acid concentrations led to more intense glucose decomposition over long reaction times. The results of the study involving fiber fractions with different lengths and crystallinity showed that the latter was more important, with hydrolysis being favored at lower crystallinities. These results provide information about the liquor and residual non-hydrolyzed pulp produced by the hydrolysis reactions, which is important for both material applications and for determining parameters that can favor glucose production. This study is part of ongoing research that searches for hydrolysis conditions that lead to good outcomes with regard to both non-hydrolyzed material and liquor quality. If this strategy is applied on a larger scale, the profitability of biorefineries can be increased because the raw material for both material applications (micro- and nanofibers, nanoparticles) and bioethanol (glucose) would be generated simultaneously.

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