



## CHEMICAL SCIENCES

# Treatment and characterization of biomass of soybean and rice hulls using ionic liquids for the liberation of fermentable sugars

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**Abstract:** We investigated the changes in the physical structure of cellulose recovered from soybean and rice hulls treated with the ionic liquids 1-butyl-3-methylimidazolium chloride ([bmim][Cl]) and 1-butyl-3-methylimidazolium acetate ([bmim][Ac]). The characterization was carried out by a combination of thermogravimetric analysis (TGA), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). Regenerated cellulose from soybean hull showed loss of crystallinity and high structural disruption caused by both ionic liquid treatments as compared to the untreated material. In contrast, rice hull presented only a small structural disruption when treated with [bmim][Ac] and was practically unaffected by [bmim][Cl], showing that this biomass residue is recalcitrant towards physico-chemical treatments, possibly as a consequence of its high composition content *in silica*. These results suggest the use of soybean hull as a substrate to be treated with ionic liquids in the preparation of lignocellulosic hydrolysates to be used in second-generation ethanol production, whereas other methods should be considered to treat rice hull biomass.

**Key words:** Biomass pretreatment, ([bmim][Cl]), ([bmim][Ac]), regenerated cellulose, structural analysis of cellulose.

## INTRODUCTION

Lignocellulosic biomass derived from agricultural wastes, grasses, and trees are abundant and renewable feedstock to produce biofuels and chemicals. Among several possible lignocellulosic residues used in industry, soybean hulls are of importance, representing up to 10 % of the seed weight (Ipharraguerre & Clark 2003), of the most cultivated oilseed worldwide, with its production reaching 350 million tons in 2018 (USDA 2018). Rice is also a very important crop, with annual global productions around 495 million tons (USDA 2018). Rice hulls represents 20 % (mass fraction) of the harvested rice, and it is one of the most abundant lignocellulosic

by-products of cereal industries, accounting for more than 120 million tons generated per year (USDA 2018).

These materials are formed by polymeric carbohydrates cellulose and hemicellulose, and by lignin (Zhang & Zhao 2010, Yoo et al. 2017, Vasheghani Farahani et al. 2016). Hemicellulose is relatively amorphous in its structure (Lee et al. 2009), composed of heterogeneous polymers of pentoses, hexoses, and uronic acids, representing around 15 to 35 % of lignocellulosic biomass (Girio et al. 2010, Vasheghani Farahani et al. 2016). Lignin, comprising 15 to 35 % of the lignocellulosic matrix (Ek et al. 2009, Vasheghani Farahani et al. 2016) is a complex aromatic polymer of phenylpropanoid units acting as

cement binding cellulose and hemicelluloses (Chandra et al. 2007). However, the most abundant component of lignocellulosic biomass is cellulose, representing approximately 35 to 50 % of plant dry (Lynd et al. 1999, Vasheghani Farahani et al. 2016). Cellulose is crystalline in its structure and is composed exclusively of glucose units, the most important sugar for ethanol fermentation (Lee et al. 2009, Yoo et al. 2017).

In order to reduce the lignocellulosic biomass recalcitrance, chemical and physical pretreatments are required to open its structure by dislocating and removing lignin, depolymerizing hemicellulose, and decreasing the crystallinity of cellulose (Swatloski et al. 2002, Murakami et al. 2007, Kumari & Singh 2018, Weerachanchai & Lee 2014). However, cellulose cannot be dissolved in water or in the majority of conventional organic solvents because of the extensive network of inter and intra-molecular hydrogen bonds (Swatloski et al. 2002, Murakami et al. 2007), representing a major challenge to its application in bioprocess.

One promising new technology that has been devised to use as cellulose solvent is the use of ionic liquids (ILs), salts typically composed of large organic cations and small inorganic anions that are liquid through a wide range of temperatures. Considered environmentally safe solvents, ILs show low vapor pressures, high thermal stabilities, relative nontoxicity and ease of recycling (Zhu et al. 2006, El Seoud et al. 2007, Hayes 2009, Aung et al. 2018, Weerachanchai & Lee 2014). The recycling and recovery of hydrophilic ILs (as 1-butyl-3-methylimidazolium chloride and 1-butyl-3-methylimidazolium acetate), however, is more difficult compared to hydrophobic ILs. The supercritical CO<sub>2</sub> and induced phase separation by adding salts (salting-out process) are cited as recovery

methods for hydrophilic ILs (Mai et al. 2014, Verma et al. 2019).

Some reports have shown the interesting results using ILs for the dissolution of cellulose from wood and other agro-residues (Fort et al. 2007, Wei et al. 2012, Yoon et al. 2012, da Cunha-Pereira et al. 2016, Aung et al. 2018, Vasheghani Farahani et al. 2016, Aid et al. 2016). Among the many possible ILs to be used, interesting results have been obtained using imidazolium-based ILs possessing short side chains, possibly because their reduced viscosity and melting point (Dadi et al. 2006, Weerachanchai & Lee 2014). Regarding anions of the ILs, the most promising are chloride and acetate because they have the ability to break down the hydrogen bonds of cellulose (Fort et al. 2007, Pu et al. 2007, Kumari & Singh 2018).

The use of the ILs for the pretreatment of lignocellulosic materials may reduce the crystallinity of cellulose and partially remove hemicellulose and lignin, at the same time avoiding the formation of degradation products, such as hydroxymethylfurfural and furfural, which inhibit enzymes and the growth of fermenting microorganisms (Lee et al. 2009, Dadi et al. 2007, Sehnem et al. 2017, Kumari & Singh 2018).

In the context of these considerations, the aim of this study was to determine the effects of application of two ILs commonly used for lignocellulosic biomass treatments, 1-butyl-3-methylimidazolium chloride ([bmim][Cl]), and 1-butyl-3-methylimidazolium acetate ([bmim][Ac]), on soybean and rice hulls. The regenerated cellulose was characterized by infrared spectroscopy, thermogravimetric analysis, X-ray diffraction, and scanning electron microscopy and their properties were discussed.

## MATERIALS AND METHODS

### Biomass and compositional analysis

Soybean hulls were kindly supplied by DuPont (Esteio, RS, Brazil), whereas rice hulls were obtained from a local rice mill (Pelotas, RS, Brazil). The hulls, processed without any further treatments, were milled to a particle size < 1 mm in diameter and stored at room temperature until further use or analysis.

### Ionic liquid preparation

We firstly synthesized [bmim][Cl], which is a precursor of ILs with carboxylate anions ([bmim][Ac]), following procedures described in the literature and characterized by <sup>1</sup>H NMR (Varian Inova 300 MHz, Agilent 500 MHz) (Dupont et al. 2002, Wu et al. 2008).

Description of the ILs used: a) [bmim][Ac], colorless viscous liquid. <sup>1</sup>H NMR (500 MHz - CDCl<sub>3</sub>) δ ppm 0.97 (t, *J* = 7.4 Hz, 3H<sup>9</sup>); 1.33-1.41 (m, 2H<sup>8</sup>); 1.83-1.89 (m, 2H<sup>7</sup>); 1.97 (s, 3H<sup>12</sup>); 4.05 (s, 3H<sup>10</sup>); 4.29 (t, *J* = 7.4 Hz, 2H<sup>6</sup>), 7.24 (s, 1H<sup>5</sup>); 7.30 (s, 1H<sup>4</sup>); 11.08 (s, 1H<sup>2</sup>); b) [bmim][Cl], white solid. <sup>1</sup>H NMR (300 MHz - D<sub>2</sub>O) δ ppm 0.89 (t, *J* = 7.4 Hz, 3H<sup>9</sup>); 1.22-1.35 (m, 2H<sup>8</sup>); 1.77-1.87 (m, 2H<sup>7</sup>); 3.86 (s, 3H<sup>10</sup>); 4.17 (t, *J* = 7.2 Hz, 2H<sup>6</sup>); 7.40 (d, *J* = 2.0 Hz, 1H<sup>5</sup>); 7.45 (d, *J* = 2.0 Hz, 1H<sup>4</sup>).

### Ionic liquid pretreatment of soybean hull

In a polypropylene tube, 2 g of IL or IL-water mixture (1 g of each) were mixed with 240 mg of the dried lignocellulosic biomass sample. The samples were incubated in an oil bath at 75 °C or 100 °C and different incubation times (2 or 6 h), as shown in detail in Table I. All experiments were performed under atmospheric pressure. After pretreatment, 10 mL of deionized water was added to samples to serve as an anti-solvent in the mixture and to regenerate the cellulose.

The resulting mixture was briefly centrifuged (7,000 g), and the supernatant was removed. The precipitate (treated biomass containing regenerated cellulose) was washed with the same amount of anti-solvent and subsequently centrifuged four times (7,000 g). The treated biomass was oven-dried at 75 °C for 24 h.

### Thermogravimetric analysis

The samples were submitted to thermogravimetric analysis (TGA), using a Shimadzu TGA-50. The samples were heated up to 600 °C at a rate of 10 °C min<sup>-1</sup> and a flow rate of 50 ml min<sup>-1</sup> of N<sub>2</sub>. Thermogravimetric data were converted into derivative thermograms (DTG).

### Fourier Transform Infrared (FTIR) analysis

The FTIR spectra of samples were obtained using a Shimadzu FTIR, model Prestige 21, in ATR mode, with a resolution of 4 cm<sup>-1</sup> and 32 cumulative scans.

### X-RAY powder diffraction (XRD) analysis

In order to determine the transformation in the crystallinity of treated cellulose, XRD studies were carried out using a Siemens D-500 powder diffractometer, equipped with Soller slits and a graphite monochromator in the secondary beam. Data were collected with CuK $\alpha$  radiation, with a wavelength of 0.15418 nm.

### Scanning Electron Microscopy (SEM)

The materials were analyzed by scanning electron microscopy (SEM) using a Jeol equipment, model JEOL JSM6060, operating at 18 kV and with magnification of 500 times.

**Table I. Lignocellulosic biomass used in this research and the treatment conditions.**

Lignocellulosic Biomass	Treatment		
	Ionic Liquid	Temperature (°C)	Time (h)
Soybean Hull	50% [bmim][Ac]+50% water	75	2 and 6
	100%[bmim][Ac]	75	
	100%[bmim][Ac]	100	
	50% [bmim][Cl]+50% water	75	2 and 6
	100%[bmim][Cl]	75	
	100%[bmim][Cl]	100	
Rice Hull	50% [bmim][Ac]+50% water	75	2 and 6
	100%[bmim][Ac]	75	
	100%[bmim][Ac]	100	
	50% [bmim][Cl]+50% water	75	2 and 6
	100%[bmim][Cl]	75	
	100%[bmim][Cl]	100	

## RESULTS AND DISCUSSION

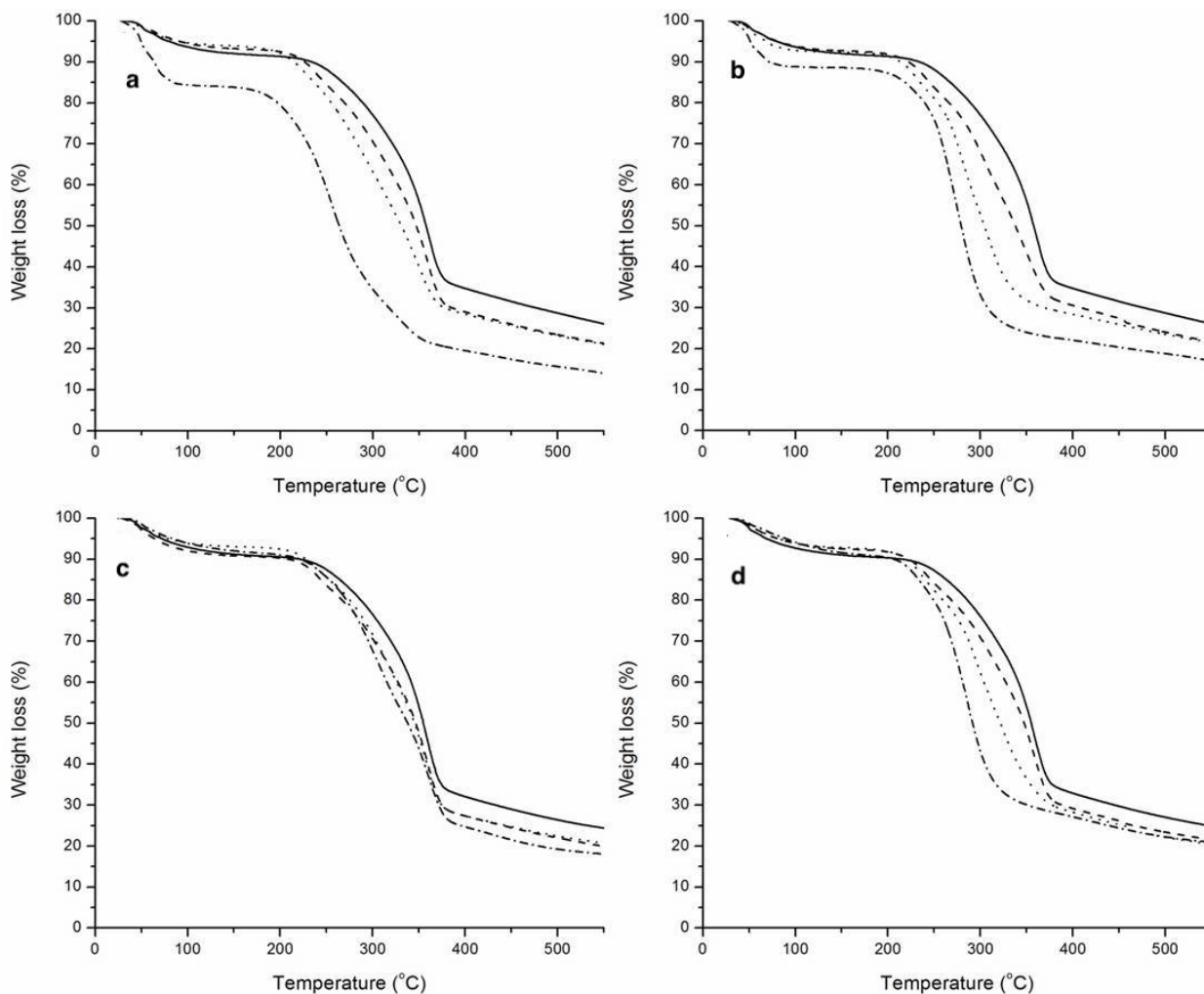
### Thermogravimetric analysis

We used thermogravimetric analysis (TGA and DTG) to obtain information on the weight loss of samples as a function of temperature, thus defining the thermal decomposition profiles of untreated and regenerated cellulose. This analysis is important to determine whether the pretreatment of soybean and rice hulls using the ILs affected the thermal stability of the lignocellulosic materials. The TGA results are shown in Figure 1 for soybean hull, and Figure 3 for rice hull. For DTG, the results are shown in Figures 2 and 4 for soybean and rice hulls, respectively.

We may divide the curves into three principal different regions, independent of the

treatment or biomass (Wei et al. 2012, Alemdar & Sain 2008). Analyzing the results of Figures 1 and 2, results show the thermal stability of the lignocellulosic biomass decreases after treatment and regeneration with water.

The first part of the TGA curve increases to approximately 200 °C, showing a slight decline of the sample weight due to the release of unbound water during the drying procedure. The second stage is between 200 °C and 380 °C, in which a great decrease in weight is caused by the thermal decomposition of hemicellulose and cellulose. Above 380 °C, the samples went to the final stage, associated with thermal decomposition of lignin, but showing weight loss not as significant as compared to the second stage (Wei et al. 2012, Alemdar & Sain 2008).

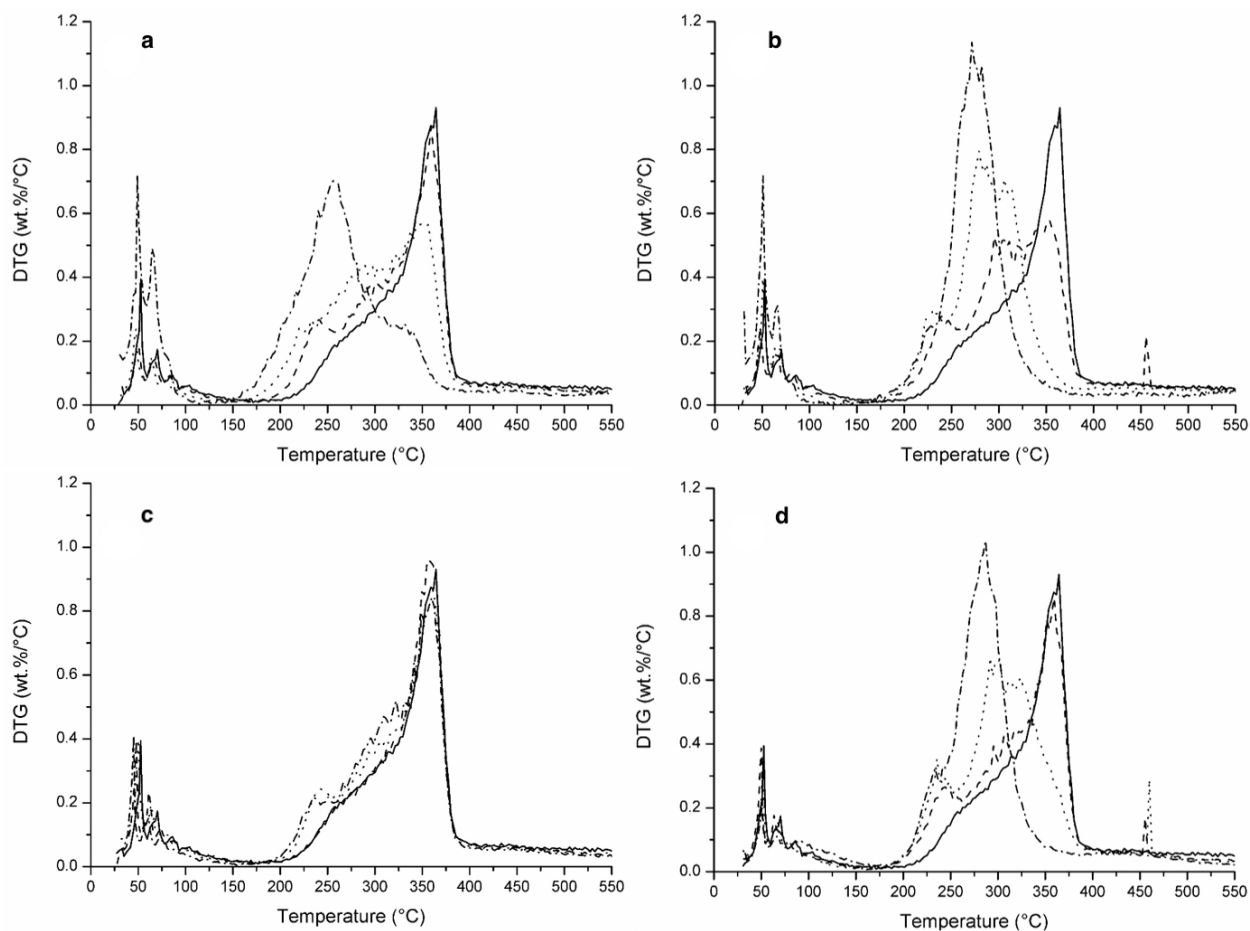


**Figure 1.** TGA thermograms of soybean hull treated with [bmim][Ac] (a and c), and [bmim][Cl] (b and d), for 2 h (a and b), and 6 h (c and d). Legend: (—) untreated biomass, (---) IL 50 % and 75 °C, (····) IL 100 % and 75 °C, and (-·-·-) IL 100 % and 100 °C.

Lignin decomposition occurs in a wide range of temperature that partially overlaps hemicellulose and cellulose fractions. Observing Figure 2, it is possible to verify that, the higher the proportion of LI and temperature, there is a displacement of the pulp peak to lower temperatures, (Figure 2 a, b, d). These results suggest that IL treatment of samples decreased the thermal stability of cellulose, which may be related to the decrease in crystallinity of the biomass, confirmed by results shown in XRD (Figure 5), where the peak 16.6° was reduced (see discussion for The XRD analysis below). Kin

et al. (2001), reported that the modification of the thermal behavior of cellulose is caused by the adherence of the anion group of IL into the surface of cellulose.

We also found that increasing LI and temperature, dissociation of the peaks occurs, differentiating hemicellulose and cellulose. The peak of hemicellulose is reduced, while that of the pulp increases. Thus, part of the hemicellulose must have been dissociated. In Figure 2d can be observed that there was no significant change in thermal stability when [Bmim] [Ac] was used for 6 h of treatment of



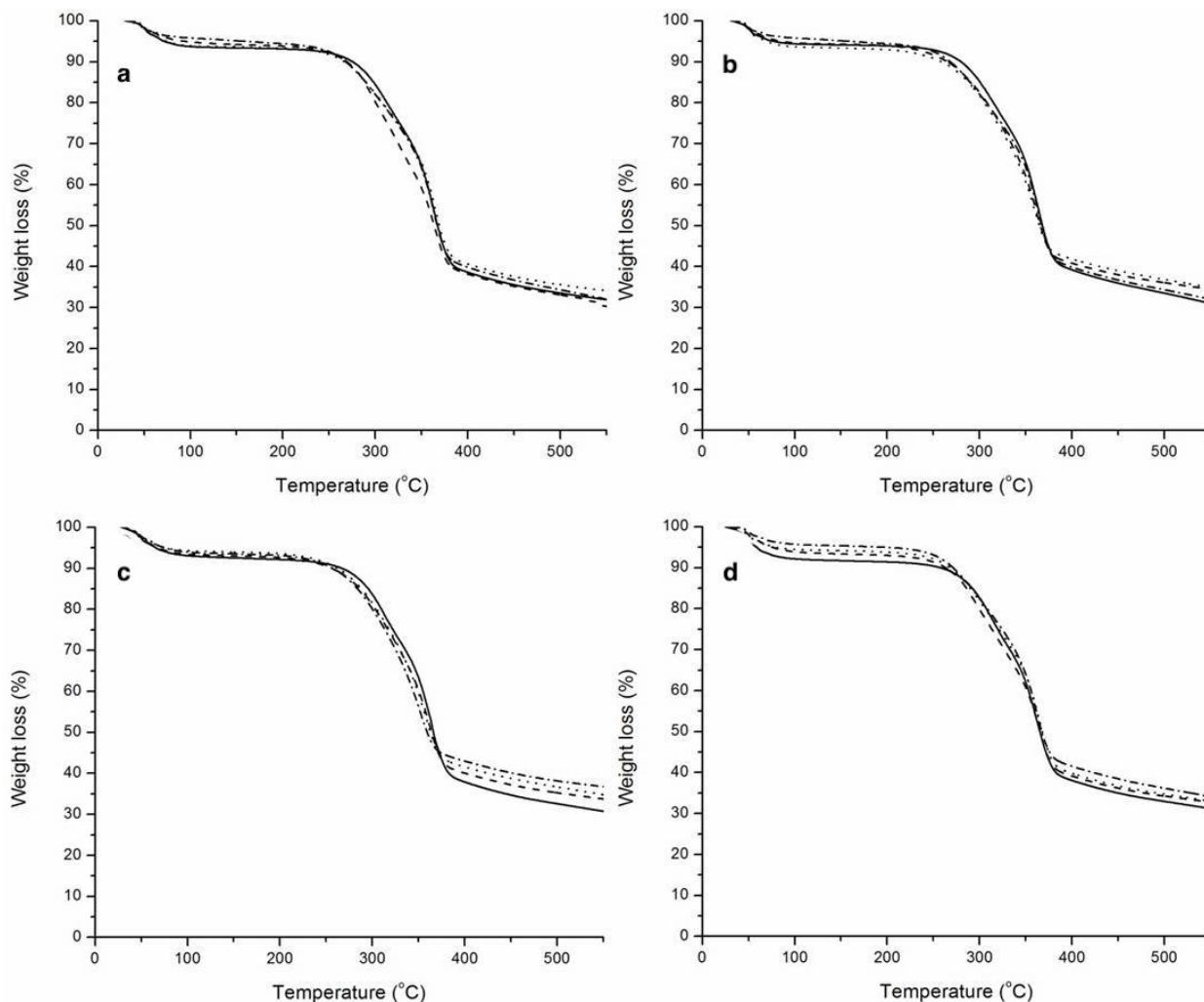
**Figure 2.** DTG of soybean hull treated with [bmim][Ac] (a and c), and [bmim][Cl] (b and d), for 2 h (a and b), and 6 h (c and d). Legend: (—) untreated biomass, (---) IL 50 % and 75 °C, (· · · ·) IL 100 % and 75 °C, and (-·-·-) IL 100 % and 100 °C.

soybean hulls. However, small differences in the values of the maximum temperature between treated and untreated biomass are probably due to changes in the crystalline structure of cellulose, as a result of partial removal of amorphous cellulose and delignification, which changed the thermal stability to a slightly lower temperature level (Financie et al. 2016).

The TGA results for rice hulls are shown in Figure 3. This biomass undergone the same three stages observed for soybean hulls. However, after treatment with [bmim][Cl] and [bmim][Ac] the regeneration of cellulose, we could not observe a decrease in decomposition temperature compared with the untreated biomass.

Observing Figure 4, we can verify that the treatment with LI rather than decreasing the thermal stability had the opposite effect (displacement of the pulp peak to regions of higher temperatures), and it is still possible to verify that increasing the proportion of LI and temperature, the dissolution of the hemicellulose (decrease of the first peak) occurs, whereas there is an increment in the cellulose peak (Zhang et al. 2014). These results are in accordance with the analyses of XRD and FTIR, where no alterations indicated a change in the cellulose present in the rice husk. Thus, it is evident that there was no decrease in the decomposition temperature of the cellulose, which did not depolymerize. For the treatment





**Figure 3.** TGA thermograms of rice hull treated with [bmim][Ac] (a and c), and [bmim][Cl] (b and d), for 2 h (a and b), and 6 h (c and d). Legend: (—) untreated biomass, (----) IL 50 % and 75 °C, (· · · ·) IL 100 % and 75 °C, and (-·-·-) IL 100 % and 100 °C.

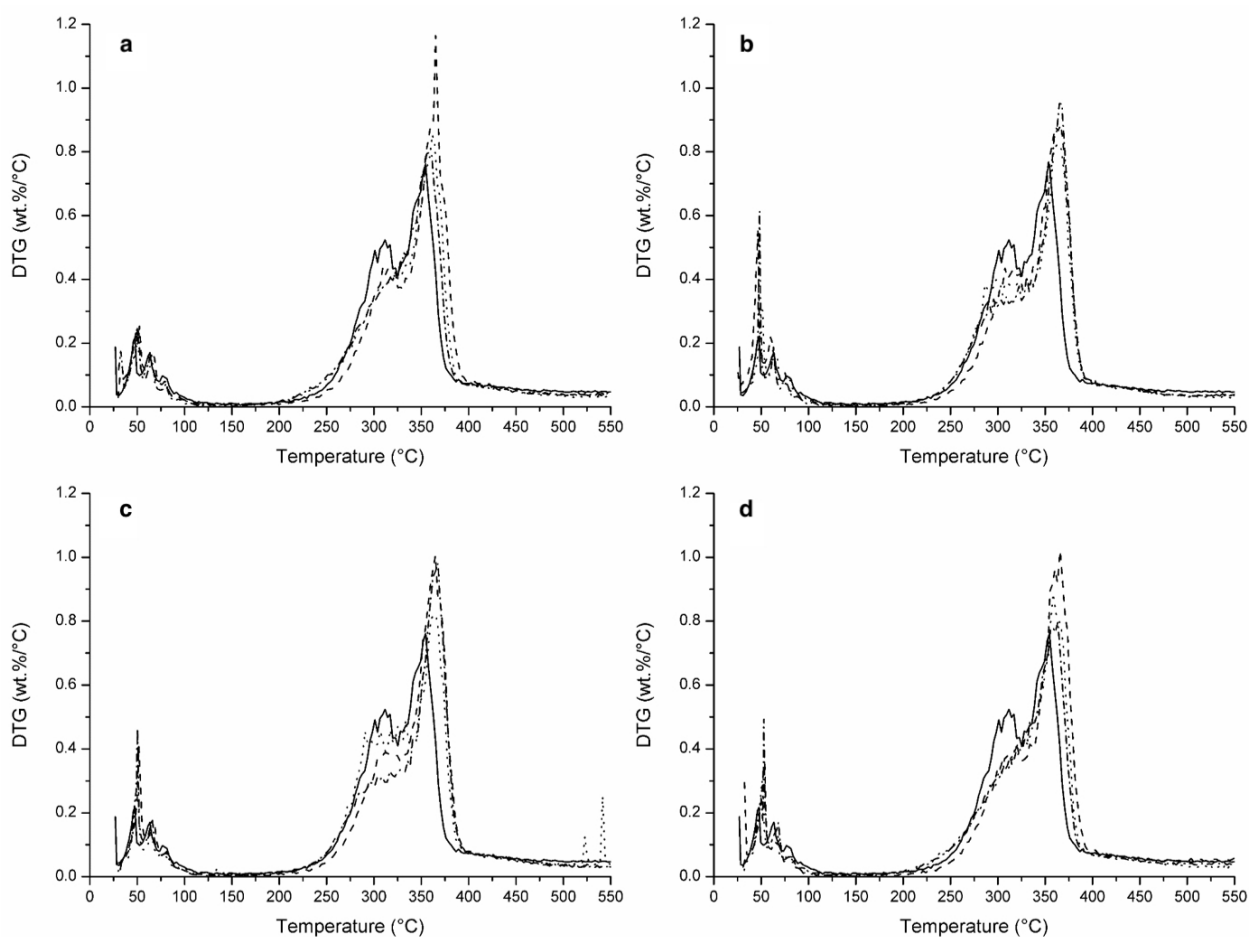
of cypress wood with [C2mim][OAc] at 80 °C for 1 h, an increase in thermal stability was reported (Moniruzzaman & Ono 2013).

The regenerate samples did not apparently exhibit any decreases in the temperature of decomposition compared to the original sample, possibly because this biomass is rich *in silica* and very recalcitrant to the decrystallization of the cellulose (Swatloski et al. 2002, Fort et al. 2007).

### Fourier Transform Infrared (FTIR) analysis

The spectra of regenerated cellulose of soybean hull after treatment with IL and its corresponding untreated sample are shown in Figure 5. For these analyses, ATR FTIR spectroscopy was used, in the region of 600 – 4000  $\text{cm}^{-1}$ , which is commonly applied to study the fine structural characteristics of cellulose (Poornejad et al. 2013).

The regenerated biomass underwent chemical and structural changes compared with untreated samples. The 3330  $\text{cm}^{-1}$  range represents the -OH stretch region present in lignin structures.



**Figure 4.** DTG of rice hull treated with [bmim][Ac] (a and c), and [bmim][Cl] (b and d), for 2 h (a and b), and 6 h (c and d). Legend: (—) untreated biomass, (----) IL 50 % and 75 °C, (····) IL 100 % and 75 °C, and (-·-·-) IL 100 % and 100 °C.

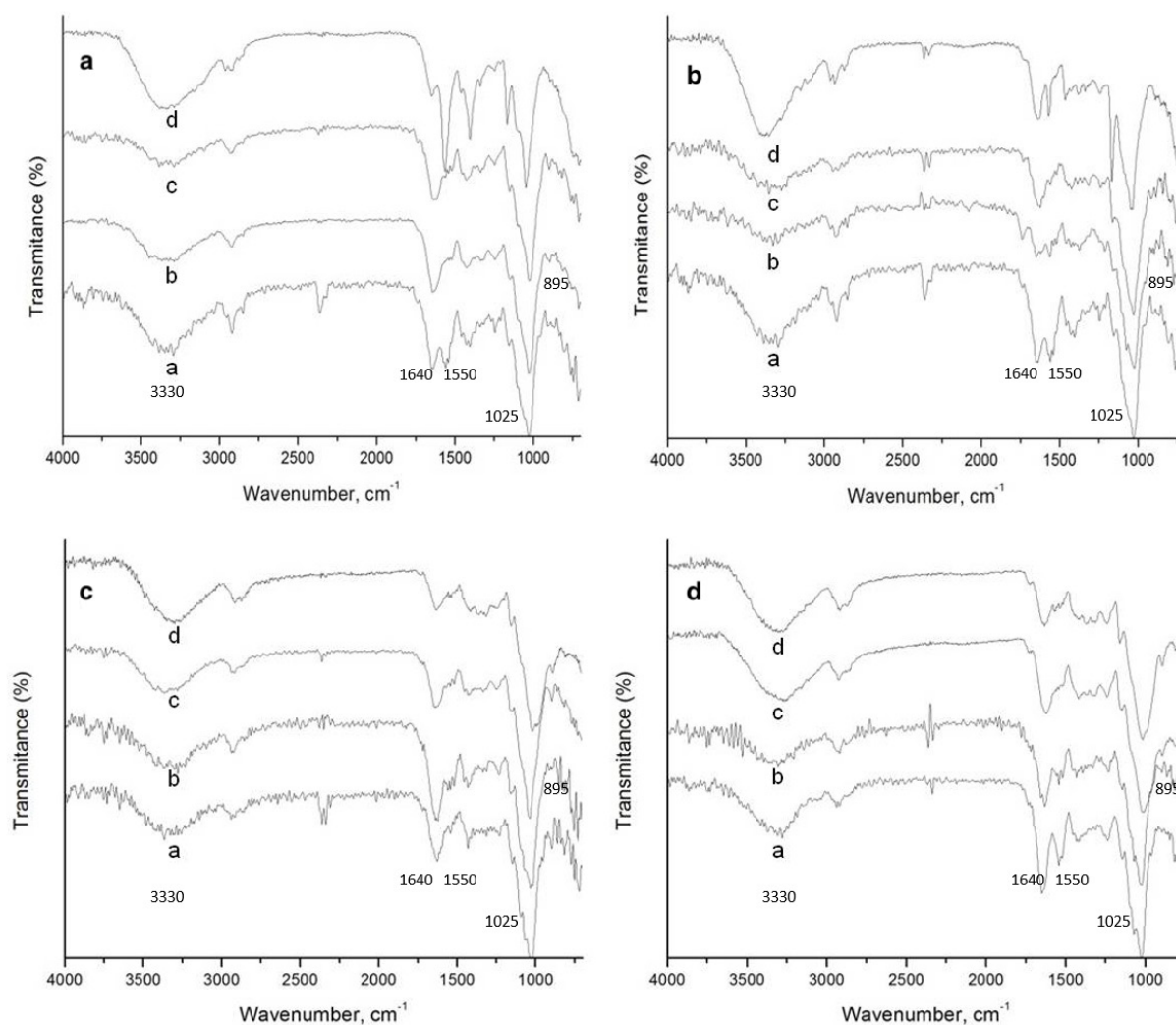
Figure 5 shows an increase of the peak between 3500 to 3000  $\text{cm}^{-1}$ , which can be attributed to the dissociation of the hemicellulosic fraction of the soybean hull, leading to a greater exposure of lignin and thus a greater vibration of the bands in -OH (Lachos-Perez et al. 2017).

The peak around of 1640  $\text{cm}^{-1}$  is assigned to the absorbed water (Sun et al. 2004, Morán et al. 2008), and the reduction of the intensity of this peak in the treated biomass was caused by the partial removal of hemicelluloses (Alemdar & Sain 2008). In the spectra of untreated samples, the peaks in the region between 1420 -1540  $\text{cm}^{-1}$  represent the aromatic C-C stretch of aromatic

rings of lignin (Sun et al. 2005, Xiao et al. 2001). In the curves of treated samples, the intensity of these peaks decreased, attributed to the partial removal of lignin (Alemdar & Sain 2008).

The 1025  $\text{cm}^{-1}$  peak corresponds to C-O-C pyranose ring skeletal vibration at cellulose/hemicellulose. The emergence of a small sharp 895  $\text{cm}^{-1}$  peak is an indication of glycosidic C-H deformation with ring vibration contribution, which is a characteristic of  $\beta$ -glycosidic linkages between glucose molecules in the cellulose structure (Wei et al. 2012). This peak also indicates that the regenerated cellulose is more amorphous and disordered than the original material. The



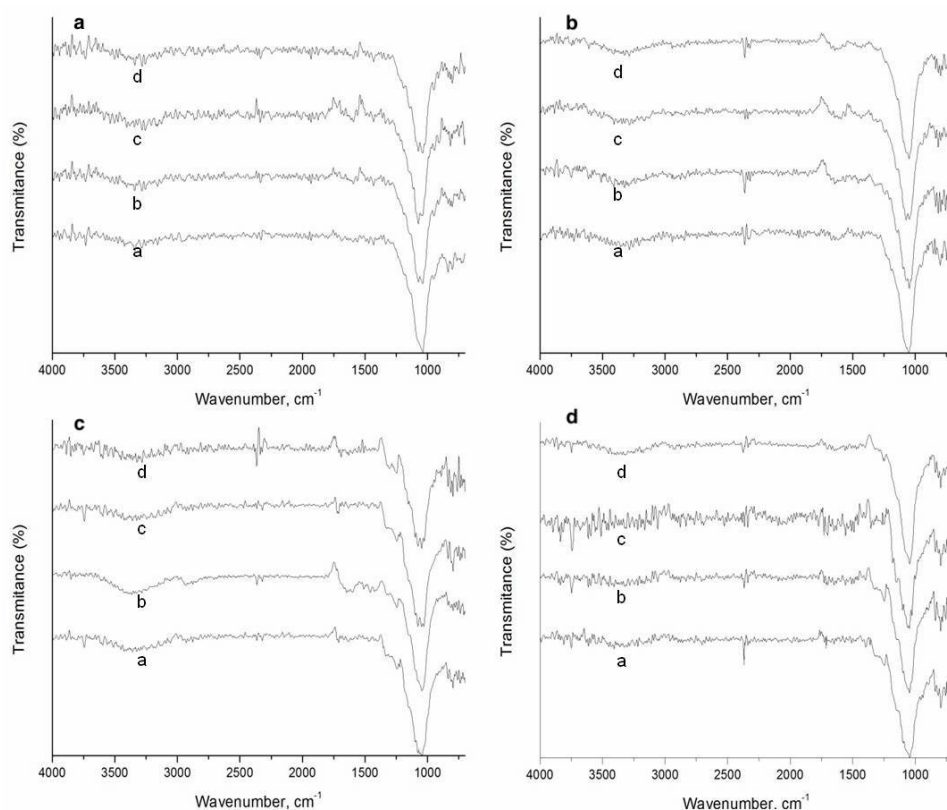


**Figure 5.** FTIR spectra of soybean hull treated with [bmim][Ac] (a and c), and [bmim][Cl] (b and d), for 2 h (a and b), and 6 h (c and d). Legend: a) untreated biomass, b) IL 50 % and 75 °C, c) IL 100 % and 75 °C, and d) IL 100 % and 100 °C.

higher degree of amorphous structure of the regenerated cellulose can be attributed to the dissolution and subsequent regeneration of the hemicellulose (Ang et al. 2012). The narrowing of the 1025  $\text{cm}^{-1}$  peak and the emergence of another in position 895  $\text{cm}^{-1}$  in the spectra of the treated samples are indicating that the regenerated material is richer in carbohydrates than the untreated biomass (Singh et al. 2009, Sun et al. 2009).

Thus, the presence of these peaks for the FTIR in our work indicates that the hemicellulose

and lignin were successfully removed during treatments and the concentration of cellulose in the treated sample was increased. However, this pattern was not observed in Figures 5a, d, because when [Bmim] [Ac] was used, at high temperatures or for a long time, the imidazolium IL cation can be trapped within the structure of the regenerated cellulose. This can be observed by the narrow peak around 1550  $\text{cm}^{-1}$ , which represents the C-C plane stretching vibration of imidazolium cation of IL (Liu et al. 2012). This observation is in accordance with the results



**Figure 6.** FTIR spectra of rice hull treated with [bmim][Ac] (a and c), and [bmim][Cl] (b and d), for 2 h (a and b), and 6 h (c and d). Legend: a) untreated biomass, b) IL 50 % and 75 °C, c) IL 100 % and 75 °C, and d) IL 100 % and 100 °C.

for the same sample in the analysis of DTG (Figures 2 a, d), in which we can see a wide peak shifted to 250 °C, which approaches the range of decomposition temperatures of hemicellulose, cellulose, and [Bmim] [Ac] (Williams et al. 2018).

Therefore, the comparison of the FTIR spectra of untreated and treated samples with [bmim] [Ac] and [bmim][Cl], strongly suggest that inter and intra-molecular hydrogen bonds of cellulose were significantly broken during the treatment with IL.

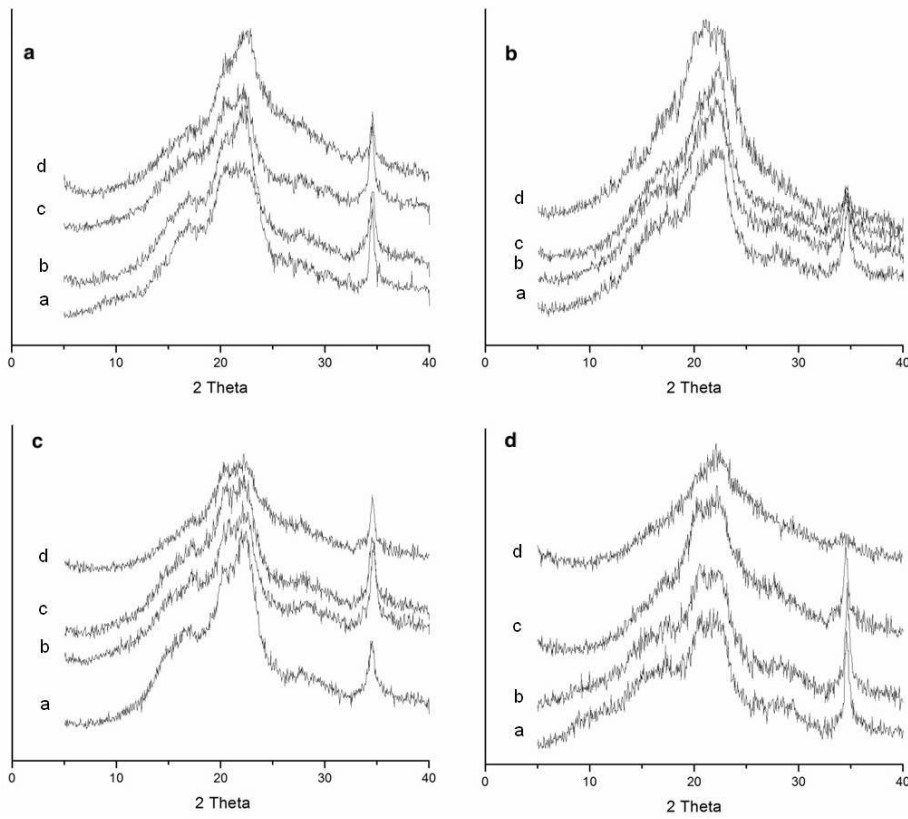
Comparing the types of ILs used (Figure 5) and the reduction of the peaks between 1420-1540  $\text{cm}^{-1}$  related to lignin, we can note that the [bmim][Ac] (Figure 5a, c) could remove lignin, whereas [bmim][Cl] (Figure 5b, d), can only partially remove this component. These results are associated with the depolymerization of lignin during treatment, consequently increasing the soluble lignin in the ionic liquid (Yoon et al. 2012, 2017).

The same analysis was performed for rice hull, results shown in Figure 6. For the conditions used in this study, there were no significant differences between the spectra of treated samples, suggesting that the tested ILs had no significant effect on the crystallinity of the rice hull cellulose, likely because of the high silica content of this material.

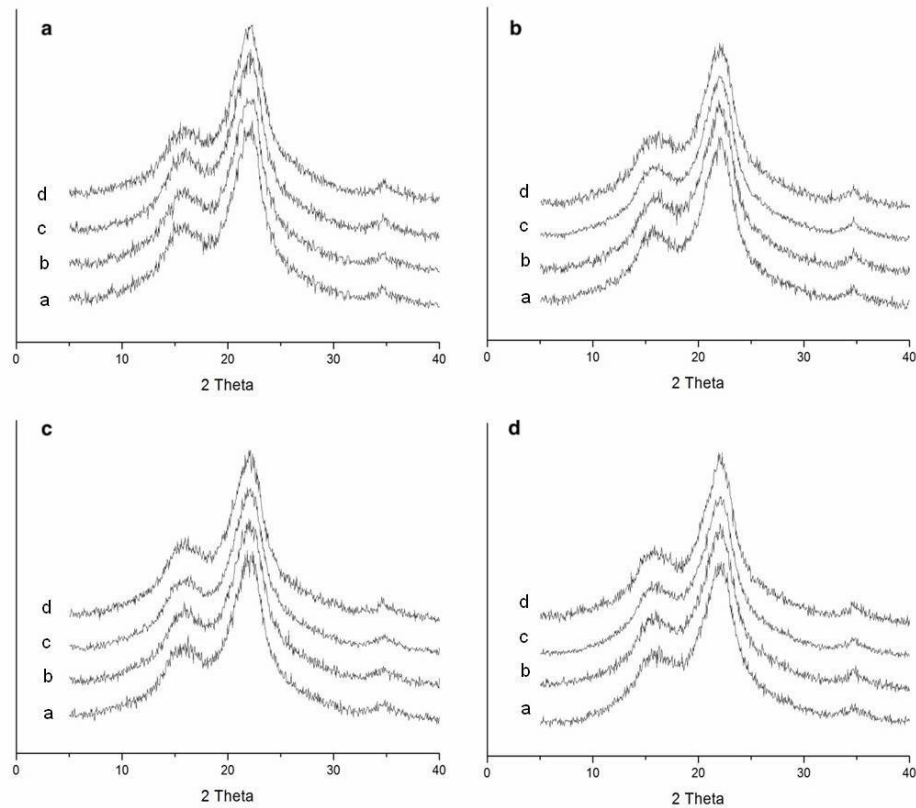
### X-RAY powder diffraction (XRD) analysis

The patterns of XRD of samples treated with ILs are compared to the untreated samples (controls) in Figures 7 and 8.

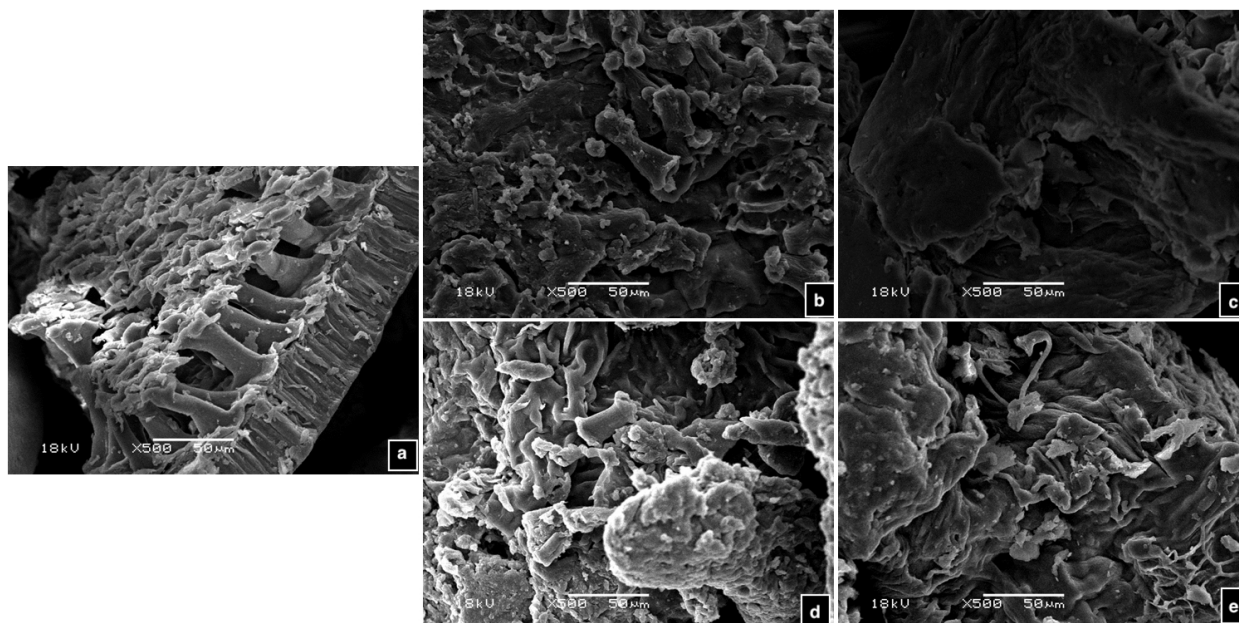
Analyzing the results of Figure 7, we can visualize the diffraction peaks in about 15° and 22° for all controls, which are typical of cellulose type I (Mansikkamäki et al. 2005). For the soybean hull, the first peak at 16.6° corresponds to the (110) planes, whereas the second peak, at 22.5°, corresponds to the (002) plane of cellulose type I. The peak around 16.6° was reduced after the



**Figure 7.** X-ray diffraction analysis of soybean hull treated with [bmim][Ac] (a and c), and [bmim][Cl] (b and d), for 2 h (a and b), and 6 h (c and d). Legend: a) untreated biomass, b) IL 50 % and 75 °C, c) IL 100 % and 75 °C, and d) IL 100 % and 100 °C.



**Figure 8.** X-ray diffraction analysis of rice hull treated with [bmim][Ac] (a and c), and [bmim][Cl] (b and d), for 2 h (a and b), and 6 h (c and d). Legend: a) untreated biomass, b) IL 50 % and 75 °C, c) IL 100 % and 75 °C, and d) IL 100 % and 100 °C



**Figure 9.** SEM comparison of soybean hull samples before and after treatment using IL (500 times magnification). (a) Untreated soybean hull outer surface; (b) treatment at 100 °C, 2 h, using [bmim][Ac]; (c) treatment at 100 °C, 6 h, using [bmim][Ac]; (d) treatment at 100 °C, 2 h, using [bmim][Cl], (e) treatment at 100 °C, 6 h, using [bmim][Cl].

treatment using [bmim][Ac] (Figure 7a, c) and was completely absent for samples after treatments with [bmim][Cl] (Figure 7b, d), whereas peak (002) became wider and less intense. These results suggest that treatment with IL can reduce the cellulose crystallinity in soybean hull. It was observed that the reduction of peak 16.6° increased proportionally with temperature and with the amount of IL used. However, increasing time of treatment did not show any significant modifications.

It has been suggested that the anion and the cation of ILs are responsible for the cellulose dissolution (Dadi et al. 2007). The free hydroxyl group of the cellulose is attacked and deprotonated by the anion of IL, while the cation interacts with the hydroxyl oxygen atoms. In this way, the hydrogen bonds in the cellulose matrix are disrupted and replaced by hydrogen bonding between the anion of ILs and cellulose hydroxyl, thus destroying the crystallinity of cellulose, which is caused by dissolution.

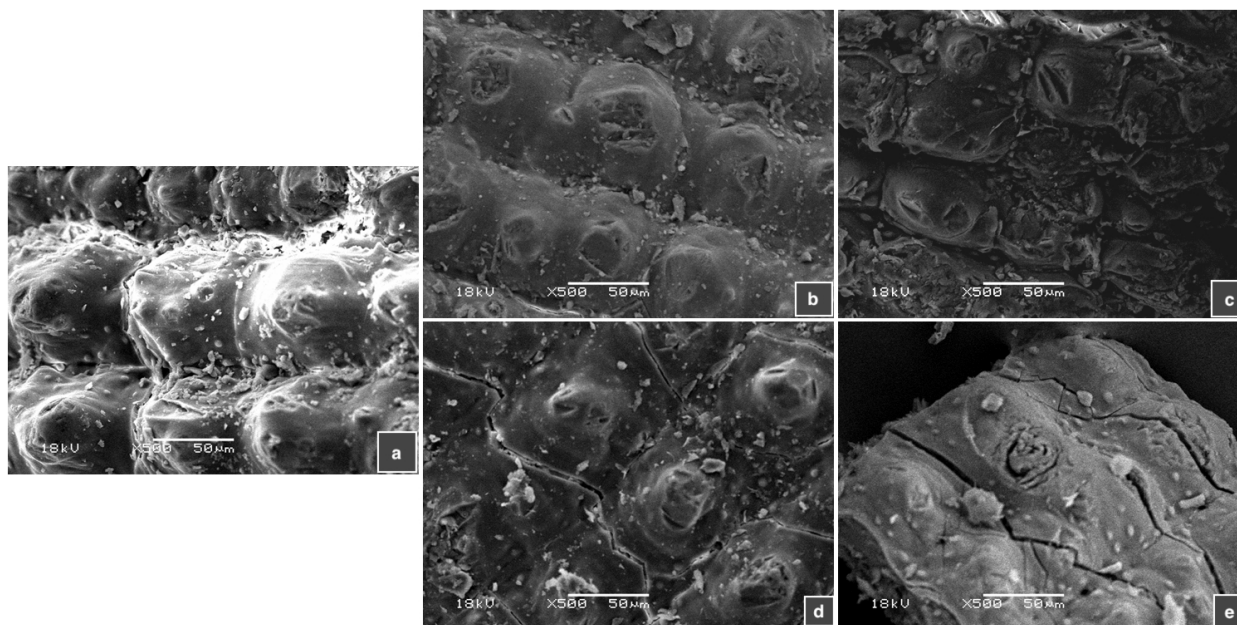
The X-ray diffraction analysis of rice hulls, shown in Figure 8, produced no differences between treated and untreated samples. Thus, the results of XRD analysis corroborate results obtained by FTIR and TGA: the modifications in the patterns for rice hull was not as pronounced as for soybean hull (reduction of peak around 16°), indicating that there was no expressive change in the crystallinity of the cellulose of this biomass.

### Scanning Electron Microscopy (SEM)

We carried out the SEM analysis to detect possible changes in the surface of the biomass, which could be related to the different treatments using the ILs. The SEM images of the soybean hull and rice hull samples are represented in Figures 9 and 10, respectively.

Swanson et al. (1985), reported that the soybean hull consists of cuticle, epidermis (palisade cells), hypodermis (hourglass cells), and parenchymal cells. The cells of the palisade





**Figure 10.** SEM comparison of rice hull samples before and after treatment with IL (600 times magnification). (a) Untreated rice hull outer surface, (b) treatment at 100 °C, 2 h, using [bmim][Ac]; (c) treatment at 100 °C, 6 h using [bmim][Ac]; (d) treatment at 100 °C, 2 h, using [bmim][Cl]; (e) treatment at 100 °C, 6 h, using [bmim][Cl].

layer are presented with a stretched position perpendicular to the surface of the hull. The hypodermis is made up of a layer of uniform cells, appearing continuously and showing a non-uniform cell thickness, with the presence of large intercellular spaces. This structure can be seen in the pictures of the untreated samples of soybean hull (Figure 9a). In contrast, the regenerated samples of soybean hull treated with [bmim][Ac] and [bmim][Cl] were both clearly modified, showing physical disorganization, which was proportional to the time of treatment (Figure 9b-e). The observed changes were both in the morphology of samples, as well as in their crystallinity, which decreased as shown in the X-ray diffraction analysis. The SEM images suggest that the [bmim][Cl] produced a more porous surface of soybean hulls. It has been suggested that these alterations are caused by the breakdown of biomass natural structures during the dissolution of cellulose in presence of IL, causing a reduction in crystallinity (Tan et al. 2011). Other studies have also reported that the

structure of the biomass became more porous and loses crystallinity after IL treatment (Yoon et al. 2012). Ang et al. (2011), for instance, suggested that the breaking of the biomass surface might be caused by the solvating action of ILs, in which the lignocellulosic matrix is swelled and dissolved.

In Figure 10 are shown the SEM images of rice hulls samples treated with [bmim][Ac] and [bmim][Cl], and the corresponding untreated control. The protuberances and silica are clearly visible in the SEM images of untreated biomass (Figure 10a). Although the results of other analysis (TGA, FTIR, and XRD) did not show any significant changes in the structure of treated rice hull compared to the untreated biomass, the SEM analysis showed that rice hull treated with [bmim][Ac] had a certain, albeit small, degree of disruption when compared against the material treated using [bmim][Cl] in 6 h (Figures 10d, e, respectively). Rice hull is a biomass with a complex composition, having silica and organic material that are combined, resulting in a fibrous and very recalcitrant material. The silica

is concentrated on protuberances and trichomes of the epidermis (Liou 2004). This composition and structure are possible the cause that hinders cellulose breakdown of rice hull when using ILs, at least under the conditions used in our work.

## CONCLUSION

The results obtained in this study suggest that ILs can be used in the treatment of lignocellulosic biomass, such as soybean hulls, but perhaps not for rice hulls because of its high content of silica. The use of ILs is an alternative technology, leaving the surface of the lignocellulosic materials exposed to subsequently carry out the enzymatic hydrolysis in order to obtain fermentable sugars. The types of IL used will also produce different results in respect of the disruption of biomass structures. Soybean hulls were easily decrystallized by both ILs used, in contrast with the very recalcitrant rice hulls, which was modified more intensely by [bmim][Ac] than by [bmim][Cl]. Our results point to the fact that the dissolution of cellulose matrix will be influenced by the type of IL used, the ratio of liquid and solid in the reaction, the temperature, and the time of treatment.

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Fernanda Cunha-Pereira and Carla Matte carried out the conceptualization, methodology, and writing original draft; Tânia Maria Haas developed some methods; Jairton Dupont was responsible for resources and funding acquisition; Marco Antônio Záchia Ayub was involved in the general resources allocation, conceptualization, methodology, writing, funding acquisition, project administration, and supervision.

