



# The use of ionic liquid pretreatment aims to enhance the enzymatic hydrolysis of green coconut fiber and produce lignin

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Received: 25 July 2023 / Revised: 1 November 2023 / Accepted: 8 November 2023  
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## Abstract

Converting green coconut waste into value-added products can be a way to increase the profit margin of coconut exploration companies. Due to its high lignin content in green coconut fiber (GCF), alkaline pretreatments have been prioritized so that investigations with ionic liquids, potential delignifying agents, are still scarce. In this sense, the present study evaluated pretreatments of GCF with choline chloride ([Ch][Cl]) to obtain pretreated biomass enriched in cellulose and high lignin content in the liquid phase. Adding sulfuric acid boosted lignin solubility in [Ch][Cl]-rich liquid phase. The increase in [Ch][Cl] concentration and the increase in incubation time favored the rise of crystallinity and cellulose content in the pretreated GCF. The condition with 75% (w/v) [Ch][Cl], 1% (w/w) sulfuric acid, and a time of 50 min promoted high GCF disorganization, leading to the best enzymatic digestibility result (22.61 g/L), and 71.21% of GCF lignin was accumulated in the liquid phase from pretreatment. The isolated lignin successfully treated oil-contaminated water, achieving 99% oil removal using 0.2 g/L of lignin. Pretreatment with [Ch][Cl] effectively fractionates GCF and appears to be an attractive 2G ethanol technology option for other lignin-rich biomasses.

**Keywords** Lignocellulosic material · Choline chloride · Saccharification · Sulfuric acid · Oil removal

## Highlights

- An ionic liquid pretreatment by choline chloride in a diluted sulfuric acid medium to depolymerize green coconut fiber.
- Hydrolysis enzymatic over the pretreated biomasses reaching a maximum glucose concentration of 22.61 g/L.
- Increase of 67% in the digestibility of cellulose in free sugars when compared the untreated fiber to the pretreated GCF with [Ch][Cl].
- Oil removal of 98.98% (w/w) from contaminated water treated with lignin particles from pretreated green coconut fiber.

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## 1 Introduction

The advance in the study of the use of lignocellulosic residues aims at not dependence on non-renewable energy resources. The great advantage of these lignocellulosic residues is the renewable energy sources, the eco-friendly environment, and the abundance availability. Coconut residues are lignocellulosic materials produced worldwide. In this perspective, Brazil occupies the fifth position globally for coconut production, and the northeast region holds more than 73.0% of national production. Brazilian coconut production reaches about 2.4 million tons, corresponding to 80.0% of the total production in South America. Some countries, such as India, Indonesia, the Philippines, and Brazil, have active coconut industries to supply coconut products [1–3].

The coconut tree is part of the class Monocotyledonea, belonging to the family Palmae, which originates from *Cocos nucifera* L. The exploitation of green coconuts is attractive for the coconut water industry and for generating other food products, including coconut oil and coconut milk. However, high volumes of waste are generated since

the green coconut shell represents 80% of the total mass of the fruit. Coastal regions are particularly affected by inadequate disposal of coconut waste. According to Cabral et al. [4], coconut waste affects the landscape of coastal regions and imposes more stress on the collection and treatment of urban solid waste. Therefore, adding value to green coconut waste is critical to mitigating these problems.

Green coconut fiber (GCF) results from the comminution of coconut shells and is mainly formed by cellulose, hemicellulose, and lignin, characterizing it as lignocellulosic biomass. Recent studies, such as Nogueira et al. [5] and Ribeiro et al. [6, 7], have pointed out the potential of GCF as a source of sugars and, consequently, of 2G ethanol. Due to its recognized lignin content (25–35%), GCF must undergo pretreatments to improve its enzymatic digestibility and fermentability [8, 9]. Alkali reagents (i.e., sodium hydroxide and sodium carbonate) promote the disruption of ether bonds within the lignin structure, facilitating biopolymer removal from the pretreated biomass [10]. Although these alkali pretreatments are successful in enzymatic digestibility, they have the disadvantages of generating undesirable secondary products and difficult-to-treat liquid phase [11].

Over the last decades, ionic liquids (ILs) have been arousing the academic community's interest in producing biofuels from lignocellulosic materials available for saccharification. ILs are organic molten salts with modulable properties capable of dissolving lignocellulosic matrix compounds. Changes in cations or anions affect not only the performance of pretreatments but also their objectives, be they delignification, hemicellulose removal, or cellulose amorphization/swelling [12, 13]. Considering coconut waste as a substrate, few studies have investigated pretreatments with ionic liquids for 2G ethanol technology. Ribeiro et al. [14] investigated the effects of incubation time and temperature on the digestion of coconut coir pretreated with *n*-butylammonium acetate. After optimization, the authors recorded glucose production of 27.06% and cellulose conversion of 33%. In 2022, Anuchi et al. reported successful pretreatments with *N,N,N*-dimethylbutylammonium hydrogen sulfate using coconut husk and shells as substrates. The pretreatment, also called *ionoSolv*, was tested at high temperatures (150–170 °C) and a wide time range (15–90 min) and promoted strong delignification for both substrates (~80% removal). Under optimal conditions, cellulose conversions were 82% and 89% for coconut husk and shell samples, respectively. Deep eutectic solvents based on ILs have also proven successful in pretreatments of coconut waste, as in the papers by Mankar et al. [15] and Muhammad et al. [16]. Without a doubt, this information is valuable for the progress of GCF technology. However, there are still no reports on the performance of choline chloride ([Ch][Cl]), one of the classic ionic liquids, as the principal pretreatment

agent. The absence of this data gives the impression that steps were missed. The merit of more unusual ILs in other biomasses does not imply that they would be promising agents for pretreatments of GCF. Furthermore, [Ch][Cl] is easily accessible for commercialization, and its use would not require greater synthesis efforts on the lab bench.

ILs generally outperform other pretreatments in some responses or possibilities (delignification, reduction of enzymatic hydrolysis time, and pretreatment-enzymatic hydrolysis integration, among others). However, their high cost is still a problem that prevents its commercialization. So, answers are required about the co-products generated and how the market can exploit them [17–19]. Lignin has proven to be a multifaceted material for multiple purposes, from cement paste modifiers [20, 21] to bioactive encapsulating agents [30]. Notably, the ability of lignin to modify liquid–liquid systems has aroused interest in their application in the treatment of contaminated water [22, 23]. Consequently, it is possible to apply the lignin to a downstream process, for example, the sedimentation of emulsified oil from an aqueous solution [24].

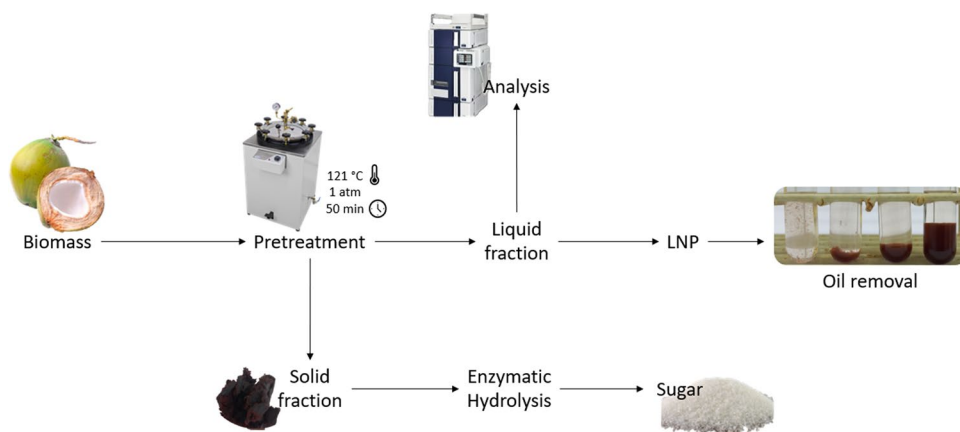
In this context, the present study proposed to value GCF using pretreatment with [Ch][Cl]. The effects of [Ch][Cl] concentration, temperature, and addition of mineral acid (sulfuric acid) were investigated on delignification and cellulose retention in the solid fraction. Pretreated GCF samples were characterized by chemical structure, crystallinity, and morphology. The success of pretreatment with [Ch][Cl] on the digestibility of GCF was evaluated in an enzymatic hydrolysis experiment. Additionally, lignin isolated from the liquid fraction was used as a destabilizer to remove oil in contaminated water.

## 2 Material and methods

The study followed the steps shown in Fig. 1 to obtain sugars and lignin from the pretreatment of GCF in an acidified [Ch][Cl] medium.

### 2.1 Chemical reagent

[Ch][Cl] was purchased from Êxodo Científica (SP, Brazil). Citric acid, sodium citrate, sulfuric acid, ethanol, yeast extract, glucose, sodium hydroxide, and ethylene glycol were acquired from Synth (Diadema, SP, Brazil). 3,5-Dinitrosalicylic (DNS) acid was purchased from Sigma-Aldrich (MO, USA). Mineral oil 20W-50 was acquired from Petronas Lubrificantes Brasil S.A. (MG, Brazil). Deionized water was obtained by the Milli-Q system. All reagents were analytical grade.

**Fig. 1** Schematic of the general process

## 2.2 Raw material

Green coconut (Fig. 1) was kindly donated by the Company Aqua Coco (Maxaranguape, Rio Grande do Norte, Brazil). The GCF was obtained from the epicarp and mesocarp of the green coconut. Fibers were washed, dried at 60 °C for 72 h, milled, and sieved to 20 mesh (0.85 mm) (Hammer Mill Willye, TE-680, Tecnal-Brazil). GCF was stored in plastic containers at room temperature.

GCF alkaline lignin was obtained from pretreatment with sodium hydroxide in the study by Padilha et al. [22]. GCF (20.0 g) was added in an 250 mL-Erlenmeyer containing 2.0% (w/v) sodium hydroxide solution. Then, the vial was incubated in an autoclave at 121 °C. After incubation (30 min), the liquid fraction and the first washing water of the pretreated material were mixed and vacuum-filtered. The lignin-rich liquid had the pH adjusted to 2.0 with 5.0 M sulfuric acid, centrifuged at 1500 × g, and washed with acidified water (pH 2.0; sulfuric acid). Lignin was dried in an air circulation oven (80 °C) for 24 h, a mortar and a pestle were used to grind the lignin. So, GCF from sodium lignin showed approximately 90% (w/w) Klason lignin and 0.8% (w/w) soluble acid lignin [31].

## 2.3 Solubilization of lignin with [Ch][Cl]

Solubilization tests were performed to investigate the amount of [Ch][Cl] required for delignification. The lignin for this experiment was obtained as described in Sect. 2.2. Thus, 50.0 mg of lignin were mixed under different concentrations of [Ch][Cl] (0%, 10%, 25%, 50%, 75%, 80%, and 90%, w/w) using acidified distilled water (1.0% sulfuric acid, w/w) as a solvent. The mixtures were incubated in an orbital shaker at 150 rpm and 25 °C for 10 h. The mixtures were centrifuged at 1500 × g for 10 min, and then, the supernatant was taken for further analysis. The response was obtained by spectrophotometer (Thermo Spectronic, Genesys 10 UV,

USA) using ethanol as a solvent (wavelength = 450 nm). This step was performed twice, and each concentration was analyzed in triplicate.

## 2.4 Pretreatments of GCF with [Ch][Cl]

Pretreatments were carried out according to Chen et al. [25], and a summary of the operating conditions is shown in Table 1. Each pretreatment factor was evaluated at two values: 50% and 75% (w/w) of [Ch][Cl], 25 and 50 min of incubation time, and 0% and 1% (w/w) of sulfuric acid. Hydrothermal pretreatments (without [Ch][Cl]) at 25 and 50 min were used as reference. Pretreatments were carried out (in duplicate) with 5% (w/w) solids in 250 mL-Erlenmeyer flasks, which were heated in an autoclave (121 °C, 1 atm). After incubation, the Erlenmeyer flasks were left on the bench to cool, and a cloth filtration was used to collect the solid and liquid fractions. The solid fractions were dried in an air circulation oven at 60 °C for 24 h and then taken for characterization and enzymatic hydrolysis. The amount of lignin dissolved in each liquid fraction was also determined by spectrophotometry.

### 2.4.1 Combined severity factor (CSF)

First, the severity factor (SF) is measured to analyze the intensity of any thermal processes (see Eq. (1)). SF evaluates time and temperature factors in a single parameter  $\text{Log } R_0$ . For this study, the hydrothermal process was performed for all the experiments by the autoclave (121 °C, 1 atm), in other words, constant temperature. Meantime, the residence time has changed for some experiments (see Sect. 2.4).

Therefore,  $\text{Log } R_0$  is a function dependent on temperature and residence time as described by Eq. (1) [26]:

$$\log R_0 = \int_0^t \exp\left(\frac{T-100}{14.75}\right) dt \quad (1)$$

**Table 1** Pretreatments with [Ch][Cl] under different operational conditions at autoclave (121 °C, 1 atm)

Run*	[Ch][Cl] (% w/w)		Time (min)		Sulfuric acid (% w/w)	
	50	75	25	50	0	1
W25m			•			
W50m				•		
C50/25 m	•		•		•	
C50/50 m	•			•	•	
C50/25/H	•		•			•
C50/50 m/H	•			•		•
C75/25 m		•	•		•	
C75/50 m		•		•	•	
C75/25 m/H		•	•			•
C75/50 m/H		•		•		•

\*Experiments were executed randomly

To measure the force of the acidified aqueous system (see Sect. 2.4), the CSF was used as a parameter (see Eq. (2)). CSF brings the contribution of hydrogen ions on the pretreatment processes. This parameter was developed by Abatzoglou et al. [27] and Chum et al. [28].

$$\log \text{CSF} = \log R_0 - \text{pH} \quad (2)$$

where, for both equations, Eq. (1) and Eq. (2),  $T$  is the pretreatment temperature (°C),  $t$  is the residence time, 100 is the temperature reference in Celsius, and 14.75 is an empirical constant.

## 2.5 Physicochemical characterization of GCF before and after pretreatment

The untreated and pretreated fibers were analyzed in terms of cellulose, hemicellulose, and lignin according to Sluiter et al. [29], solid recovery (SR), reducing sugars [29], ash [30], and moisture according to Rocha et al. [31]. Each analysis was conducted in triplicate.

## 2.6 Structural analysis of untreated and pretreated GCF

The structural analysis by Fourier-transform infrared (FTIR) of untreated and pretreated GCF has enabled the identification of functional groups present in the main constituents of biomass, such as cellulose, hemicellulose, and lignin. Also, the analysis by scanning electron microscopy (SEM) makes it possible to verify the conformation of the untreated fiber and the changes or breakages in the

pretreated biomass fiber. X-ray diffraction (X-RD) protocol measures the crystallinity and amorphous part of the cellulose.

### 2.6.1 FTIR

The FTIR analysis was conducted at the Universidade Federal do Ceará (Fortaleza, CE, Brazil). The Cary 630 (Agilent, USA) was used to analyze pretreated and untreated GCF. The samples were measured in transmittance mode between 650 and 4000  $\text{cm}^{-1}$ .

### 2.6.2 SEM

The analyses of SEM (HITACHI model TM 3000, To, Japan) of pretreated and untreated GCF were performed at the Universidade Federal do Rio Grande do Norte (Natal, RN, Brazil). It allowed to visually observe the modification of the structure. An amplification of tenfold-hundred times electronic micro-images was obtained, and the resolution of images was 640 × 480 pixels.

### 2.6.3 X-RD analysis

The X-RD analyses were conducted at the Universidade Federal do Rio Grande do Norte (Natal, RN, Brazil). Cellulose crystallinity of pretreated GCF was analyzed in an X-ray diffractometer (model Miniflex II desktop, Rigaku Corporation, Japan). The operating voltage and current were 30 kV and 15 mA, respectively. The wavelength is around 260 nm based on voltage. The crystallinity index (CrI) was defined by using Eq. (2).

$$CrI = \frac{I_{22.6^\circ} - I_{18.7^\circ}}{I_{22.6^\circ}} \cdot 100 \quad (3)$$

where  $I_{22.6^\circ}$  is the maximum intensity (2 theta) of the (22.6°) lattice diffraction and  $I_{18.7^\circ}$  is the intensity of the amorphous diffraction (2 theta).

## 2.7 Enzymatic hydrolysis

The pretreated GCF was used in the enzymatic hydrolysis as substrate. The operational condition was done in 50 mL-Erlenmeyer flasks with 5 mL of usable volume and 5.0% (w/v) pretreated GCF at 50 °C using Cellic CTec2® (Novozymes, 113 FPU/mL initial activity). The enzymatic loading was 20.0 FPU [32] per gram of pretreated GCF (all samples were performed in duplicate). The mixture was maintained in sodium citrate buffer (50 mM, pH 4.8) with 0.01% (w/v) sodium azide to prevent microbial growth. The flasks were kept at 150 rpm for 48 h. The samples were collected at the final time of 48 h due to outcomes from Gonçalves et al. [33], which reached the maximum glucose concentration from 48 h. Sugar concentrations were determined by the DNS method (see Sect. 2.4.1). Equation (4) was employed to calculate the hydrolysis yield. All assays were performed in triplicate.

$$\text{Hydrolysis yield}(\%) = \frac{C_g \times V_R}{m_{pGCF}} \quad (4)$$

In Eq. (4),  $C_g$  means free sugar concentration;  $V_R$  is the reaction volume;  $m_{pGCF}$  is the pretreated green coconut fiber (GCF) mass.

## 2.8 Recovery of lignin and lignin characterization

After choosing the best pretreatment, the liquid fraction of the respective pretreatment was used to obtain lignin. The first liquid fraction (R1-1) was collected from this pretreatment, and the volume was measured. The liquid fraction was added to water in a 1:10 (v/v) ratio, and the mixture stood for 3 h. Then, the supernatant was removed. Afterward, the decanted material was washed with water, and this step was repeated three times. Lignin was recovered from the aqueous solution by centrifugation at 1500 × g. Finally, the decanted lignin was transferred to Petri dishes and dried in an oven with air circulation (Tecnal TE-394/2-MP, SP/Brazil) at 60 °C temperature for 24 h. The recovery of lignin was carried out in duplicate.

The recovered lignin dissolved 4.0 g of lignin in 350 mL of ethylene glycol. This ethylene glycol/lignin (EG/L) mixture was dropped into the water at a 1:10 (v/v) ratio while vigorously stirring to obtain lignin nanoparticles. After 30 min of mixing, the supernatant was removed by

centrifugation (1500 × g, 3 min), the precipitate was resuspended with deionized water, and sodium azide was added at 0.01% (v/v).

The chemical characterization of the lignin obtained was verified according to the protocol provided by the NREL [28]. Lignin characteristics were also evaluated from the FTIR procedures and thermogravimetric analyses (TGA, TA Instruments, US).

### 2.8.1 Oil removal from contaminated water

Oil/water (O/W) emulsions (4:96, v/v) were prepared under three different pHs (3; 5.5; 8) into a mechanical shaker (Tecnal TE 139, SP, Brazil) for 5 min at 2650 rpm (see Fig. 2). After, the emulsions were kept in the separation funnel for almost 18 h. Different concentrations of lignin (0.5, 1.0, 2.0, and 4.0 mg/mL) were added to the aqueous phase obtained from the O/W emulsion in 1:1 (v/v) ratio followed by vortex mixing (30 s). After 2 h, the results were assessed by imaging.

Thus, this procedure was performed again with the best pH condition but with more concentrations of lignin (0.05, 0.1, 0.2, 0.5, and 1.0 mg/mL) to verify the minimum concentration of lignin as the optimal concentration for removing oil in the aqueous phase of the W/O emulsion. For the best condition, the aqueous layer was removed. Then, the aqueous layer was mixed with hexane solvent in a 1:1 (v/v) ratio. Next, the samples were centrifuged (4838 × g, 5 min, Solab SL-703). Finally, the supernatant was extracted, and the measurements were carried out in the spectrophotometer (wavelength = 360 nm). The percentage of oil removal expressed the response of the process. All assays were performed in triplicate.

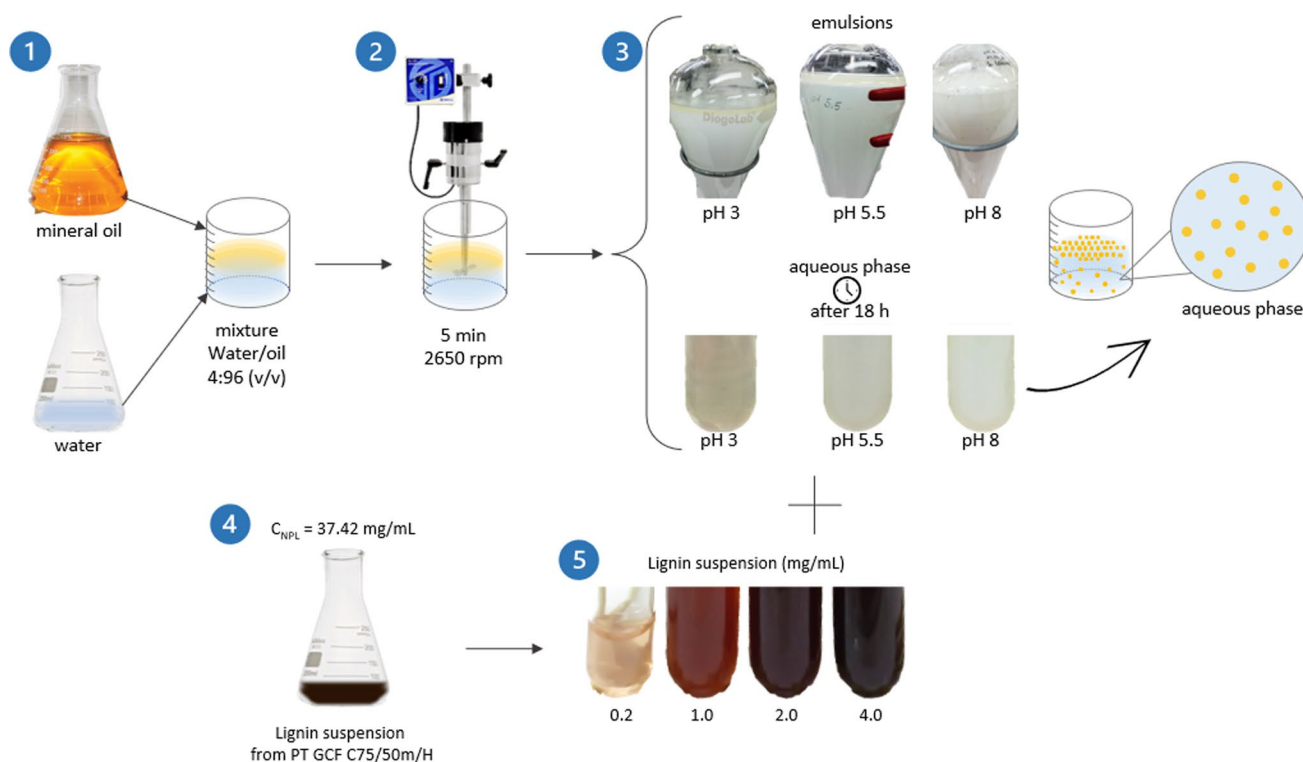
## 2.9 Statistical analysis

Each stage of this study was carried out at least in duplicate. All samples were performed in triplicate. Then, all responses were expressed as mean and standard deviation. Statistical calculations (Statistica 7.0 Inc., Tulsa, USA) were conducted using a one-way ANOVA with Tukey's post-test, considering the 95% confidence level ( $p < 0.05$ ) as a significant result.

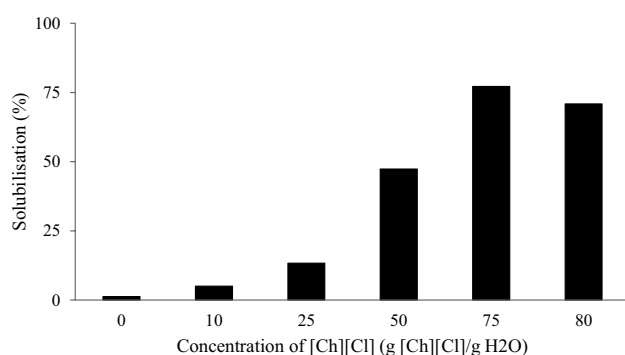
## 3 Results and discussion

### 3.1 GCF lignin solubility in [Ch][Cl] mixture

In the preparation of the mixtures, it was only possible to handle the maximum concentration of [Ch][Cl] in the aqueous phase until 80.0% (w/w) because when carried beyond that point, the mixture of [Ch][Cl]-water-sulfuric acid has



**Fig. 2** Schematic of the oil removal from contaminated water using lignin



**Fig. 3** Extraction of GCF lignin through an acidified mixture of [Ch][Cl] under different concentrations ( $t = 10 \text{ h}$ ;  $T = 25 \text{ }^\circ\text{C}$ )

presented crystalline shapes, not being a homogeneous phase solution. Although the sulfuric acid/[Ch][Cl] mixture is not considered a DES, high solubility of lignin was observed in this mixture, indicating a new solvent for delignification biomass. It was possible to extract 77.17% of lignin from the solution in the presence of [Ch][Cl] (Fig. 3). Further analysis should be taken to investigate the thermodynamic properties of that mixture [19, 34]. One proposition is the attraction between the aromatic rings from the lignin and the choline. Choline has a positive density on the nitrogen due to the molecule configuration, and lignin has a negative density

due to the diversity of monomer groups with aromatic rings (H, G, S) [34].

Even though the interaction between lignin and [Ch][Cl] is quite complex to explain, some studies on biomass delignification presented the DES solvent ([Ch][Cl]/lactic acid (LA); [Ch][Cl]/carboxylic acid; [Ch][Cl]/urea; [Ch][Cl]/glycerol) as a new strategy in lignocellulosic fractionation. Besides, there is little literature concerning the formation of DES with [Ch][Cl] and sulfuric acid. Thus, this study has supposed that [Ch][Cl] could be the hydrogen bond acceptor while sulfuric acid would be the hydrogen bond donor [19, 25, 35].

Furthermore, some hypotheses have been raised about the lignin-DES interaction: high solubility [36], rupture of intramolecular bonds via chloride ions [37], and disruption of connections from lignin-carbohydrate complexes [38]. Additionally, it is quite complex to define the interaction of [Ch][Cl] with lignin due to its aromaticity with several functional groups. Also, there are multiple mechanisms attributed to biomass delignification [39].

On one hand, Smink et al. [40] have studied the relationship of [Ch][Cl] solubility with lactic acid to solubilize lignin. They commented that only the solution with lactic acid provided higher lignin solubility. Also, Francisco et al. [36] have noted that lignin solubility increased with the decrease in the concentration of [Ch][Cl]. The present study observed the increment of lignin solubility with the increase

of [Ch][Cl] concentration. However, it should be considered that the lignin quantification methodology is quite different from Smink et al. [40] and Francisco et al. [36]. Herein, it kept the fixed lignin mass and changed the [Ch][Cl] concentration. In addition, [Ch][Cl]/sulfuric acid might be treated as a DES (1:75 or 1:50, acid sulfuric:[Ch][Cl], w/w).

### 3.2 Physicochemical characterization

The composition of untreated and pretreated biomass used (% dry weight) is presented in Table 2. As it is seen, the data showed the untreated GCF as a source of lignocellulosic content. As expected for GCF, lignin content has reached over 30.0%, which indicates a recalcitrant structure. Furthermore, cellulose content suggests potential biomass for converting cellulose into glucose. Therefore, the GCF has the potential to provide glucose and lignin due to suitable pretreatment. The data for GCF corroborates the composition values obtained by Nogueira et al. [41] and Gonçalves et al. [42] (see Table 2). Changes in chemical composition may occur due to changes in climate, soil, biomass maturation time, and residue processing [25, 42, 43].

Physicochemical characterization of the fiber after pretreatment was also carried out. As can be seen in Table 2, hemicellulose contents have diminished significantly after pretreatment with [Ch][Cl]. Hemicellulose is an amorphous polysaccharide susceptible to breakdown when exposed to ionic liquids and high temperatures [44]. In turn, cellulose

contents have increased for all pretreatments to balance the hemicellulose loss. Besides, acidic pretreated GCF has twice the cellulose content of untreated GCF. The lignin contents have also increased for most pretreatments except for C75/50 m and C75/50 m/H.

The operating conditions W25m and W50m did not present statistically significant changes in lignin and hemicellulose content compared to untreated biomass, GCF, indicating low severity due to autohydrolysis pretreatment (see Table 2). In the literature, for pretreatment to be considered a hydrolysis pretreatment, it is necessary to surpass the minimum limit of SF ( $> 150\text{ }^{\circ}\text{C}$ ,  $\text{SF} > 3.0$ ) to obtain a meaningful change in the biomass structure. Therefore, the severity imposed by the autoclave is insufficient to modify the lignocellulose composition of biomass because the temperature only reaches  $121\text{ }^{\circ}\text{C}$  (see Table 2) [25, 45, 46].

According to Rambo et al. [47] and based on the literature, the SF values for acid pretreatment stay from 1.4 to 2.8, as occurred in this study. However, log CSF is taken by reference because it adds the combined contribution of time, temperature, and acid concentration parameters. The same interpretation is taken to log CSF; the greater the number, the greater the severity of the pretreatment. The best conditions were perceived: C50/50 m/H and C75/50 m/H. This interaction between sulfuric acid and [Ch][Cl] may bring a new path for the biorefinery concept. Also, the acidified medium brought a greater disruption of bondage from lignin and hemicellulose.

**Table 2** Chemical composition (% dry weight) of untreated and pretreated biomass of GCF with [Ch][Cl]

Run*	Solid recovery (%)	Cellulose (%)	Hemicellulose (%)	Klason lignin (%)	Ash (%)	Moisture (%)	CrI (%)	pH	Log CSF
GCF**	100	30.68 ± 0.52 <sup>cd</sup>	18.45 ± 1.02 <sup>d</sup>	30.64 ± 1.05 <sup>ab</sup>	2.71 ± 0.15 <sup>f</sup>	9.83 ± 0.12 <sup>g</sup>	27.63	3.95	-
W25m	74.68	40.13 ± 1.24 <sup>a</sup>	19.52 ± 0.76 <sup>e</sup>	37.30 ± 1.61 <sup>abc</sup>	1.27 ± 0.06 <sup>5e</sup>	6.47 ± 0.24 <sup>ad</sup>	33.27	3.67	-1.65
W50m	70.21	40.52 ± 1.77 <sup>a</sup>	21.06 ± 0.82 <sup>f</sup>	36.07 ± 3.31 <sup>bc</sup>	0.80 ± 0.01 <sup>d</sup>	5.92 ± 0.55 <sup>ad</sup>	30.51	3.62	-1.30
C50/25 m	43.18	33.08 ± 0.64 <sup>c</sup>	1.71 ± 0.24 <sup>abc</sup>	34.95 ± 3.51 <sup>abc</sup>	0.74 ± 0.03 <sup>abc</sup>	6.27 ± 0.48 <sup>bce</sup>	30.91	2.39	-0.37
C50/50 m	63.07	41.86 ± 0.15 <sup>b</sup>	0.99 ± 0.01 <sup>abc</sup>	40.94 ± 2.12 <sup>c</sup>	0.69 ± 0.04 <sup>c</sup>	8.30 ± 0.55 <sup>f</sup>	30.68	3.97	-1.65
C50/25 m/H	53.68	54.07 ± 0.38 <sup>e</sup>	0.47 ± 0.00 <sup>4ab</sup>	40.28 ± 0.54 <sup>c</sup>	0.52 ± 0.08 <sup>a</sup>	5.13 ± 0.25 <sup>ab</sup>	38.07	2.90	-0.88
C50/50 m/H	46.36	59.57 ± 0.30 <sup>f</sup>	8.53 ± 0.09 <sup>ab</sup>	35.69 ± 3.52 <sup>abc</sup>	0.80 ± 0.03 <sup>bc</sup>	7.09 ± 0.01 <sup>e</sup>	44.37	2.50	-0.18
C75/25 m	80.12	41.22 ± 1.11 <sup>b</sup>	2.91 ± 0.01 <sup>c</sup>	34.91 ± 2.74 <sup>abc</sup>	0.71 ± 0.01 <sup>ab</sup>	5.32 ± 0.18 <sup>abc</sup>	32.91	3.63	-1.61
C75/50 m	66.16	39.94 ± 0.52 <sup>bd</sup>	2.17 ± 0.03 <sup>bc</sup>	28.65 ± 0.54 <sup>ab</sup>	0.60 ± 0.02 <sup>ab</sup>	3.91 ± 0.33 <sup>d</sup>	30.97	3.75	-1.43
C75/25 m/H	65.11	68.63 ± 3.08 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	32.30 ± 3.36 <sup>abc</sup>	0.76 ± 0.06 <sup>abc</sup>	6.36 ± 0.19 <sup>ce</sup>	42.16	2.96	-0.94
C75/50 m/H	40.10	62.91 ± 1.80 <sup>ae</sup>	0.43 ± 0.08 <sup>ab</sup>	26.32 ± 2.53 <sup>a</sup>	0.63 ± 0.04 <sup>ab</sup>	5.25 ± 0.15 <sup>abc</sup>	39.36	2.54	-0.22

\*Experiments were carried out randomly. \*\*Data provided by Ribeiro et al. [12]. <sup>a, b, c, d, e, f</sup>Values in the same row followed by the same letter are not significantly different ( $p < 0.05$ ),  $n = 3$ . W25m, pretreatment of GCF for 25 min without [Ch][Cl] and sulfuric acid; W50m, pretreatment of GCF for 50 min without [Ch][Cl] and sulfuric acid; C50/25 m, pretreatment with [Ch][Cl] (50.0%, w/w) in nonacidified aqueous solution for 25 min; C50/50 m, pretreatment with [Ch][Cl] (50.0%, w/w) in nonacidified aqueous solution for 50 min; C50/25 m/H, pretreatment with [Ch][Cl] (50.0%, w/w) in acidified aqueous solution (sulfuric acid, 1.0%, w/w) for 25 min; C50/50 m/H, pretreatment with [Ch][Cl] (50.0%, w/w) in acidified aqueous solution (sulfuric acid, 1.0%, w/w) for 50 min; C75/25 m, pretreatment with [Ch][Cl] (75.0%, w/w) in nonacidified aqueous solution for 25 min; C75/50 m, pretreatment with [Ch][Cl] (75.0%, w/w) in nonacidified aqueous solution for 50 min; C75/25 m/H, pretreatment with [Ch][Cl] (75.0%, w/w) in acidified aqueous solution (sulfuric acid, 1.0%, w/w) for 25 min; C75/50 m/H, pretreatment with [Ch][Cl] (75.0%, w/w) in acidified aqueous solution (sulfuric acid, 1.0%, w/w) for 50 min

The pretreatment with [Ch][Cl] was chosen due to the excellent lignin solubility (see Fig. 3). Thus, from the mild-est pretreatment (C50/25 m, solid recovery of 43.18%) to the most intense pretreatment (C75/50 m/H, solid recovery of 40.10%), clearly, pretreatments with [Ch][Cl] directly modified the structures of lignin, hemicellulose, and cellulose. However, two points surprisingly called the attention: the sudden loss of hemicellulose and the maintenance of lignin even in acidic conditions (see Table 2). Both facts could be explained by the formation of pseudo lignin due to the diluted acid medium. According to Rambo et al. [47], the action of the acid is more directed toward the opening of the biomatrix. In addition, the pseudo lignin might be formed through the acid-catalyzed condensation reactions where carbohydrates were released from the hemicellulose portion after pretreatment [48, 49]. All fibers have exhibited an acidic solid medium lower than pH 4.0.

Several studies have shown the increment of lignin content of pretreated biomass compared to untreated biomass, especially for diluted acid pretreatments [49–51]. Pseudo lignin behaves like Klason lignin and cannot be distinguished from natural lignin via traditional quantification. For more than 30 years, researchers have been observing this formation of pseudo lignin, but there is a lack of knowledge about the chemical route [52, 53]. There is a complexity to correlate the interactions due to the lignocellulosic matrix, the lignin, the heterogeneous environment, etc. Despite all explanations, the [Ch][Cl] pretreatment must favor the access of cellulase enzymes to the cellulose portion to deliver monomers of glucose, cellobiose, and oligomers.

Focusing on the solid recovery of the pretreated experiments, a few correlations could be pointed out as contributors to the low content of pretreated fiber—for instance, filtration step, degradation reactions, acid acting, and solid transport. However, the solid recovery for acid pretreatments tends to be less than other pretreatments. For this study, the minimum solid residue was 40.1% for C75/50 m/H, and the maximum solid residue was 80.1% for C75/25 m (see Table 2).

Based on the outcomes of chemical characterization, this study has chosen three experimental conditions to proceed with subsequent analysis: C50/50 m/H, C75/25 m/H, and C75/50 m/H. They presented better cellulose contents, higher crystallinity indexes, reasonable acidic pHs, and higher combined severity factors (log CSF).

### 3.3 Effect of pretreatment on the morphological and chemical properties of GCF

X-RD analysis will indicate the quality of the cellulose component through the ratio between the difference of intensity peaks at 22.6° and 18.7° divided by the peak intensity at 22.6° (Fig. 3, Figure S1, in the Supplementary

material, images of the diffractogram can be seen). The cellulose polymer consists of homogeneous monomers of D-glucopyranose, high molecular weight, containing crystalline, and amorphous portions. The amorphous part of cellulose is known to be more accessible to the digestibility of enzymes [54]. Thus, the decrement of CrI indicates the accessibility of cellulolytic enzymes to cellulose (see Table 2), because the crystalline portion of cellulose has an ordered structure that lays down a natural barrier to external agents and other biomass characteristics, for example, fiber porosity, intertwining, distribution and composition of lignin/hemicellulose, particle size, and polymerization degree [54, 55].

Taking untreated GCF as a point of reference, it could be noticed that all acidified pretreatments had increased the CrI values. So, it was possible to induce the removal of the amorphous portions of the cellulose and the lignin portions [8, 54]. Also, the non-acidified pretreatments had little increment of CrI values, whereas the untreated GCF. As could be noted, three experimental conditions had the highest CrI values: C50/50 m/H (solid recovery 46.36%); C75/25 m/H (solid recovery 65.11%); and C75/50 m/H (solid recovery 40.10%), showing that the combined effect between time and acid may modify structural conformation of fiber.

In addition, it was not feasible to note any change visually from the structure of pretreated GCF by SEM analysis (see Figures S1 and S2). Figure 4 shows the best outcome of SEM, where it contains the untreated and pretreated GCF at 75% (w/w) of [Ch][Cl] concentration over 50 min.

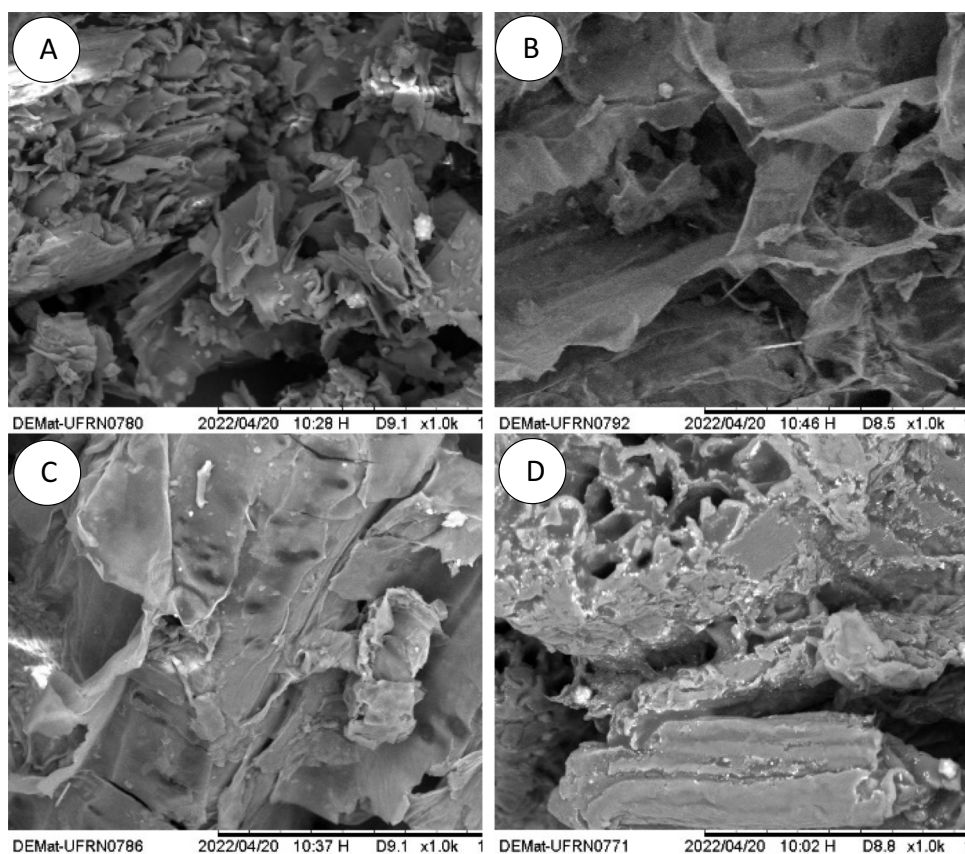
The absence of eruptions or breakage along the fibers for the untreated GCF was observed, even though they were crushed (see Fig. 4A). Compared to tests with water only (see Fig. 4B), no changes are noticed visually. Some modifications can be observed in the acidified condition for the longest time. The C75/50 m/H pretreatment (see Fig. 4D) showed wear along the fiber and eruptions along it. Also, irregularities on the surface indicated viable interaction between the lignin and the [Ch][Cl] solvent. White spots can be seen in Fig. 4D, which may be referred to as the chloride presence [56].

The SEM analysis for the C75/50 m/H condition has given some indications about more opened fibers that may favor the accessibility of cellulolytic enzymes to the cellulose. It was observed that only [Ch][Cl] use slightly modified the fiber structure morphologically without signals on the surface. On the other hand, superficial changes were observed along the fiber in the presence of the acidified aqueous solution. Rambo et al. [47] commented on this effective action of diluted acid in IL pretreatments.

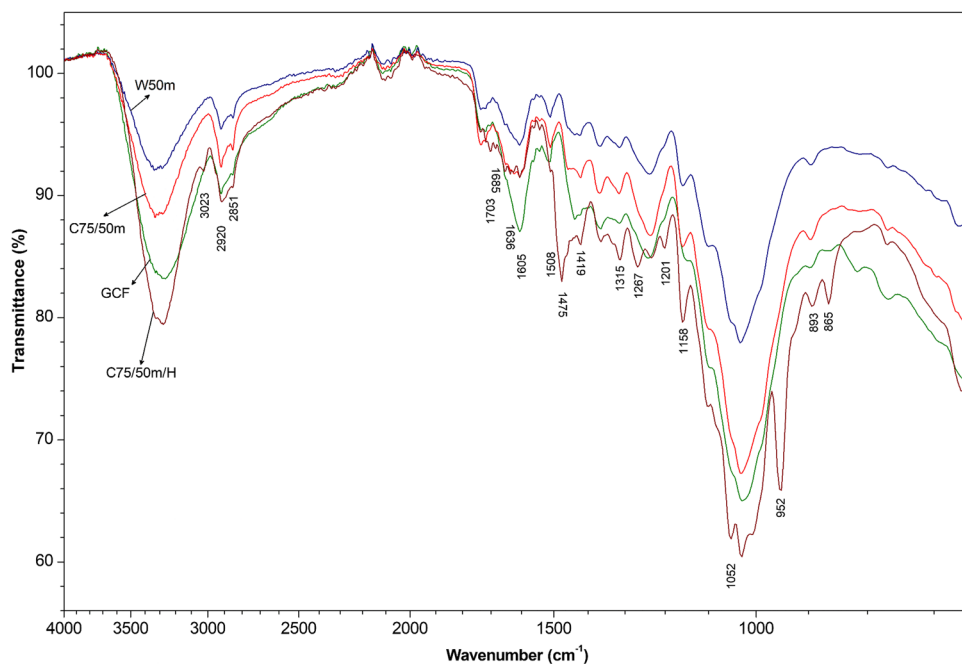
Then, the chemical changes from the untreated GCF to the pretreated GCF were analyzed by FTIR images (see Fig. 5). Hemicellulose can be characterized by the following patterns in the spectrum: 893, 1042, 1160, 1266, 1623,



**Fig. 4** Scanning electron microscopy (SEM) of GCF (A), W50m (B), C75/50 m (C), and C75/50 m/H (D). Images with amplification of tenfold-hundred. **A** GCF: green coconut fiber; **B** W50m: autohydrolysis of GCF for 50 min; **C** C75/50 m: autohydrolysis of GCF with [Ch][Cl] (75.0%, w/w) in nonacidified aqueous solution for 50 min; **D** C75/50 m/H: pretreatment with [Ch][Cl] (75.0%, w/w) in acidified aqueous solution (sulfuric acid, 1.0%, w/w) for 50 min



**Fig. 5** FTIR images of green coconut fiber (GCF), W50m, C75/50 m, and C75/50 m/H at autoclave (121 °C, 1 bar). GCF, green coconut fiber; W50m, autohydrolysis of GCF for 50 min; C75/50 m, autohydrolysis of GCF with [Ch][Cl] (75.0%, w/w) in nonacidified aqueous solution for 50 min; C75/50 m/H, autohydrolysis of GCF with [Ch][Cl] (75.0%, w/w) in acidified aqueous solution (sulfuric acid, 1.0%, w/w) for 50 min



and  $2920\text{ cm}^{-1}$  [6, 57]. These peaks were found in the GCF, in the GCF with hydrothermal treatment (W50m), and in the GCF with [Ch][Cl] (C75/50 m and C75/50 m/H) where differences in the widening or narrowing of the bands were

observed. These results corroborated the presence of hemicellulose portion on the pretreated GCF.

The broad band between  $3500$  and  $3100\text{ cm}^{-1}$  stands out for lignin and cellulose, related to the stretching vibrations

O—H. For the region  $900\text{--}1150\text{ cm}^{-1}$ , the presented bands are the elongation of C—O—C (stretching of amorphous cellulose), C—O, and C=O (vibration of crystalline cellulose) which are presented. Finally, the C—H stretching bonds in the spectrum range  $2900\text{--}2910\text{ cm}^{-1}$ . With respect of lignin, there is no absorption in the region between  $2800\text{--}1800\text{ cm}^{-1}$  for all pretreated GCFs; the identified patterns of lignin were  $865$ ,  $953$ ,  $1158$ ,  $1260$ , and  $1510\text{ cm}^{-1}$  [25, 58, 59].

From the lack of data correlating [Ch][Cl] as a pretreatment, the regions used as reference were based on different studies involving the use of [Ch][Cl] [60, 61]. Assigning the following patterns to [Ch][Cl]:  $3222\text{ cm}^{-1}$  (stretching of O—H),  $3010\text{ cm}^{-1}$  (stretching of C—H),  $1482\text{ cm}^{-1}$  (strain of  $\text{CH}_3$  and  $\text{CH}_2$ ),  $1085\text{ cm}^{-1}$  (stretching of C—N), and  $865\text{ cm}^{-1}$  (symmetric stretching of C—N+). It has sought to identify whether [Ch][Cl] had been impregnated into the GCF after pretreatment based on the qualitative FTIR analysis. By the way, a change in intensity was observed at the  $865\text{ cm}^{-1}$  peak for the C75/50 m/H assay, indicating a supposed stretching of carbon and nitrogen. This result should be further analyzed to deepen the understanding. Well, two hypotheses were raised: first, the choline was grafted onto the fiber, and second, more washing steps would be necessary to remove all the choline attached superficially to the fiber.

Comparing the untreated GCF with the hydrothermal pretreatment (W50m), the absence of the  $815\text{ cm}^{-1}$  band was verified, which was not related to any lignocellulosic component. Regarding the hydrothermal pretreatment (W50m) with C75/50 m pretreatment, the peak formation for the spectrum region  $1645$  till  $1615\text{ cm}^{-1}$  was indicated as an identifier of absorbed water [57]. For hemicellulose, the  $1623\text{ cm}^{-1}$  peak has disappeared concerning the other spectra. Comparing the C75/50 m pretreatment with C75/50 m/H pretreatment, some peaks were observed:  $865\text{ cm}^{-1}$  might refer to [Ch][Cl] [51];  $1475\text{ cm}^{-1}$  is related to the C—H bond and the methyl groups of the substituent [62];  $1260\text{ cm}^{-1}$  points to the vibrations of the aromatic ring from guaiacyl units.

From the perspective of hemicellulose removal (see Table 2), changes on the spectrum were identified at wavelengths  $1728$ ,  $1248$ ,  $1158$ ,  $1052$ , and  $893\text{ cm}^{-1}$ ; these peaks represent hemicellulose patterns. For the C75/50 m/H pretreatment (see Fig. 5), the appearance of a peak at  $1158\text{ cm}^{-1}$  was observed, which implies the presence of sulfur. That shows a molecular interaction between the solvent ([Ch][Cl]/sulfuric acid) and the GCF. Except for the untreated GCF, this peak was present ( $1158\text{ cm}^{-1}$ ) for all other conditions shown in the image (see Fig. 5) [63].

To cellulose, some peaks were observed:  $900\text{ cm}^{-1}$  represents the C—O—C bond—the stretching of the amorphous part of cellulose;  $1103\text{ cm}^{-1}$  means the C—O bond—the vibration of the crystalline portion of cellulose;  $1035$  and

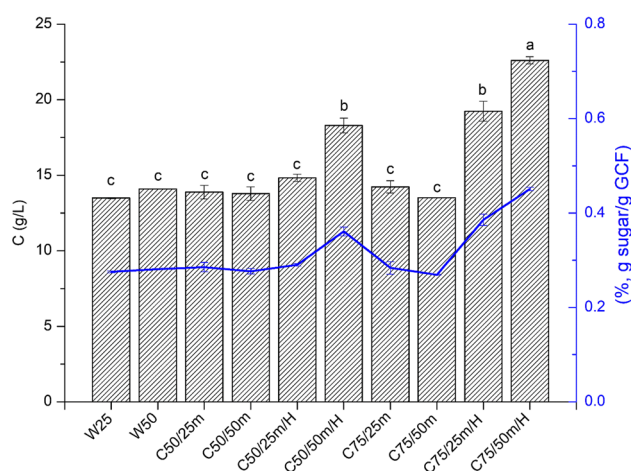
$1056\text{ cm}^{-1}$  signal C—O bond—the stretching of cellulose [25]. In summary, the [Ch][Cl] pretreatment has greatly interacted with the lignocellulosic components. The structural and morphological analyses demonstrated a less aggressive pretreatment, however, with a good removal of lignin on the solid fraction.

### 3.4 Enzymatic hydrolysis

Only the insertion of [Ch][Cl] was insufficient to open the fiber to enrich glucose concentration from enzymatic hydrolysis, as seen in Fig. 6. But for acidified conditions, the increase in free sugars has risen to the maximum of  $22.61 \pm 0.24\text{ g/L}$  for the pretreatment C75/50 m/H. This condition had a gain of 60% of sugar when compared to the pretreatment using only water (W50m), and it had an increase of 67% of sugar when compared to a similar non-acidified condition, C75/50 m. Regarding glucose concentration as a response, two more conditions stood out: C75/25 m/H and C50/50 m/H with  $19.24 \pm 0.65\text{ g/g}$  and  $18.29 \pm 0.49\text{ g/g}$  of glucose from pretreated GCF. Statistically, they represent the second-best result in obtaining glucose. Consequently, they had increased by 36% and 39% of glucose concentration, whereas the condition with only water (W25m and W50m).

When the influence of time was evaluated, there were no statistical differences among digestibility results. According to the SF, the mildest condition (25 min) indicated 2.02, and the most severe condition (50 min) indicated 2.32.

Regarding SF, only water usage is insufficient to cause solubilization of the hemicellulose and to modify the lignin structure (see Table 2). Regarding the concentration of [Ch][Cl] working together with the heating time, it did not give more sugar as expected due to the cellulose portion and CSF.



**Fig. 6** Free sugar concentration obtained from enzymatic hydrolysis of pretreated GCF. a, b, and c mean values followed by the same letter are not significantly different ( $p < 0.05$ ,  $n = 2$ )

Also, it has brought a good enrichment of the cellulose portion. However, the acidic medium had a better effect.

A straightforward explanation was the act of acid under the GCF-favored fiber breakage, and the contact time of the mixture over the GCF granted a good interaction among components [61, 64]. Consequently, this scenario showed the great potential of the pretreatment with [Ch][Cl] in an acidic medium to produce ethanol in a further process, because it was possible to have 45.0% (g sugar/g pretreated GCF) free sugar yield for the most severe pretreatment, C75/50 m/H.

A similar pretreatment condition was performed by Rambo et al. [47]. They optimized the pretreatment of coconut husks by several routes, including acid and IL pretreatments. They obtained less than 40.0% free sugar for IL pretreatment and more than 49.0% free sugar for acid pretreatment. They use 4% sulfuric acid to pretreat coconut husks and 1-ethyl-3-methylimidazolium chloride ([EMIM] Cl) as IL in the pretreatment of coconut husks. Once more, the pretreatment via acidic conditions could break down the GCF recalcitrance while the IL was not strong enough to disrupt the biomass.

Some studies have confirmed the deconstruction of biomass due to diluted acid. For example, Santana et al. [65] investigated acid pretreatments of coconut shells under different conditions. They achieved maximum sugar release performance of 27.2 g/L and hydrolysis yield of 41.9% (g sugar/g pretreated biomass). However, Nogueira et al. [66] found that hydrolysis yields equal to 43.0% (g total sugar/g pretreated fiber) were obtained in the diluted alkaline pretreatment of GCF using Tween 80.

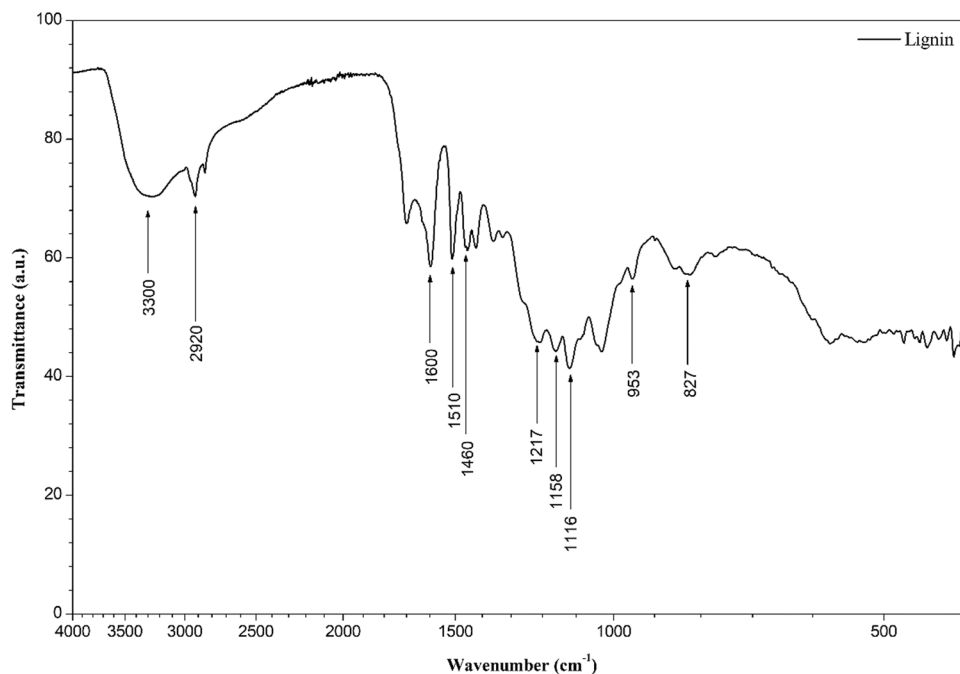
In conclusion, the contact time, the mild heating, and the acidic medium associated with the [Ch][Cl] demonstrated the good depolymerization of GCF to obtain sugars. Also, with the result of this study, it is possible to predict a good outcome of fermentation from the pretreated C75/50 m/H GCF. However, it is necessary to check the absence of inhibitory substances which might have been generated from the pretreatment.

### 3.5 Lignin

71.21 ± 6.26% of lignin from the R1-1 of the pretreatment was obtained. Then, this recovered lignin was characterized as 77.18 ± 1.58% (w/w) of Klason lignin and 0.88% of carbohydrate residues. This shows an excellent approach for lignin recovery due to its recalcitrant structure from the GCF. Because lignin was extracted from the biomatrix in only one cycle of pretreatment, its characterization showed good purity. In comparison, the work carried out by Padilha et al. [67] have recovered lignins from GCF organosolv pretreatment in one cycle and three cycles. The obtained Klason-lignin was 93.10% and 92.74% of the one-cycle and three-cycle, respectively.

The functional groups were evaluated using FTIR with transmittance (%) versus wavelength ( $\text{cm}^{-1}$ ) (see Fig. 7). The 3100 to 3500  $\text{cm}^{-1}$  region indicates the hydroxyl group (O—H) associated with vibrational stretching. The alkane group, which is related to the C—H stretch, is indicated in the region 3000 to 2900  $\text{cm}^{-1}$ , and it is also pointed out in the region 1460  $\text{cm}^{-1}$ , which can be linked to methyl and methylene [25, 56, 57, 65].

**Fig. 7** FTIR for lignin obtained from pretreated GCF



As previously mentioned, for identifying the functional groups present in lignin, there is no absorption in the region between 2800 and 1800  $\text{cm}^{-1}$  (see Fig. 7). The peak at 1600  $\text{cm}^{-1}$  assumes the presence of vibrational stretching of the C=C double bond in acetone, lactone, carboxyl, and aldehyde groups. There is an indication of the presence of C=O, which comes from the vibrational stretching of the aromatic structure in the region 1512  $\text{cm}^{-1}$ . Finally, the peaks indicate the presence of the syringyl group (1217  $\text{cm}^{-1}$ ) and the guaiacyl group (1116  $\text{cm}^{-1}$ ) [25, 58, 59, 68].

For lignin to be used in industrial processes, it is necessary to know the thermal profile of the material during the pyrolysis process (see Fig. 8) [47]. The thermal profile behavior grants excellent thermal stability to apply the GCF lignin into the fabrication of products based on lignin and use it as chemical transformation [9].

The first threshold represents the loss of moisture trapped in the LNP (0–120 °C). Then, the lignin burning profile was observed, which occurs from 120 to 800 °C, with the highest degradation rate between 200 and 400 °C. Lignin presents a complex structure due to the high degree of branching and the formation of highly condensed and aromatic structures [9, 69]. The ash resulting from lignin pyrolysis was 36.1%, and the maximum value of DTG was around 300 °C. According to Collard et al. [70], the rupture of the most known aliphatic bonds ( $\alpha$ -O-4,  $\beta$ -O-4, etc.) of monomers and dimers occurs after 120 °C. Padilha et al. [22] pointed out the range of rupture of bonds: from 240 to 280 °C, dehydration of aliphatic hydroxyl groups and C—C bonds of the

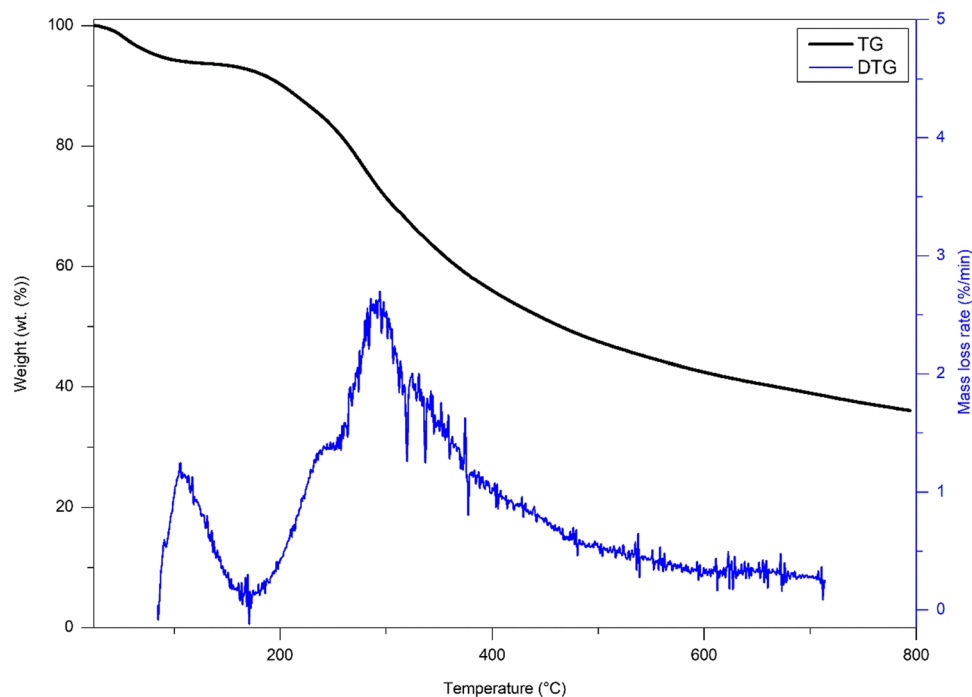
aliphatic chain; from 270 to 300 °C, rupture of alkyl-aryl and C—O—C bonds; from 300 to 385 °C, cleavage of C—C bonds. From 350 °C, the conversion of the minor substituents of the benzene rings begins. And finally, over 500 °C, the carbonization process takes place [70]. In this stage, the volatile compounds released by the rearrangement of the carbon chain have low molecular weight, being mostly non-condensable gases [22, 50, 69–71].

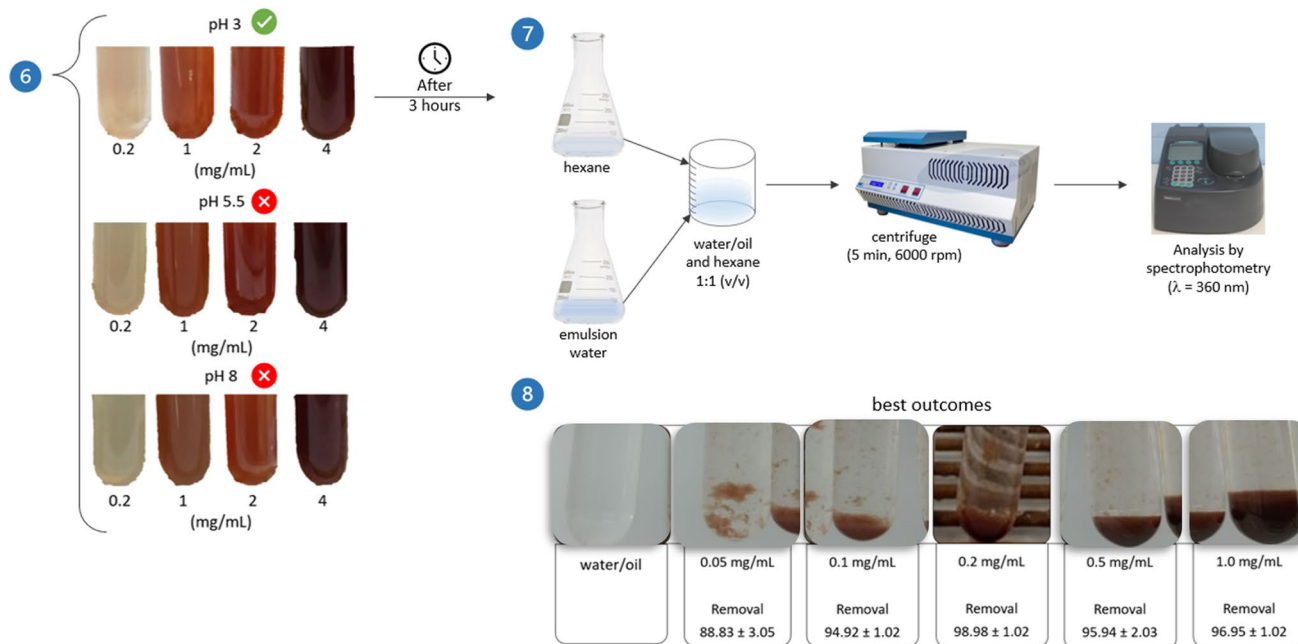
### 3.6 Oil removal performance

The ability of lignin to interact with interfaces was noticed, mainly to destabilize them in specific scenarios. It was noted that at pHs higher than 3 the zeta potential would be high enough to improve the stabilization of the oil droplets [35]. Therefore, for this region (pH > 3), the surface charge must be very high to the point of repulsion between the lignins. In this way, the coagulation process is only helpful at low pH. In this environment, lignin particles interact with each other and clots are generated. Lignin hydroxyls are protonated in acidic environments, so that electrostatic repulsion is minimized and hydrophobic interactions and  $\pi$ - $\pi$  stacking are maximized [22]. They became heavier than their separated and scattered component in the aqueous phase. Consequently, the agglomeration of lignin and oil promotes the removal of the oil by sedimentation (see Fig. 9).

Another aspect observed was the speed of decantation of the lignin clots with oil. With a minimum of 2 h, there was an oil removal from the aqueous phase of at least 88.0%

**Fig. 8** Pyrolysis curve of lignin nanoparticle (LNP) in thermogravimetry analyses (TGA)





**Fig. 9** Removal of oil through lignin from green coconut fiber (GCF)

for all the samples (see Fig. 9). However, the decantation time was insufficient for the experiments with 0.05 mg/mL and 0.1 mg/mL of lignin, which reached removals of  $88.83 \pm 3.05\%$  and  $94.92 \pm 1.02\%$ , respectively. This would be a weakness of these concentrations whether the process depended on the continuous oil removal at the bottom of the decanter. On the other hand, the concentration of 0.2 mg/mL of lignin showed an excellent oil removal of 99%, but visually, it is still possible to see flocs dispersion on the reaction vessel. Therefore, the experimental condition of 1.0 mg/mL of lignin exhibited an oil removal of 97% and a very low dispersion of the clots on the aqueous phase, being taken as the best condition.

## 4 Conclusion

This work has observed the affinity of lignin in the presence of [Ch][Cl], a scenario that favored the delignification process. Among all the pretreatments performed, the most severe condition was highlighted, which contained 75.0% of [Ch][Cl] (w/w) and 1.0% of sulfuric acid (w/w); it was possible to obtain a hydrolysis yield of 45.0%, which may promote the fermentation stage on further analysis. In this way, bioethanol production from pretreated GCF is feasible. It was noticed that pretreatment with [Ch][Cl] was not aggressive enough to modify the lignin structure via SEM. However, there was an excellent molecular interaction between lignin and [Ch][Cl], which made it possible

to extract the lignin from the solid fraction to apply it as a coagulant agent in removing emulsified oil. Also, it can be researched in the future the solvent recycling during pretreatment and its consequence on hydrolysis enzymatic and lignin removal. In addition, it would be necessary to check the viability of the fermentation stage by investigating ethanol yields and the influence of inhibitory substances. Another suggestion is to apply the GCF lignin in other contaminated water situations to assess the multiple facets of the lignin. Overall, this study utilized the pretreated GCF to remove the lignin and provide fermentable sugars. The GCF lignin was used in the oil removal process.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s13399-023-05127-3>.

**Author contribution** JR: conceptualization, experiments execution, and writing—original draft preparation. VR: experiments execution, software, and statistics. JCF: software and statistic. ALdS: experiment execution. JC: experiment execution. CP: conceptualization, reviewing, and editing. ES: writing—original draft preparation, funding acquisition, and supervision writing.

**Funding** The authors gratefully acknowledge the Brazilian research funding agencies Brazilian National Council of Research-CNPq (Grant number: 141528/2019–2), the Coordination for the Improvement of Higher Education Personnel-CAPES (Finance code: 001), and CNPq (National Council for Scientific and Technological Development—no. 304844/2020–9) for financial support.

**Data availability** Data and materials are available on request.

## Declarations

**Ethical approval** This article does not contain any studies with humans or animals performed by any of the authors.

**Competing interests** The authors declare no competing interests.

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