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ORIGINAL PAPER

Valorization, Comparison and Characterization of Coconuts Waste and Cactus in a Biorefinery Context Using $NaClO_2$–$C_2H_4O_2$ and Sequential $NaClO_2$–$C_2H_4O_2$/Autohydrolysis Pretreatment

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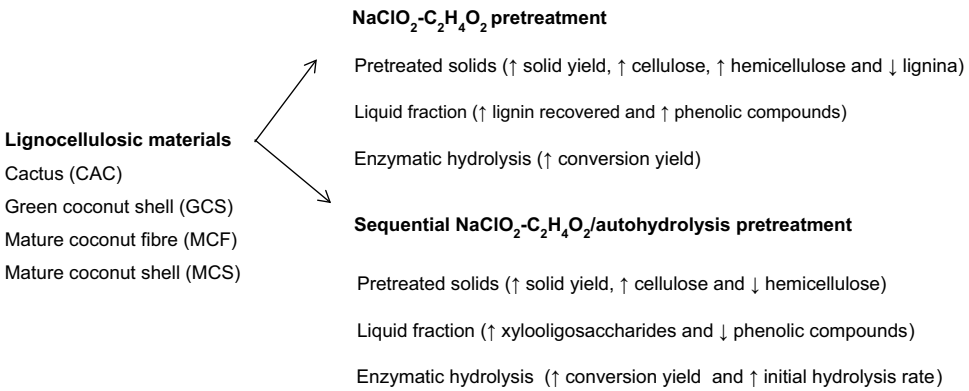
Abstract

The search for new sources of lignocellulosic raw materials for the generation of energy and new compounds encourages the search for locations not well known and with a high potential for biomass availability as is the case of the Northeast Region of Brazil. Thus, the cactus (CAC), green coconut shell (GCS), mature coconut fibre and mature coconut shell were pretreated by $NaClO_2$–$C_2H_4O_2$ and sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis aiming at the obtention of high added-value compounds in the liquid fraction and solid phase. The yield of the solid phase was between 61.42 and 90.97% and the reduction up to 91.63% of lignin in the materials pretreated by $NaClO_2$–$C_2H_4O_2$. After $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment the obtained solids yield was between 43.57 and 52.08%, with a solubilization of the hemicellulose content up to 81.42%. For both pretreatments the cellulosic content remained almost unchanged. The pretreated solids were characterized by SEM, X-ray and crystallinity indexes showing significant modifications when submitted to pretreatments. These results were further confirmed by the enzymatic conversion yields of 81.68–90.03 and 86.97–90.36% of the LCMs pretreated by $NaClO_2$–$C_2H_4O_2$ and pretreated by $NaClO_2$–$C_2H_4O_2$/autohydrolysis, respectively. The resulting liquors had a total phenolic compounds content between 0.20 and 3.05 g/L, lignin recovered up to 7.40 g/L (absence of sulphur) and xylooligosaccharides between 16.13 and 20.37 g/L. Thus, these pretreatments showed an efficient fractionation of LCMs, especially in the GCS, being an important requirement for the generation of products and byproducts in the context of the biorefinery.

Graphical Abstract



Keywords Hydrothermal · Xylooligosaccharides · Phenolic compounds · Lignin recovered · Enzymatic hydrolysis · Biorefinery

Extended author information available on the last page of the article

Introduction

The use of fossil fuels strengthens world energy matrix and generates negative environmental consequences. Therefore, the research in this field has motivated to find new alternatives for feedstock in the production of renewable energy. The lignocellulosic materials (LCMs) are considered as viable, sustainable and may be based on the concept of biorefineries.

LCMs are mainly composed of cellulose, hemicellulose and lignin in different proportions, which are tightly bonded by chemical and physical interactions [1, 2]. The bioconversion processes of LCMs to bioethanol usually involve the following steps: (i) milling, (ii) pretreatment, (iii) enzymatic hydrolysis, (iv) fermentation and (v) distillation. These steps may be conducted separately or in combination [3]. The pretreatment stage strongly influences the other steps in the production of bioethanol [1, 4], because the complex structure of cellulose combined with hemicelluloses and lignin restricts the enzymatic hydrolysis efficiency on LCMs, besides the generation of inhibitory compounds as phenolics and oligomers from hemicellulose.

The pretreatments commonly used on LCMs possess chemical, physical or biological action and there is the possibility of combining these actions and pretreatments [5].

The sequential processes are possibilities based in the biorefinery concept, mainly in the fractionation of the main components as lignin, hemicellulose and the production of a substrate rich in cellulose for subsequent production of biofuels. The delignification with sodium chlorite–acetic acid ($NaClO_2$–$C_2H_4O_2$) is based on the oxidative action of chlorine dioxide, disrupts the lignin structure and breaks the linkage between lignin and the carbohydrates, being selective in removing lignin with low dissolution of hemicellulose [6, 7]. As lignin is one of the most abundant polymers in nature, it arises from the their physical and chemical properties a high potential for various industrial applications, including the obtention of bioactive compounds for pharmaceutical or veterinarian purposes [8], production of thermoplastic matrices [9] and gasification or pyrolysis for energy production [10]. Thus, it is important the recovery of the lignin present in the liquor coming from the pretreated LCMs.

On the other hand, autohydrolysis pretreatment allows the solubilization and depolymerization of hemicelluloses and therefore the formation of soluble sugars [1], without the addition of chemical reagents. Autohydrolysis pretreatment generates protons from auto-ionisation of water that act as catalysts, disrupting the acetyl groups present in the xylans, forming acetic acid that improves the hydrolysis of hemicelluloses [2, 11, 12]. The LCMs with high amount of xylan can be submitted to autohydrolysis to obtain xylo-oligosaccharides (XOS) as the main product in the liquid phase [13]. XOS are known to have beneficial health properties and are considered to be functional food ingredients used for pet feed and food [13, 14].

In this sense, cactus (CAC), green coconut shell (GCS), mature coconut fibre (MCF) and mature coconut shell (MCS) were pretreated by sodium chlorite and acetic acid ($NaClO_2$–$C_2H_4O_2$) and by a sequential processes of sodium chlorite and acetic acid with autohydrolysis ($NaClO_2$–$C_2H_4O_2$/autohydrolysis) for the fractionation of lignin and hemicelluse, respectively. The liquors resulting from the pretreatments were analysed and recovered lignin evaluated in quantity and quality. Also, the enzymatic hydrolysis of pretreated solids obtained by $NaClO_2$–$C_2H_4O_2$ and $NaClO_2$–$C_2H_4O_2$/autohydrolysis were performed for the production of fermentable sugars according to the concept of a biorefinery.

Materials and Methods

Raw Materials and Chemical Characterization

CAC, GCS, MCF and MCS were obtained from the agroindustries and urban locations in the Northeast of Brazil. The chemical composition of the raw materials was obtained according to TAPPI—T222 om-98; T212 om-98; T264 cm-97; T211 om-93; T207 om-93; T204 cm-97—(http://www.tappi.org) and Sluiter et al. [15].

Pretreatment Process

Preparation of Raw Materials Before the Pretreatment

LCMs were washed five times with water at 70 °C for removal of residual compounds. After this procedure, the LCMs were dried in an oven with air circulation at 40 °C for 24 h. The LCMs were milled and selected on the particle size of 48 mesh ($\leq$0.3 mm).

Sodium Chlorite and Acetic Acid ($NaClO_2$–$C_2H_4O_2$) Pretreatment

LCMs were pretreated with sodium chlorite and acetic acid for up to 4 h. For each gram of LCMs were added 0.3 g of sodium chlorite (0.93% w/v), 0.1 mL of anhydrous acetic acid (0.31% v/v) and 32 mL of water, kept in a hot bath at 75 °C. After each 1 h of pretreatment, 2 of the flasks was withdrawn from the hot bath, and the same amount of sodium chlorite and acetic acid was added to the other flasks that remained in a hot bath. This procedure was performed

for up to 4 h, yielding eight pretreated samples in total. Subsequently, solid and liquid (liquor) were separated via vacuum filtration, both have been characterized. The solid samples were washed with water (1 L) and acetone (200 mL). The experiments were performed in duplicate [7].

Sequential Pretreatment of Sodium Chlorite and Acetic Acid ($NaClO_2$–$C_2H_4O_2$) and Autohydrolysis

The pretreated solids from each LCM in the pretreatment with $NaClO_2$–$C_2H_4O_2$ by 4 h were transferred to stainless steel cylinders reactors and mixed with water in order to obtain a ratio 10:1 liquid/solid (water and LCM), the process was carried out in 50 mL total volume. The reactor was closed and mounted vertically and then submerged in a Julabo oil bath open heating circulator (Julabo, Germany) with PID temperature control, previously heated to the desired reaction temperature (200 °C) [12]. At the end of the desired reaction time (50 min), the reactor was removed from the oil bath and cool down by soaking in an ice-water bath for 5 min [12]. Solid and liquid were separated via vacuum filtration, both have been characterized [12]. The experiments were performed in duplicate.

Statistical Analyzes in the Pretreatment Process

Statistical significance was evaluated by Fisher F-test for analysis of variance (ANOVA) and Student *t* test with a confidence level of 95%. Statistical analyzes were performed in the Statistica software.

Characterization of Pretreated Solids

Chemical Composition After Pretreatment

The chemical composition was performed as described above (see "Raw Materials and Chemical Characterization").

X-ray Diffraction Analysis for Crystallinity Determination

Cellulose crystallinity of GCS untreated and pretreated were analyzed an X-ray diffractometry (Bruker D8 Discover, USA), using the radiation from copper Kα, with voltage of 40 kV, electrical intensity of 40 mA and speed of 2°/min using continuous scanning angle 2θ from 4 to 70. The crystallinity index (CI) was defined using the Eq. 1 [16].

$$CI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

where I_{002} = maximum intensity (2θ, 22.6°) of the (002) lattice diffraction; I_{am} = intensity of the amorphous diffraction (2θ, 18.7°).

Scanning Electron Microscopy (SEM)

SEM surface of GCS untreated and pretreated were visualized by a scanning electron microscope (Nova NanoSEM 200, Netherlands) and photographed. Samples were initially coated with a gold layer by a cathodic sputtering process on voltage of 15 kV and afterwards visualized by SEM.

Characterization of Liquors

Liquor Composition After Sodium Chlorite and Acetic Acid ($NaClO_2$–$C_2H_4O_2$) Pretreatment

The glucose, hemicellulose (concentration of sugars coming from the hydrolysis of the hemicellulosic fraction of the lignocellulosic material), HMF and furfural contained in the liquors were analyzed by high performance liquid chromatography (HPLC) (see "Analysis of Samples in High Performance Liquid Chromatography").

Liquor Composition After Sequential Pretreatment of Sodium Chlorite and Acetic Acid with Autohydrolysis ($NaClO_2$–$C_2H_4O_2$/Autohydrolysis)

The liquors of LCMs pretreated by $NaClO_2$–$C_2H_4O_2$/autohydrolysis were analyzed by HPLC (see "Analysis of Samples in High Performance Liquid Chromatography") for glucose, hemicellulose, HMF and furfural. A second aliquot of liquors (20 mL) was subjected to quantitative posthydrolysis (with 4% H_2SO_4 at 121 °C during 1 h), before HPLC analysis [17, 18], for glucooligosaccharides (GO), xylooligosaccharides (XO), arabinooligosacharides (ArO) and acetyl groups linked to oligosaccharides (AcO).

Total Phenolic Compounds

Total phenolic contents of liquor samples were determined by spectrophotometer method using Folin–Ciocalteu reagent [19]. 100 µL of liquor were added in 2 mL of sodium carbonate solution (75 g/L), 500 µL of Folin–Ciocalteu reagent and 7.5 mL of distilled water. The tubes were placed in a water bath at 50 °C for 5 min and then cooled to room temperature and vortexed. The absorbance of the samples was measured at 700 nm. Gallic acid was used as a standard, with a seven point standard curve (0–2000 mg/L) and the levels of total phenolic contents were determined in triplicate.

Acid Precipitation of Insoluble Lignin in the Liquors Obtained of the Pretreatment with Sodium Chlorite and Acetic Acid ($NaClO_2$–$C_2H_4O_2$)

Acid precipitation was used to quantitate approximately the insoluble lignin present in liquors. This fraction could be

precipitated together with hemicellulose causing impurities. For that purpose the liquids were acidified to pH 2 with sulfuric acid at 72% (w/w) and stored by 48 h to decant the insoluble lignin. Sequentially the insoluble lignin was washed and vacuum dried at 50 °C until constant weight (modified from Egüés et al. [11]).

Theoretical solubilized lignin (TSL) was calculated by the Eq. 2, modified from Gonçalves et al. [20]:

$$\mathrm{TSL} = \mathrm{IL} - \mathrm{YL}/100 \times \mathrm{PL} \quad (2)$$

where IL = % lignin of LCM untreated; YL = % unsolubilized LCM; PL = % lignin of LCM pretreated.

Characterization of Lignin of GCS Pretreated

Fourier-Transform Infrared (FTIR) of Lignin

FTIR spectra of lignin of GCS pretreated (higher percentage of lignin recovered from the liquor) was obtained on an FTIR spectrophotometer (FTLA 2000 series, ABB Bomem Inc., Quebec, Canada). The conditions of analysis were as resolution 4 cm^{-1}, using 20 scans and frequency range of 500–4000 cm^{-1}. Commercial lignin (Sigma) was used as standard.

Thermogravimetric Analysis (TGA) of Lignin

TGA was performed using a Shimadzu TGA-50 equipment (Shimadzu, Japan), with thermal software TASYS. Samples were weighed (between 10 and 15 mg) in aluminum sample pans. The experiments were conducted under N_2 atmosphere, at heating rate of 10 °C/min over a temperature range between 20 and 600 °C. Commercial lignin (Sigma) was used as standard.

Enzymes

Enzyme solutions, cellulases, β-glucosidase and hemicellulases (Cellic CTec2) and endoxylanase (HTec2) were kindly supplied by Novozymes A/S (Bagsvaerd, Denmark). The initial enzyme activities were 126 FPU/mL of cellulase, 269 CBU/mL of β-glucosidase for Cellic CTec2 and 1654 IU/mL of endoxylanase for Cellic HTec2 [12].

Enzymatic Hydrolysis

Enzymatic Hydrolysis Yield

The pretreated solids obtained in the sequential pretreatment were used as substrate in the enzymatic hydrolysis. Enzymatic hydrolysis were performed with 4% (w/v) of pretreated solids from each LCM, in an Erlenmeyer flask with a volume of 48 mL at 50 °C using Cellic CTec2 and HTec2 with an enzymatic load of 10 FPU, 30 CBU and 40 IU xylanase per gram of pretreated solid, in 50 mM sodium citrate buffer (pH 4.8) with 0.02% (w/v) sodium azide to prevent microbial growth. The agitation was maintained at 150 rpm for 96 h. The samples were taken at 6 h intervals for the first 12 h and at 24 h intervals until a total time of 96 h [21, 22]. All determinations were performed in duplicate. Sugars concentrations were determined by HPLC (see "Analysis of Samples in High Performance Liquid Chromatography"). The yield of enzymatic hydrolysis was calculated using Eq. 3 [21].

$$\text{Hydrolysis yield}\,(\%) = \frac{\{[\text{glucose}] + 1.053\,[\text{cellobiose}]\}}{\{(1.111)\,\text{f}[\text{biomass}]\}} \times 100 \quad (3)$$

where [glucose] = glucose concentration (g/L); [cellobiose] = cellobiose concentration (g/L); [biomass] = concentration of dry biomass initial of enzymatic hydrolysis (g/L); f = constitutes of the cellulose fraction of dry biomass (g/g); 1.111 = consists in the conversion factor of cellulose to equivalent glucose; 1.053 = consists in the conversion factor of cellobiose to equivalent glucose.

Statistical Analysis of Enzymatic Hydrolysis

Statistical significance was evaluated on the enzymatic hydrolysis (Eq. 3) by Fisher F-test for ANOVA and Student *t* test, with a confidence level of 95%. For the data analyses, Statistica software was used.

Analysis of Samples in High Performance Liquid Chromatography (HPLC)

For the sugar determination, the samples obtained from LCMs were centrifuged and filtered through a 0.2 µm sterile membrane filter for glucose, xylose, arabinose, HMF, furfural and acetic acid quantification. Chromatographic separation was performed using a Metacarb 87H column (300 × 7.8 mm, Varian, USA) under the following conditions: mobile phase 0.005 mol/L sulfuric acid, flow rate 0.7 mL/min and column temperature 60 °C using a Jasco chromatograph 880-PU pump (Jasco, Japan) equipped with a Jasco 830-IR refraction-index detector (Jasco, Japan) and a Jasco AS-2057 Plus auto sampler (Jasco, Japan).

Results and Discussion

Chemical Compositions of Raw Materials

The composition of raw materials used is presented in Table 1. The initial moisture content of CAC, GCS, MCF and MCS was 12.60, 8.99, 6.14 and 5.52%, respectively. The major component present in these materials was cellulose with 38.12,

Table 1 Chemical composition (% dry weight) of untreated, $NaClO_2$–C_2H_4O pretreatment and sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment for MCF, GCS, MCS and CAC

Pretreatment	Solid yield (%)	Components (%)			
		Cellulose	Hemicellulose	Lignin	Ash
(A) MCF					
Untreated	100 ± 0.00	32.18 ± 0.12	27.81 ± 0.74	25.02 ± 0.01	3.31 ± 0.02
$NaClO_2$–$C_2H_4O_2$					
1 h	88.92 ± 0.40	36.05 ± 0.57	25.93 ± 0.33	24.83 ± 0.10	2.54 ± 0.08
2 h	76.87 ± 0.31	45.95 ± 0.47	26.95 ± 0.78	10.05 ± 0.85	2.83 ± 0.07
3 h	69.03 ± 0.32	52.21 ± 0.61	27.38 ± 0.24	3.51 ± 0.15	3.24 ± 0.09
4 h	64.05 ± 1.05	57.13 ± 0.47	29.31 ± 0.25	2.32 ± 0.10	3.61 ± 0.12
$NaClO_2$–$C_2H_4O_2$/autohydrolysis	48.50 ± 0.32	71.25 ± 0.24	6.67 ± 0.19	4.25 ± 0.27	3.79 ± 0.15
(B) GCS					
Untreated	100 ± 0.00	33.23 ± 0.24	29.14 ± 0.22	25.44 ± 0.12	2.34 ± 0.11
$NaClO_2$–$C_2H_4O_2$					
1 h	90.97 ± 1.03	34.65 ± 0.16	23.81 ± 0.26	23.62 ± 0.16	2.35 ± 0.07
2 h	83.43 ± 0.42	41.60 ± 0.39	24.43 ± 0.17	13.26 ± 0.21	2.67 ± 0.17
3 h	73.09 ± 0.31	49.51 ± 0.35	25.56 ± 0.69	6.58 ± 0.18	2.93 ± 0.15
4 h	69.92 ± 0.85	51.48 ± 0.43	27.35 ± 0.15	2.88 ± 0.23	3.04 ± 0.16
$NaClO_2$–$C_2H_4O_2$/autohydrolysis	52.08 ± 0.45	74.28 ± 0.57	5.31 ± 0.28	4.67 ± 0.25	3.15 ± 0.15
(C) MCS					
Untreated	100 ± 0.00	29.58 ± 0.50	27.77 ± 0.79	31.04 ± 0.10	3.84 ± 0.08
$NaClO_2$–$C_2H_4O_2$					
1 h	85.55 ± 0.39	33.64 ± 0.21	28.80 ± 0.21	24.61 ± 0.37	2.25 ± 0.20
2 h	76.09 ± 0.47	42.46 ± 0.28	31.38 ± 0.45	14.27 ± 0.18	2.55 ±
3 h	65.89 ± 1.21	50.84 ± 0.17	32.95 ± 0.57	5.40 ± 0.26	2.60 ± 0.15
4 h	61.42 ± 0.43	52.07 ± 0.16	33.09 ± 0.24	2.60 ± 0.11	2.82 ± 0.13
$NaClO_2$–$C_2H_4O_2$/autohydrolysis	43.57 ± 0.26	71.03 ± 0.36	7.39 ± 0.39	5.19 ± 0.20	3.09 ± 0.21
(D) CAC					
Untreated	100 ± 0.00	38.12 ± 0.75	23.50 ± 0.42	19.51 ± 0.13	5.64 ± 0.21
$NaClO_2$–$C_2H_4O_2$					
1 h	87.32 ± 0.19	40.92 ± 0.48	26.80 ± 0.31	18.55 ± 0.44	4.16 ± 0.11
2 h	81.44 ± 0.63	42.82 ± 0.37	29.17 ± 0.20	16.35 ± 0.36	4.36 ± 0.07
3 h	70.22 ± 0.46	46.18 ± 0.45	30.69 ± 0.38	5.45 ± 0.26	4.58 ± 0.18
4 h	66.75 ± 0.52	47.78 ± 0.57	31.43 ± 0.33	3.02 ± 0.05	4.83 ± 0.14
$NaClO_2$–$C_2H_4O_2$/autohydrolysis	46.17 ± 1.16	65.93 ± 0.50	5.84 ± 0.16	3.49 ± 0.15	5.05 ± 0.13

33.23 and 32.18% for CAC, GCS, and MCF, respectively, followed by hemicellulose and lignin, except for MCS, where lignin has a higher percentage compared with cellulose and hemicellulose. This peculiarity of the MCS provides the high rigidity and hardness to the LCM. To our knowledge there are few reports regarding the composition of CAC, GCS, MCF and MCS. In a recent study, Gonçalves et al. [22] reported the chemical composition of CAC, obtaining 38.33, 22.19 and 19.51% of cellulose, hemicellulose and lignin respectively; GCS contains the chemical composition of 32.88, 26.50 and 25.44% of cellulose, hemicellulose and lignin respectively; MCF obtained 31.60, 26.33 and 25.02% of cellulose, hemicellulose and lignin respectively; MCS obtained 30.47, 25.42 and 31.04% of cellulose, hemicellulose and lignin respectively. Ding et al. [23] reported the chemical composition for coconut husk as cellulose (21.26%), hemicellulose (17.33%) and lignin (46.36%) in a dry basis. Thus, the chemical composition of these LCMs suggests their use as raw material for the production of bioethanol and into value-added products.

Effect of Pretreatment

Effect of Pretreatment with Sodium Chlorite and Acetic Acid ($NaClO_2$–$C_2H_4O_2$)

The purpose of using $NaClO_2$–$C_2H_4O_2$ was to improve the efficacy of the delignification process on the fractionation of LCMs. The solid yields after this pretreatment were between 61.42 and 90.97%. In Table 1, the chemical composition of the pretreated solids is presented. The main effect of this process was the reduction of the lignin content in the pretreated solids. The values for percentage of remaining lignin are 5.94, 7.92, 5.14 and 10.33 for MCF, GCS, MCS and CAC respectively, indicating a good efficacy of $NaClO_2$–$C_2H_4O_2$ pretreatment during 4 h. The $NaClO_2$–$C_2H_4O_2$ pretreatment carried out on the MCF, GCS and MCS resulted in the maintenance of cellulose, solubilization and depolymerization of hemicellulose between 10.7 and 34.4%, demonstrating the selectivity of the pretreatment, being the residual content of lignin in the LCMs between 2.32 and 3.02% (see Table 1). The CAC presented a reduction in the cellulose content during pretreatment, from 93.7% for 1 h pretreatment to 83.7% for 4 h of pretreatment. Furthermore, the MCF, GCS and CAC showed low reduction of lignin when pretreated with $NaClO_2$–$C_2H_4O_2$ for 1 h, MCF and GCS showing depolymerization and solubilization of hemicellulose. The LCMs pretreated with $NaClO_2$–$C_2H_4O_2$ for 2, 3 and 4 h showed a significant reduction of lignin up to 91.63%, compared to the initial chemical composition of the LCMs. The greatest removal of lignin in the LCMs pretreated with $NaClO_2$–$C_2H_4O_2$ occurred between 2 and 3 h. The MCF, GCS and MCS pretreated with $NaClO_2$–$C_2H_4O_2$ showed low depolymerization of cellulose (see Table 1).

These results are in agreement with other values found in the literature. Siqueira et al. [7] of the reduction in lignin using sugarcane bagasse pretreated with $NaClO_2$–$C_2H_4O_2$, maintaining the cellulosic and hemicellulosic fraction almost unchanged. Gulati et al. [24] reported reduction of lignin for sawdust pretreated with $NaClO_2$–$C_2H_4O_2$ after the pretreatment with NaOH with a high solubilization of hemicellulose in the liquid phase. This high solubilization of hemicellulose reported by Gulati et al. [24], diverges of results obtained in this work and also by Siqueira et al. [7].

Chemical composition of MCF pretreated with $NaClO_2$–C_2H_4O for 1, 2, 3 and 4 h was evaluated by ANOVA and significant differences in the level of confidence of 95%, being the highest cellulose content in the solid phase obtained in $NaClO_2$–$C_2H_4O_2$ pretreatment for 4 h (Table 1). Similar results were obtained for GCS, MCS and CAC.

Effect of Autohydrolysis Pretreatment in the LCMs Pretreated by Sodium Chloride and Acetic Acid

The solids pretreated with $NaClO_2$–$C_2H_4O_2$ for 4 h showed an efficient delignification, showed low reduction of cellulose and hemicellulose (see "Effect of Pretreatment with Sodium Chlorite and Acetic Acid ($NaClO_2$–$C_2H_4O_2$)"). Thus, sequential pretreatment ($NaClO_2$–$C_2H_4O_2$/autohydrolysis) was used to solubilize the hemicellulose fraction. The autohydrolysis is considered an environmentally friendly pretreatment, that enables fractionation of LCMs based on the biorefinery concept and modifies composition and structure of LCMs, besides improving enzymatic susceptibility of LCMs.

The chemical composition of the different LCMs after $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment is presented in Table 1. The main effect of this process was the reduction of the hemicellulose content in the pretreated LCMs, in comparison to the LCMs pretreated with $NaClO_2$–$C_2H_4O_2$ by 4 h. After $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment, the solid yields obtained were 46.17, 52.08, 48.50 and 43.57% for CAC, GCS, MCF and MCS, respectively, with reduction of the hemicellulose content up to 81.42%.

The cellulose content in the pretreated solids after this sequential process was between 65.93 and 74.28%, especially in the pretreated GCS. This effect can be explained by the solubilization/depolymerization of lignin in the $NaClO_2$–$C_2H_4O_2$ pretreatment, solubilization of hemicellulose and byproducts formation (acetic acid, formic acid and furfural) in the $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment, revealing that the cellulose contained in the MCF, GCS and MCS was almost not affected by the pretreatments, consequently, pretreated solids with high cellulose content were obtained.

In recent work, Ertas et al. [3] reported 93.9% of hemicelulose solubilization and 42% of lignin in wheat straw pretreated by autohydrolysis, maintaining the cellulosic content unchanged. According to Ertas et al. [3], the cellulose contained in the wheat straw remains unaffected in the autohydrolyzed solids as the polymer chains in cellulose are tightly packed in highly crystalline structures. These results are in agreement with the results obtained in this work for the cellulose and hemicellulose of solids pretreated in $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment; however, they differ from the lignin content as, in this work the permanence of lignin is observed, while Ertas et al. [3] reported that wheat straw pretreated by autohydrolysis allowed lignin solubilization.

Chemical composition of MCF in $NaClO_2$–$C_2H_4O_2$ pretreatment for 4 h and sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment were evaluated by t-test and significant differences with 95% of confidence level were observed in

chemical composition of pretreated MCF. Similar results were obtained for GCS, MCS and CAC.

Characterization of Pretreated Solids

Scanning Electron Microscopy (SEM)

The SEM images allow to visualize the effects promoted for the pretreatments on the LCMs. Differences between the fibre structure of untreated, $NaClO_2$–$C_2H_4O_2$ pretreatment for 4 h and sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment of GCS are presented in Fig. 1a–c (GCS was selected by the higher enzymatic digestibility, see "Enzymatic Hydrolysis"). The untreated GCS showed the fibre rigid surfaces intact, contiguous and highly ordered (Fig. 1a). However, SEM images of GCS pretreated with $NaClO_2$–$C_2H_4O_2$ (Fig. 1b) and $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreated (Fig. 1c) clearly show modified structures and destruction of the fibres, fibre separation and the appearance of disordered fibres, which could be explained by the solubilization/depolymerization of lignin and hemicelluloses of pretreated GCS. These structural features may provide greater susceptibility of pretreated GCS to enzymatic action. In recent works, Gonçalves et al. [12] and Xiao et al. [4] reported similar results in GCS and bamboo residues untreated and pretreated by autohydrolysis, respectively, when observed by SEM. Gonçalves et al. [22] also reported similar results in GCS untreated and submitted to alkaline pretreatment.

X-ray Diffraction Analysis and Crystallinity

The analysis of X-ray diffraction (Fig. 2) and the CI determination were carried out in the GCS untreated and pretreated ($NaClO_2$–$C_2H_4O_2$ pretreated by 4 h and $NaClO_2$–$C_2H_4O_2$/autohydrolysis) aiming to analyze the crystalline structure

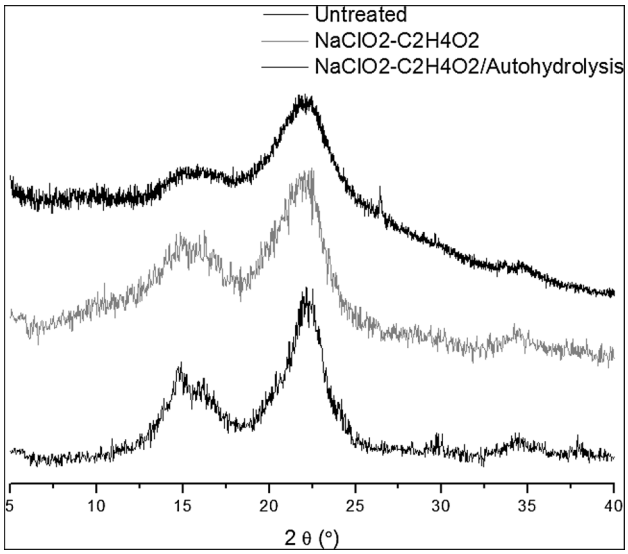


Fig. 2 X-ray diffraction curves of untreated, $NaClO_2$–$C_2H_4O_2$ pretreatment for 4 h and sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment of GCS

(GCS was selected by the higher enzymatic digestibility, see "Enzymatic Hydrolysis").

The crystallinity indexes in the GCS increased from 29.35% (untreated) to 54.87% ($NaClO_2$–$C_2H_4O_2$ pretreatment) and 60.49% ($NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment). Crystallinity consists of numerous inter and intra chain hydrogen bonds in the cellulose fibre and the pretreatment change this crystallinity, such as the pretreatments ($NaClO_2$–$C_2H_4O_2$ and $NaClO_2$–$C_2H_4O_2$/autohydrolysis) used in GCS [4].

In recent work, Gonçalves et al. [22] reported an increase in the CI of GCS untreated (29.31%) submitted to pretreatment with alkaline hydrogen peroxide (49.18%) and sequential alkaline hydrogen peroxide sodium hydroxide pretreatment (49.89%). Gonçalves et al. [12] reported similar results

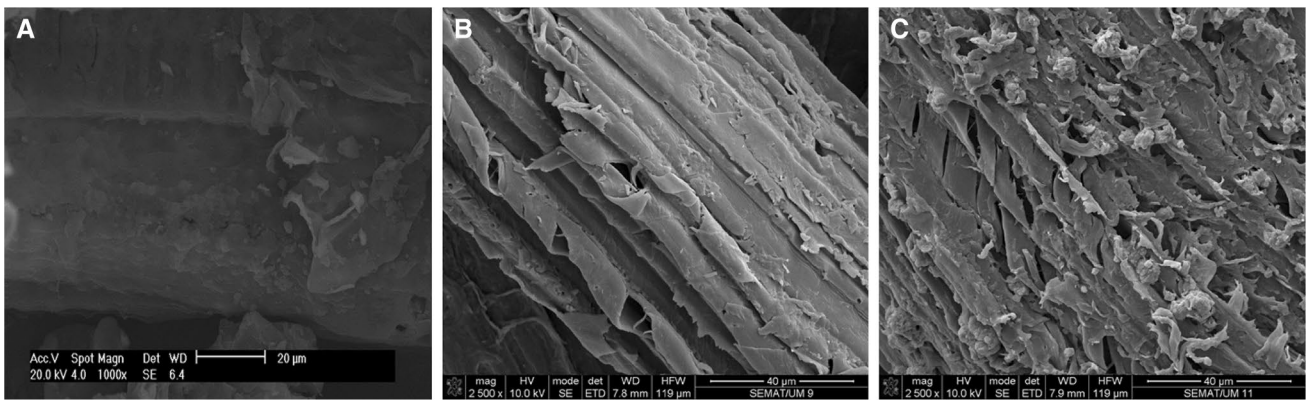


Fig. 1 SEM images of GCS: **a** untreated, **b** $NaClO_2$–$C_2H_4O_2$ pretreatment for 4 h, **c** sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment

in GCS untreated (29.31%) and pretreated by autohydrolysis (43.47%). Xiao et al. [4] also reported an increase in the CI of bamboo residues untreated (45.5%) and pretreated by autohydrolysis (50.2%). Thus, the pretreated LCMs showed increase in CI due to the significant increase in cellulose content. These results corroborate with the results obtained in this work.

Characterization of the Liquor

Liquor Composition

Liquor separated by filtration after the LCMs pretreatment by $NaClO_2$–$C_2H_4O_2$ and sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis presented pH variations during the process (Table 2). Liquors of solids pretreated with $NaClO_2$–$C_2H_4O_2$ presented a correlation between the reduction of the pH and increasing concentration of glucose, hemicelullulose, HMF and furfural in the liquor. These concentrations were low due to reduced solubilization of cellulose and hemicellulose contained in the LCMs pretreated with $NaClO_2$–$C_2H_4O_2$. It is important to mention that these liquors allowed a high concentration of recovered lignin (until 7.40 g/L).

The liquors of solids pretreated with $NaClO_2$–$C_2H_4O_2$/autohydrolysis presented increasing pH and concentration of HMF and furfural in the liquor, when compared to the liquors of solids pretreated with $NaClO_2$–$C_2H_4O_2$ for 4 h due to pentose degradation that leads to formation of furfural and hexose degradation leading to formation of HMF. High concentrations of GO, XO, AcO and ArO in the liquor deriving mainly from hemicellulose were observed (see Table 2). Also, a low amount of cellulose was degraded into GO and glucose. Similar results were obtained by Gullón et al. [25] using rye straw pretreated with autohydrolysis.

Furthermore, Buruiana et al. [1] reported that the liquor of corn stover pretreated by autohydrolysis resulted in 12.0 g/L of XO's. In comparison, the high values obtained in this work (16.13–20.37 g/L of XO) indicate the efficiency of the pretreatment used, making possible to consider the application of these oligomers, in pet feed and food as well as in biofuel production under the biorefinery concept [1, 13, 14]. Also, the others oligosaccharides, monosaccharides, acetic acid, HMF, furfural, total phenolic compounds and lignin present in these liquors may be applied in other processes.

Total Phenolic Compounds

The $NaClO_2$–$C_2H_4O_2$ pretreatment promotes the delignification of the LCMS, making important to determine the total phenolic compounds present in the liquor and evaluate the possibility of recovering them for further application in technological processes such as antioxidant in food additives [26]. Moreover, these phenolic compounds could be redeposited on the surface of the pretreated solid acting as a barrier for enzymatic saccharification [27].

In this sense, the liquors derived from pretreated LCMs with $NaClO_2$–$C_2H_4O_2$ were shown to have between 0.39 and 3.05 g/L of total phenolic compounds (corresponds to total phenolic compounds between 13.01 and 101.68 mg/g of LCM dry) (see Table 2). Faustino et al. [28] analyzed black liquor of *Eucalyptus globulus* in kraft pretreatment obtaining a value for total phenolic compounds between 91.6 and 293.5 mg/g of LCM dry.

Acid Precipitation of Insoluble Lignin in the Liquors

The pretreatment step performed on the LCMs may result in the release of lignin and the recovery of lignin contained in this liquor will allow its use in technological processes, for example, in the production of resin, polymer formulations, converted into synthesis gas, and as an additive for the production of renewable biofuels by ozonolysis [22, 29–31]. In addition, lignin could be burned to supply heat and power [32]. Acid precipitation is the most common method for recovering lignin from black liquor [33].

In this context, liquors obtained in the LCMs pretreated with $NaClO_2$–$C_2H_4O_2$ were precipitated by the addition of sulfuric acid at 72% (w/w), resulting in the obtention of between 0.72 and 7.40 g/L of lignin, with a yield respect to TSL (Eq. 2) between 80.98 and 92.07%. In comparison, Egüés et al. [11] carried out lignin recovery from liquors obtained from corn residues pretreated with sodium hydroxide and obtained 2.5 g/L of lignin. Schorr et al. [34] reported the lignin recovery from liquors provided by the pulp and paper industries, resulting in a yield of recovered lignin from 65% (pulp industries) and 68% (paper industries). These results reported by Egüés et al. [11] and Schorr et al. [34] were lower than the results obtained in this work, indicating the potential of lignin recovery contained in the liquor of LCMs pretreated with $NaClO_2$–$C_2H_4O_2$.

FTIR of Lignin of Pretreated LCM The analyzes of FTIR were carried with the purpose of obtaining information of the chemical groups present in the lignin [35]. The FTIR spectra in the region between 500 and 4000 cm^{-1} of standard lignin (Sigma-Aldrich) and lignin recovered of liquor from GCS pretreated with $NaClO_2$–$C_2H_4O_2$ for 4 h are shown in Fig. 3a. In general, the spectra of standard lignin and lignin recovered showed similarity.

The lignins showed significant absorption around wavelength of 3300 cm^{-1} due to aliphatic and phenolic OH groups. Small peaks in the wavelength of 2900 and 2800 relate with methyl and methylene groups [36, 37]. The absence of peak on wavelength of 1739 cm^{-1} is assigned to CO stretching of unconjugated ketone, carbonyl and ester groups, indicating the presence of hydroxycinnamates [37].

Table 2 Chemical composition of the liquid phase (liquor) of $NaClO_2$–C_2H_4O pretreatment and sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreatment for MCF, GCS, MCS and CAC

Pretreatment	pH	Lignin recovered (%)	Lignin recovered[a]	Phenolic compounds[a]	Liquid phase (g/L)							
					Glucose	Hemicellulose	HMF	Furfural	[GO]	[XO]	[ArO]	[AcO]
(A) MCF												
$NaClO_2$–$C_2H_4O_2$												
1 h	3.8	88.84	0.72 ± 0.12	0.39 ± 0.03	0.04	1.05	0.02	0.12	–	–	–	–
2 h	2.8	84.52	4.26 ± 0.56	2.18 ± 0.22	0.12	1.17	0.02	0.18	–	–	–	–
3 h	2.5	85.87	5.80 ± 0.29	3.02 ± 0.46	0.16	1.78	0.03	0.19	–	–	–	–
4 h	2.4	80.98	5.71 ± 0.37	2.80 ± 0.34	0.20	1.94	0.03	0.20	–	–	–	–
$NaClO_2$–$C_2H_4O_2$/autohydrolysis	3.0	–	–	0.28 ± 0.01	0.37	1.75	0.27	0.85	0.61	16.78	0.71	1.03
(B) GCS												
$NaClO_2$–$C_2H_4O_2$												
1 h	3.1	92.07	1.09 ± 0.16	0.59 ± 0.18	0.02	2.08	0.01	0.15	–	–	–	–
2 h	2.8	87.95	3.80 ± 0.61	1.96 ± 0.36	0.18	2.19	0.02	0.17	–	–	–	–
3 h	2.8	89.89	5.48 ± 0.59	2.90 ± 0.35	0.24	2.49	0.04	0.18	–	–	–	–
4 h	2.7	85.92	6.04 ± 0.74	3.05 ± 0.41	0.40	2.59	0.04	0.20	–	–	–	–
$NaClO_2$–$C_2H_4O_2$/autohydrolysis	3.1	–	–	0.26 ± 0.01	0.26	1.47	0.33	0.91	0.83	16.13	0.60	1.12
(C) MCS												
$NaClO_2$–$C_2H_4O_2$												
1 h	3.3	88.04	2.44 ± 0.14	0.73 ± 0.31	0.00	1.08	0.01	0.13	–	–	–	–
2 h	3.1	89.91	5.22 ± 0.24	1.57 ± 0.59	0.12	1.30	0.01	0.15	–	–	–	–
3 h	3.1	87.00	7.12 ± 0.69	2.13 ± 0.76	0.12	1.44	0.01	0.15	–	–	–	–
4 h	3.1	83.92	7.40 ± 0.30	2.22 ± 0.46	0.24	1.56	0.02	0.15	–	–	–	–
$NaClO_2$–$C_2H_4O_2$/Autohydrolysis	3.3	–	–	0.38 ± 0.01	0.34	1.72	0.64	1.35	0.61	20.37	0.58	0.78
(D) CAC												
$NaClO_2$–$C_2H_4O_2$												
1 h	3.6	90.92	1.01 ± 0.20	0.57 ± 0.21	0.20	0.07	0.01	0.05	–	–	–	–
2 h	3.4	90.33	2.07 ± 0.31	1.15 ± 0.12	0.24	1.94	0.01	0.17	–	–	–	–
3 h	3.4	86.02	4.10 ± 0.93	2.18 ± 0.37	0.25	2.36	0.01	0.17	–	–	–	-
4 h	3.2	84.51	4.46 ± 0.39	2.31 ± 0.38	0.29	2.47	0.01	0.17	–	–	–	–
$NaClO_2$–$C_2H_4O_2$/Autohydrolysis	3.9	–	–	0.20 ± 0.01	0.24	2.77	0.29	0.83	0.86	18.44	0.63	1.05

[a]g/L of liquor; *GO* glucooligosaccharides, *XO* xylooligosaccharides, *ArO* arabino-oligosaccharides, *AcO* acetyl groups linked to oligosaccharides, – unrealized

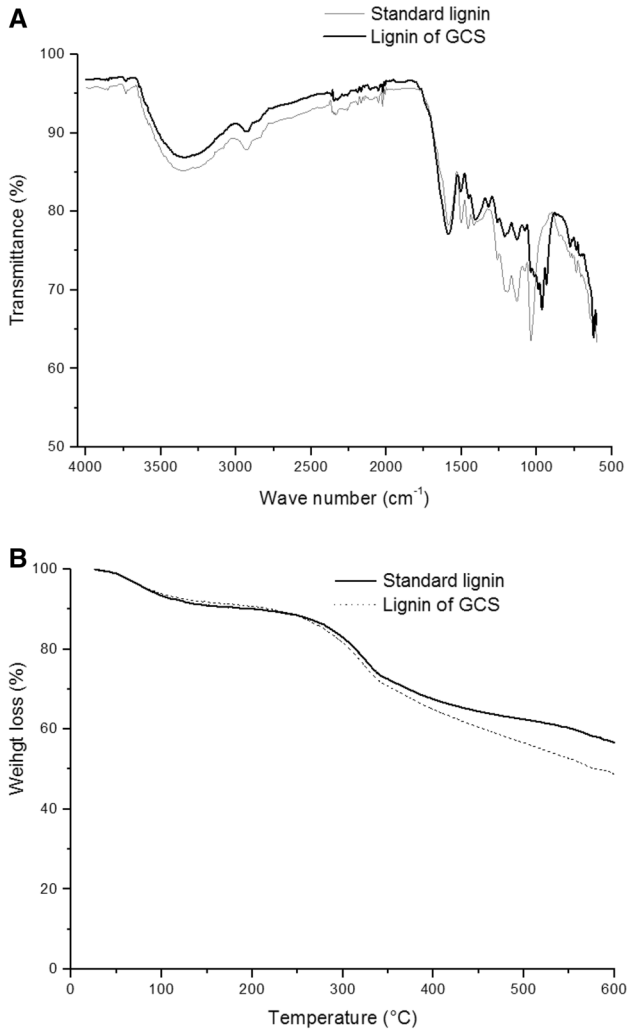


Fig. 3 GCS pretreated with $NaClO_2$–$C_2H_4O_2$ for 4 h and sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis process: **a** FTIR of standard lignin and recovered lignin of liquor of GCS pretreated, **b** TGA of standard lignin and recovered lignin of liquor of GCS pretreated

The peak at wavelength of 1590 cm^{-1} was attributed to the aromatic skeleton vibration plus C=O stretchings, whereas absence of peak in the wavelength of 1328 cm^{-1} could be due to vibration of syringyl with guaiacil rings [36, 37]. Vibrations of aromatic molecules can be related to peaks at wavelength of 1604, 1590, 1516, 1500, 1495, 1330, 1260, 1120, 1030 and 800 cm^{-1} [37–39].

The peak in the wavelength of 1454 cm^{-1} is assigned to CH deformations and aromatic ring vibrations and the peak in the wavelength of 1440 cm^{-1} to aromatic skeletal vibrations. The peak in the wavelength of 1370 cm^{-1} may be attributed to the O–H in plane deformation of alcohols and phenols, peak in the wavelength of 1284 cm^{-1} is assigned to guaiacyl ring breathing with CO stretching, the peak in the wavelength of 1210 cm^{-1} to the C–O vibrations of primary alcohols and the peak in the wavelength of 1039 cm^{-1} to aromatic CH guaiacyl type and CH deformation of primary alcohol [37, 39]. Besides these there are peaks observed at 1201, 1070, 753, 667 and 570 cm^{-1} wavelength.

The peaks on wavelength of 1170–1164 cm^{-1} were not observed in the spectra of standard lignin and lignin recovered. The absence of this peaks indicates the absence of sulphur in the lignin and becomes an important characteristic, because sulphur in the lignin is a contaminant and interferes with its use [40].

TGA of Lignin of LCM Pretreated The thermal stability of the standard lignin and recovered lignin from GCS pretreated liquor with $NaClO_2$–$C_2H_4O_2$ for 4 h were investigated in TGA method and results are shown in Fig. 3b. The lignin degradation profiles of standard lignin and sample of the recovered lignin of liquor from GCS pretreated with $NaClO_2$–$C_2H_4O_2$ for 4 h were different after 350 °C. The residue weight at 600 °C of recovered lignin was 49.23% of the initial weight, whereas that of the standard lignin was 56.71% of the initial weight.

Both lignins showed the same behavior until 350 °C. According to Rencoret et al. [41], between 25 and 150 °C, it occurs the dehydration of lignin due to the absorbed water by the lignin, and up to 350 °C the fragmentations of internal linkages between lignin units takes place. The major products in this stage are coke, organic acid and phenolic compounds together with the production of gases. The different behavior between the lignins after 350 °C was due to pyrolytic degradation of lignin, decomposition and condensation of the aromatic rings [42].

In a recent work, Schorr et al. [34] performed TGA in the lignins derived from liquors provided by the pulp and paper industries, Kruger Wayagamack and Domtar Windsor, obtaining weights of residual lignin at 600 °C of 50 and 48%, respectively. Thus, the result obtained in the present work is in the range of results reported by Schorr et al. [34].

Enzymatic Hydrolysis

The pretreated solids are rich in cellulose content that can be enzymatically converted into glucose. However, the association of cellulose with lignin and hemicellulose limits the enzymatic action. Thus, the conversion yields (%) of untreated CAC, GCS, MCF and MCS were 23.01, 20.24, 16.15 and 13.27%, respectively, after 96 h of hydrolysis. These results demonstrate the need to reduce the recalcitrance of LCMs, and according to Xiao et al. [4] the main criterion for the evaluate efficiency of this pretreatment consists in the cellulose conversion rate.

In this sense, enzymatic hydrolysis of pretreated solids with $NaClO_2$–$C_2H_4O_2$ for 4 h and $NaClO_2$–$C_2H_4O_2$/autohydrolysis were performed to evaluate the susceptibility of the pretreated solids when submitted to enzymatic

action and the corresponding results are shown in Fig. 4a, b. The conversion yields (%) of the CAC, GCS, MCF and MCS pretreated with $NaClO_2$–$C_2H_4O_2$ were 89.28, 90.03, 81.68 and 87.59%, respectively, (Fig. 4a). The conversion yields (%) of the CAC, GCS, MCF and MCS pretreated with $NaClO_2$–$C_2H_4O_2$/autohydrolysis were 89.79, 90.36, 86.97 and 89.83%, respectively, (Fig. 4a). These results demonstrate the susceptibility of the pretreated solids by $NaClO_2$–$C_2H_4O_2$ and $NaClO_2$–$C_2H_4O_2$/autohydrolysis, with a significant increase in the CI, to enzymatic action.

Moreover, a high conversion of cellulose to glucose was obtained in the first 12 h of enzymatic hydrolysis (see Fig. 4b). This kinetic behavior is in agreement with the work reported by Siqueira et al. [7] using sugarcane bagasse pretreated with $NaClO_2$–$C_2H_4O_2$ and is due to the higher initial susceptibility of pretreated solids and enzyme availability.

The results obtained the this work are higher than those reported by some authors as Gonçalves et al. [22] using GCS pretreated with alkaline hydrogen peroxide where the enzymatic conversion yield to glucose was 70.20%. Xiao et al. [4] reported an enzymatic conversion yield of 75.7%, using bamboo residues pretreated by autohydrolysis at 200 °C for 120 min. Ding et al. [23] using coconut husk submitted to the pretreatment of autohydrolysis at 121 °C for 15 min, obtained a conversion into reducing sugars during the enzymatic hydrolysis of 62.46%, in 48 h. Kim et al. [43] reported

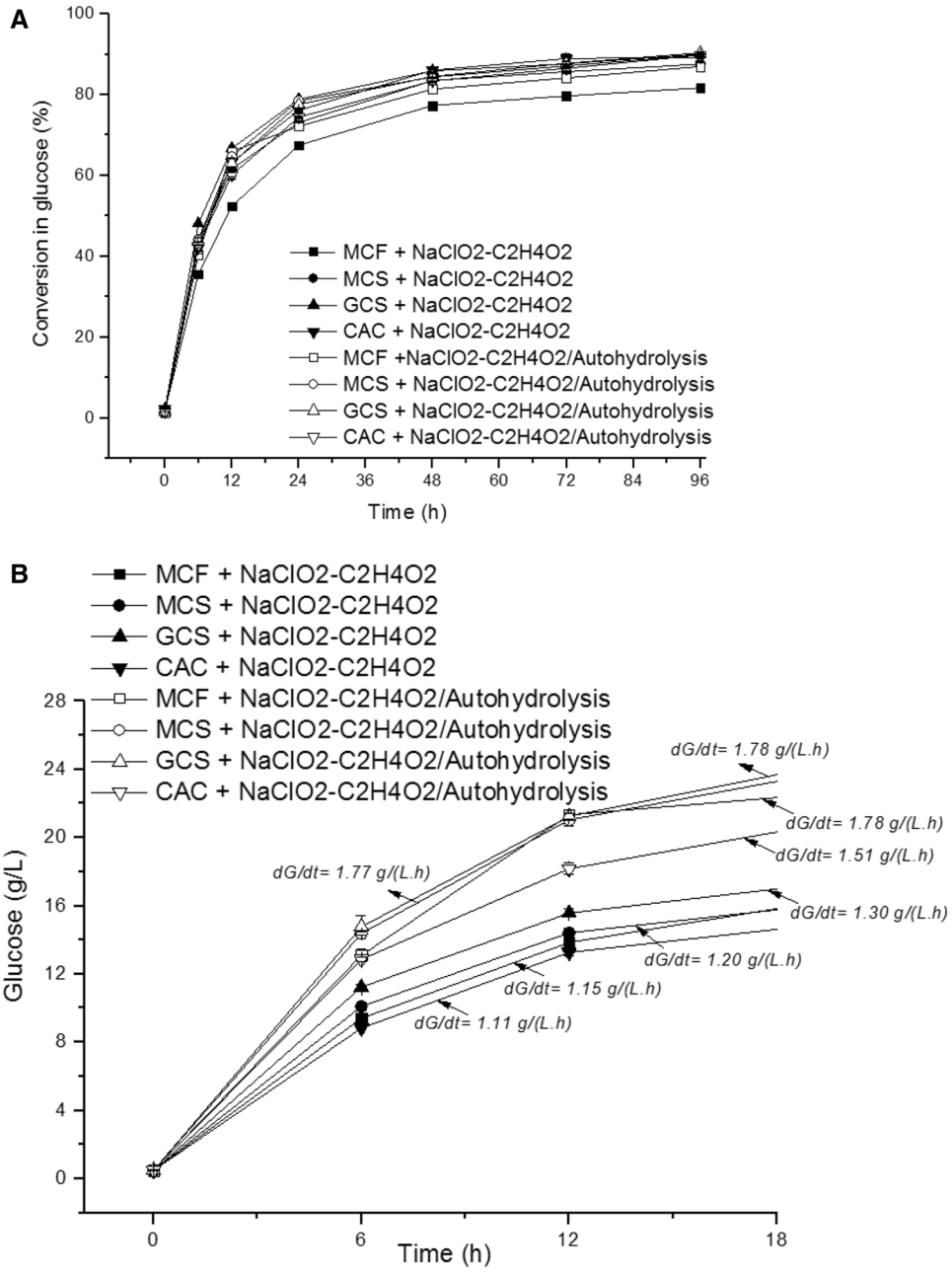


Fig. 4 Enzymatic hydrolysis of GCS, CAC, MCF and MCS pretreated with $NaClO_2$–$C_2H_4O_2$ by 4 h and sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis. **a** Conversion yield (%), **b** initial hydrolysis rate at 12 h

an enzymatic conversion yield of 77.9%, using coffee residues pretreated by $NaClO_2$–$C_2H_4O_2$ at 80 °C for 1 h.

The results obtained in the enzymatic hydrolysis of solids pretreated with $NaClO_2$–$C_2H_4O_2$ and $NaClO_2$–$C_2H_4O_2$/autohydrolysis were evaluated by ANOVA and significant differences in the level of confidence of 95% were observed, being the highest sugar yield obtained for GCS in sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis. The comparison between the results obtained in the enzymatic hydrolysis of GCS pretreated with $NaClO_2$–$C_2H_4O_2$ and GCS pretreated with sequential $NaClO_2$–$C_2H_4O_2$/autohydrolysis showed significant differences, when evaluated by the *t* test analysis. Similar results were also showed for MCF, MCS and CAC. Thus, these results emphasize the importance of autohydrolysis ($NaClO_2$–$C_2H_4O_2$/autohydrolysis) after of pretreatment with $NaClO_2$–$C_2H_4O_2$ on the LCMs.

Regarding the maximum initial hydrolysis rate (*dG*/*dt*) of pretreated solids with $NaClO_2$–$C_2H_4O_2$ measured during the first 12 h (the slope of glucose concentration vs time) the highest, initial hydrolysis rate was obtained for GCS [1.30 g/(L h)] and MCS [1.20 g/(L h)], while lower values were reported for MCF [1.15 g/(L h)] and CAC [1.11 g/(L h)] (Fig. 4b). The LCMs pretreated with $NaClO_2$–$C_2H_4O_2$/autohydrolysis showed an initial hydrolysis rate for GCS [1.78 g/(L h)], MCF [1.78 g/(L h)], MCS [1.77 g/(L h)] and CAC [1.51 g/(L h)] (Fig. 4b). The initial maximum hydrolysis rate was higher for $NaClO_2$–$C_2H_4O_2$/autohydrolysis pretreated solids when compared to LCMs pretreated with $NaClO_2$–$C_2H_4O_2$. In comparison, Gonçalves et al. [22], reported an initial hydrolysis rate of GCS pretreated with alkaline hydrogen peroxide of 1.28 g/(L h) using 30 FPU/g of LCM. In addition, Gonçalves et al. [12] reported an initial hydrolysis rate of GCS pretreated by autohydrolysis of 0.95 g/(L h) using 30 FPU/g of LCM. These results demonstrate the susceptibility of the pretreated solids with $NaClO_2$–$C_2H_4O_2$ and $NaClO_2$–$C_2H_4O_2$/autohydrolysis to enzymatic action.

Conclusion

The CAC, GCS, MCF and MCS pretreated by $NaClO_2$–$C_2H_4O_2$ and $NaClO_2$–$C_2H_4O_2$/autohydrolysis showed efficient fractionation and higher enzymatic digestibility, with the best results obtained for the GCS pretreated by $NaClO_2$–$C_2H_4O_2$/autohydrolysis, when compared to the other LCMs. SEM and X-ray characterization and crystallinity indexes of the pretreated solids showed significant modifications when LCMs were submitted to these processes. The liquors were shown to have a significant amount of total phenolic compounds and XOS, besides enabling the recovery of lignin absent of sulphur. The LCMs evaluated in this work with the pretreatments applied showed to comply with the requirements for the generation of products and byproducts from LCMs in the context of the biorefinery.

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