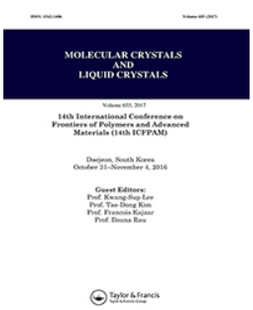

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Characterization of the pre-treated biomass of *Eichhornia crassipes* (water hyacinth) for the second generation ethanol production

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ABSTRACT

Eichhornia crassipes (water hyacinth), is considered an aquatic pest. An alternative to solve the excess water hyacinth problem is to use the biomass for second generation ethanol. This process can be divided into: collection of biomass pre-treatment, enzymatic hydrolysis, fermentation and distillation. Some chemical processes for pre-treatment of biomass water hyacinths were evaluated to determine which procedure degrades the greater amount of lignin and hemicellulose which least affects the cellulose. Characterization was made from the analysis techniques: TG-DTA, XRD and FTIR. The results revealed that the biomass to water hyacinth the most efficient pre-treatment is chemical hydrolysis with sulfuric acid.

KEYWORDS

Chemical characterization; chemical hydrolysis; pre-treatment; second-generation ethanol; water hyacinth

1. Introduction

The *Eichornia crassipes* reproduction, water hyacinth, turns those methods, asexual and sexual, what makes it difficult to control this growth and plant one growth rate has values very high, 220 kg/ha/ day. These features provides that the vegetables produces a significant amount of biomass which can cover a wide area of the surface of water bodies, causing many environmental and economic problems. For these reasons the removal of water hyacinth of water bodies and that biomass can be used as feedstock for second generation ethanol production [1, 2, 3, 5, 6, 7]. In technology used for second generation ethanol production from biomass there are two main steps: The pre-treatment of biomass and enzymatic hydrolysis. The process of pre-treatment is intended to prepare the biomass for an enzymatic hydrolysis step, expanding and breaking as the fibers and solubilizing the complex components lignin-hemicellulose-cellulose that might disrupt the performance fibers enzymes. Some types of pre-treatments, their characteristics, advantages and disadvantages are described in Table 1.

The enzymatic hydrolysis step is responsible for hydrolyzing the cellulose to glucose, so that fermentation can take place in the later stage. The method used in pre-treatment depends on each biomass and the proportions of the lignin-cellulose-hemicellulose complex. Therefore there are several possible methods for hydrolysis production and can be classified into: physical, chemical, biological or even a combination between them. Some conditions for a

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Table 1. Characteristics of some categories of pre-treatments: physical, chemical, biological and combined.

	Compositional characteristics					
Pre-treatments	Type	Lignin	Cellulose	Hemicellulose	Advantages	Disadvantages
Physical	Ball mill	Intensive Decrease of crystallinity degree	No remove	No remove	Reduction of crystallinity	High energy consumption
Chemical	Dilute acid	Low depolymerization	80–100% removal	Low removal but occur structure changes	Medium conditions, high xylose production	Difficult acid recovery, corrosive and relatively costly
	Sodium hydroxide	Significant swelling	Considerable solubility	Considerable solubilization > 50%	Effective removal of esters	High cost reagent, alkaline recovery
	Organo-solv	Considerable swelling	Almost complete solubilization	Almost complete solubilization	High xylose production, effective delignification	Solvent recovery, high cost
Biological	Microbiological	20–30% depolymerization	Over 80% solubilization	Approximately 40% delignification	Low energy requirement, effective delignification	Cellulose loss, low hydrolysis rate
Combined	Steam explosion	Low depolymerization	80–100% removal	Low removal but structure change	Energy efficient, no cost of recycling	Degradation of xylan as an inhibitory product

Source: Adapted from [8].

pre-treatment to be effective and economically feasible can be highlighted, such as: producing cellulose fibers for enzymatic attack; prevent the destruction of hemicellulose and cellulose; avoid formation of possible inhibitors of hydrolytic enzymes and fermenting microorganisms; minimize energy expenditure; reduce the cost of the process of reducing the size of the raw material; produce few residues; consume little or no chemical inputs to the environment and use a small amount of water [8–12]. Thus, the objective of this work was to evaluate the best pre-treatment for later ethanol production for water biomass hyacinth.

2. Materials and methods

The *Eichhornia crassipes* (water hyacinth) was collected manually in the Tietê River, in São Manuel – SP. The plants were collected by simple random sampling, at different times of the year and in the same area of the river, at a distance of not more than 2 m from the right border. The water plants were washed with tap water, cut into small pieces which were oven dried, at 60°C for approximately 50 h. After drying, the dried water hyacinth was ground in a Willey-type knife mill with 20 mesh (0.841 mm) sieve.

The water hyacinth *in natura* was submitted to four pre-treatments (sulfuric acid, acetic acid, hydrogen peroxide and water), in order to evaluate the efficiency of the hemicellulose and lignin removal process. The methodology used for the pre-treatment processes of the water hyacinth, so called "aguapé" in Brazil, was developed based on the research of Ganguly; Chatterjee; Dey, 2012, Bayrakci; Koçar, 2014, Das *et al.*, 2016 [3, 4–5]. Table 2 shows the parameters used in each pre-treatment. In all processes, the concentration of the reactants in the solutions was established at 1.7 mol/L, always using deionized water. The amount of

Table 2. Amount of moisture in the natural water hyacinth and statistical analysis of the results.

Water hyacinth	Mean (%)	Standard deviation	Standard error	Coef. Variation	Coef. Confidence	Lower limit	Upper limit
Dry material	2.4655	0.1828	0.0746	0.0303	0.1463	2.4611	2.4960
Humidity	97.5345	0.1828	0.0746	0.0008	0.1463	97.5300	97.5649

biomass used was also established in 30 g. All pre-treatments of the water hyacinth were performed in a vertical autoclave, under pressure of 2.5 kgf / cm^2 for 1 hour. After this period, the valve was opened and the steam abruptly released, in a process known as a steam explosion.

After the pre-treatments, washings were performed in order to remove the reagents and components from the plant wall. Initially, the samples were filtered (200 mesh sieve) and washed with deionized water. Thereafter, they were again filtered and immersed in a 20% acetone solution for 15 minutes. After this period, the samples were washed with deionized water and sonicated in small quantities (40 mL in each cycle) using the ultrasonic cell disruptor for 9 minutes. Finally, the samples were oven dried at 50°C.

2.1. Analysis

2.1.1. Moisture of water hyacinth In Natura

The moisture content of the water hyacinth *in natura* was determined by the TAPPI method 264 cm-97. In a scale of 0.0001 g precision, the wet biomass (mu) was weighed in a pre-weighed crucible. Oven dried for 4 h at 105°C, the crucible was placed in a desiccator until cooled and weighed again (ms). The test was performed in triplicate and the calculation of the moisture content was calculated according to Equation (1).

$$\text{Humidity}\ (\%) = ((\text{mu} - \text{ms})\ /\text{mu}) * 100 \tag{1}$$

2.1.2. Chemical characterization

Cellulose, hemicellulose, lignin, extractives and biomass ash contents without pre-treatment and pre-treated water hyacinth samples were qualified based on the TAPPI (Technical Association of the Pulp and Paper Industry) standards. Prior to the analysis, the moisture content of samples were taken, using a moisture analyzing scale.

2.1.2.1. Extraction content. The total extractive content was determined by the method of standard TAPPI 204 om-88. It was weighed in extracting cartridges (filter paper sachets) the equivalent of 2 g (dry weight). It was extracted into Soxhlet with alcohol-toluene (1:2) for 8 h. The alcohol-toluene mixture was replaced with 96% alcohol and extracted again for 8 h. The sachets were removed from the Soxhlet and extracted with hot water for 3 h. The samples air dried inside the bag for a week. Samples were removed from the sachets and weighed in pre-weighed beakers. Calculations were performed according to Equation (2).

$$\text{Extractive}\ (\%) = ((\text{extractive mass})\ /2) * 100 \tag{2}$$

2.1.2.2. Ash content. The ash content analysis followed the TAPPI T211 om-02 standard. The porcelain crucible was placed in the muffle at a temperature of 575° C for 1 hour. The crucible was removed and, after cooling in desiccator with silica, it was weighed in analytical balance. In the crucible was weighed 2 g of biomass (total mass 1) and it was kept in an oven for 12 h at 105°C for the determination of dry mass. After this time, the crucible was removed from the oven and kept in a desiccator until room temperature. The sample was weighed (total

mass 2). The crucibles returned to the muffle at 575°C for at least 4 h. The test was performed in duplicate and the ash content was determined by Equation (3).

$$\begin{aligned} \text{Ashes (\%)} &= ((\text{ash mass})/(\text{dry mass})) * 100 \\ \text{Dry mass} &= \text{total mass (1)} - \text{mass crucible} \\ \text{Ash mass} &= \text{total mass (2)} - \text{mass crucible} \end{aligned} \tag{3}$$

2.1.2.3. Lignin content. The standard used for the determination of lignin was TAPPI 222 om-83. Samples of 1 g (dry weight) were placed in filter paper bags and the extractives were extracted following the methodology described in the item "extractive content". The samples were transferred to a beaker (18–20 °C) and 15 mL of 72% H_2SO_4 was added and homogenized periodically for 2 h. After this process, the sample was transferred to 1 L Erlenmeyer using 560 mL of deionized water. The solution was boiled for 4 h, keeping the volume constant. After allowing the lignin to fully sediment, it was filtered through a sintered glass crucible (no 2) with the aid of a vacuum pump. The crucible was oven dried and weighed. The analysis was performed in duplicate and the content was determined by Equation (4).

$$\begin{aligned} \text{Residue (\%)} &= ((\text{residue mass})/1) * 100 \\ \text{Mass residue} &= \text{total mass of crucible} \end{aligned} \tag{4}$$

The remaining residue in the sintered glass crucible was transferred to a pre-tared porcelain crucible. This was placed in the muffle at a temperature of 575°C for 1 hour. This procedure was performed to calculate the ash content present in lignin, which was calculated according to Equation (3). The actual value of the lignin content was determined by Equation (5).

$$\text{Lignin (\%)} = \text{Residue (\%)} - \text{Ash content in lignin (\%)} \tag{5}$$

Holocellulose content. Holocellulose determination was performed using TAPPI standard T257 om-85. For this analysis we weighed 2 g of the sample (dry weight). After extractive extraction, the sample was transferred to Erlenmeyer and 55 mL of deionized water, 3 mL of 20% sodium chlorite solution and 2 mL of acetic acid (1:5) were added. The sample was placed in thermostatic bath at 70°C and every 45 minutes another 3 mL of $NaClO_2$ and 2 mL of acetic acid were added, totaling five additions. After the last treatment, it was filtered through a weighted synthesized glass crucible (no 2) and washed with 250 mL of deionized water. The material trapped in the filter was oven dried is weighed. The test was performed in duplicate and the percentage of residue was calculated through Equation (6):

$$\begin{aligned} \text{Residue (\%)} &= ((\text{residue mass})/2) * 100 \\ \text{Residue mass} &= \text{total mass of crucible} \end{aligned} \tag{6}$$

For the calculation of the ash content in holocellulose the same procedure was used for the ash content in the lignin. The remaining residue in the sintered glass crucible was transferred to a pre-tared porcelain crucible. This was placed in the muffle at a temperature of 575°C for 1 hour. The actual value of the holocellulose content was determined by Equation (7)

$$\text{Holocellulose (\%)} = \text{Residue (\%)} - \text{Ash content in holocellulose (\%)} \tag{7}$$

2.1.2.5. Cellulose content. The analysis for the determination of the cellulose content followed the methodology of TAPPI 203 om-99. 1 g of the dry holocellulose obtained in the item "holocellulose content" was weighed dryed. 15 mL of a 17.5% NaOH solution was added, 2 minutes of contact between the solution and the cellulose was expected before starts to

grind the material for 8 minutes. After grinding, 40 mL of deionized water was added and the contents quantitatively transferred to the funnel where it was filtered with the aid of vacuum cellulose. The precipitate in the funnel was oven dried and weighed in analytical balance. For the calculation of the cellulose residue content, Equation (8) was used.

$$\text{Residue (\%)} = ((\text{residue mass}) / (\text{holocellulose mass})) * 100 \tag{8}$$

$$\text{Residue mass} = \text{total mass of crucible}$$

$$\text{Hemicellulose mass} = \text{holocellulose sample mass}$$

The remaining residue in the sintered glass crucible was transferred to a pre-tared porcelain crucible. This was placed in the muffle at a temperature of 575°C for 1 hour. This procedure was performed to calculate the ash content present in the cellulose, which was calculated according to Equation (3). The actual value of the cellulose content was determined by Equation (9).

$$\text{Lignin (\%)} = \text{Residue (\%)} - \text{Ash content in lignin (\%)} \tag{9}$$

2.1.2.6. Hemicellulose content. The hemicellulose content was calculated based on the results of holocellulose and cellulose content, according to Equation (10)

$$\text{Hemicellulose (\%)} = (\text{holocellulose mass} - \text{cellulose mass}) * 100 \tag{10}$$

2.1.3. Thermogravimetric analysis (TG-DTA)

The thermogravimetry (TG) and differential thermal analysis (DTA) analyzes were used to observe and compare the thermal stability of the water hyacinth *in natura* and the different pre-treatments and the degradation temperature of the biomass. The change in mass was measured in relation to temperature and time. The equipment used in this process was SDT 2960, the 7 mg sample was placed in an aluminum oxide crucible and heated progressively at 10°C/min with an air flow of 100 mL/min.

2.1.4. X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis allows the determination of the degree of crystallinity of the lignocellulosic material and the efficiency of the treatments in lignin removal through the analysis of the diffractogram profile. The crystallinity indices (IC) of fresh and pre-treated water were calculated according to the Buschle-Diller-Zeronian equation (Equation 11) that allows calculate the degree of crystallinity of the samples.

$$\text{IC} = (1 - \text{I1}/\text{I2}) * 100 \tag{11}$$

where: I1 is the minimum intensity related to the amorphous material and I2 is the maximum intensity referring to the peak of crystallinity of the graph. The analyis were performed in a Rigaku Ultima IV diffractometer, with the X-ray being generated in a copper tube (CuKα1) with a voltage of 40 kV and a current of 30 mA, and with the length of where of $\lambda = 1.54$ Å.

2.1.5. Infrared spectroscopy with fourier transform (FTIR)

Fourier transform infrared spectroscopy (FTIR) was used to determine the functional groups in the samples, and the changes in the carboxyl groups in the pre-treated biomass. In this analysis, the Nicolet IS10 FTIR equipment was used, and the samples were detected in the region of 4000 to 400 cm^{-1}.

Table 3. Mass losses related to pre-treatment processes.

Pre-treatment	Mass Water hyacinth (g)	Mass wateer hyacinth pre-treated (g)	Mass losses (g)	Mass losses (%)	Yield (%)
Water	27.2425	17.2204	10.0221	37%	63%
Hydrogen peroxide	27.2425	14.1240	13.1185	48%	52%
Acetic acid	27.5140	15.5477	11.9663	43%	57%
Sulfuric acid	27.9666	8.1276	19.8390	71%	29%

3. Results and discussion

3.1. Chemical characterization

3.1.1. *Moisture of water hyacinth* in natura

This analysis is performed to calculate the amount of dry matter present in the natural water hyacinth without any physical treatment. Table 3 shows the dry matter value and moisture content of the water hyacinth *in natura*.

In the literature, the reported value for the moisture of the water hyacinth *in natura* is in the range of 93–96%, with the most usual value being 95% [13, 14]. Therefore, the found value of 97.5% of moisture content is similar to that observed in the literature. This result shows that *Eichhornia crassipes* has low amount of dry matter, only 2.5%. In other words, in 1 kg of water hyacinth there is only 25 g of dry matter and this fraction is composed of organic matter (cellulose, hemicellulose, lignin, among others) and inorganic matter (ashes).

In all the analysis and pre-treatment procedures, dried water hyacinth was used, that is, only the dry matter fraction.

3.1.2. *Efficiency of pre-treatments*

In the pre-treatment process one of the standard is to remove or solubilize the compounds which may impair the enzymatic hydrolysis step, i.e., extractives, lignin and hemicellulose. For this reason, in this work, the efficiency was calculated based on what was eliminated from the material, that is, the pre-treatment that obtained the largest mass loss was considered the most efficient.

According to Table 4, the most efficient pre-treatment is with sulfuric acid, followed by hydrogen peroxide, acetic acid and finally self-hydrolysis. However, these data do not show which components were solubilized, whether lignin and hemicellulose alone or whether a quantity of cellulose was eliminated.

3.1.3. *Components of Eichhornia crassipes*

In the present study, the following values were found for the dry mas of the water hyacinth: 26% cellulose, 27% hemicellulose, 9% lignin, 22% ash and 19% extractive. These values are

Table 4. Cellulose content and statistical analysis of the results.

		Pre-treatments			
	Without pre treatment	Sulfuric acid	Acetic acid	Hydrogen peroxide	Water
Celullose content (%)	26.0157	11.3321	14.9237	12.1854	17.9605
Standart deviation	1.8549	0.0330	0.4812	1.8767	1.2862
Default error	1.3116	0.0233	0.3403	1.3270	0.9095
Coef. variation	0.0504	0.0021	0.0228	0.1089	0.0506
Coef. confidence	2.5707	0.0457	0.6669	2.6009	1.7825
Inferior limit	23.6314	11.3313	14.7633	9.7448	16.8142
Upper limit	28.3999	11.3328	15.0842	14.6260	19.1068

Table 5. Hemicellulose content and statistical analysis of the results.

		Pre-treatments			
	Without pre treatment	Sulfuric acid	Acetic acid	Hydrogen peroxide	Water
Hemicellulose content (%)	27.4272	8.5130	22.1236	22.3209	26.9738
Standart deviation	1.6942	0.4016	1.9100	4.4005	4.3977
Default error	1.1980	0.2008	0.9550	2.2003	2.1988
Coef. Variation	0.0437	0.0236	0.0432	0.0986	0.0815
Coef. confidence	2.3480	0.3935	1.8718	4.3124	4.3097
Inferior limit	25.4383	8.4735	21.2299	17.5766	22.2357
Upper limit	29.0512	8.5525	23.0174	27.0651	31.7120

Table 6. Lignin content and statistical analysis of the results.

		Pre-treatments			
	Without pre treatment	Sulfuric acid	Acetic acid	Hydrogen peroxide	Water
Lignin content (%)	8.7522	4.5700	8.9584	7.3690	8.1977
Standart deviation	1.1926	0.2815	0.4125	1.6170	1.6480
Default error	0.5963	0.1990	0.2063	0.8085	0.8240
Coef. variation	0.0681	0.0436	0.0230	0.1097	0.1005
Coef. confidence	1.1688	0.3901	0.4043	1.5847	1.6150
Inferior limit	8.4037	4.5151	8.9167	6.7284	7.5323
Upper limit	9.1007	4.6088	9.0001	8.0096	8.8631

similar to the averages described for these components in the literature: 25% cellulose, 35% hemicellulose, 10% lignin and 25% ash [3, 14, 15]. The ash content is high, compared to other vegetables, because of the property of this plant remove inorganic substances from water bodies such as heavy metals.

The contents of the components of the pre-treated biomass were calculated based on the loss of mass of each pre-treatment, as presented in Table 4, in item 5.1.2 "Efficiency of pre-treatments".

The results of the calculations for the untreated and pre-treated components of the water are shown in the following tables, with Table 5 for cellulose content, Table 6 for hemicellulose, Table 7 for lignin content, Table 8 shows the ash content and Table 9 shows the extractive content.

Comparing the values of the cellular components (cellulose, hemicellulose, lignin, extractive and ash) of the water hyacinth with the pre-treated biomasses, it is possible to observe that in all the items there was reduction of the contents, as shown in Table 10, which synthesizes the results from the previous tables: Table 4, Table 5, Table 6, Table 7, Table 8 and Table 9. For these data, the most efficient pre-treatment is with sulfuric acid, as it was able to eliminate

Table 7. Ash content and statistical analysis of the results.

		Pre-treatments			
	Without pre treatment	Sulfuric acid	Acetic acid	Hydrogen peroxide	Water
Ash content (%)	22.3962	4.0689	7.0954	5.8923	9.0847
Standart deviation	2.1590	0.8143	0.4196	0.4151	1.1996
Default error	1.5266	0.4071	0.2098	0.2075	0.5998
Coef. variation	0.0682	0.1001	0.0296	0.0352	0.0660
Coef. confidence	2.9922	0.7980	0.4112	0.4068	1.1755
Inferior limit	19.1661	3.9064	7.0522	5.8501	8.7322
Upper limit	25.6263	4.2313	7.1385	5.9345	9.4372

Table 8. Extractive content and statistical analysis of the results.

		Pre-treatments			
	Without pre treatment	Sulfuric acid	Acetic acid	Hydrogen peroxide	Water
Extractive content (%)	19.4921	3.8644	3.9386	3.9264	3.8366
Standart deviation	1.6333	0.7646	1.2212	0.3882	1.4694
Default error	1.1549	0.3823	0.6106	0.2241	0.7347
Coef. Variation	0.0592	0.0989	0.1550	0.0571	0.1915
Coef. confidence	2.2635	0.7493	1.1968	0.4393	1.4400
Inferior limit	17.6437	3.7212	3.5732	3.8696	3.3076
Upper limit	21.3406	4.0077	4.3040	3.9833	4.3656

a larger amount of lignin and hemicellulose. However, this procedure also eliminated a percentage of cellulose, which is hazardous to the production of second generation ethanol. In the auto-hydrolysis the percentage of lost cellulose was lower in comparison with the other pre-treatment, however, when compared to untreated water hyacinth there was no reduction in hemicellulose content and only 1% reduction in lignin.

3.1.4. Thermogravimetry and differential thermal analysis (TG-DTA)

In Figure 1 it is possible to observe a similarity in mass loss of all samples. Therefore, it can be observed that the biomass of water hyacinth has four stages of mass loss. The first period of thermal decomposition of the water takes place in the range of 25°C to 200°C, referring

Table 9. Comparison of the vegetal components of the biomass without pre-treatment and of the pre-treated biomasses.

	Vegetal componentes					
	Mass loss (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractive (%)	Ashes (%)
Without pre-tretament	—	26	27	9	19	22
Sulfuric acid	71	11	9	5	4	4
Acetic acid	43	15	22	9	4	7
Hydrogen peroxide	48	12	22	7	4	6
Water	37	18	27	8	4	9

Table 10. Results of the TG-DTA analysis of the pre-treated water hyacinth with (a) sulfuric acid; (B) acetic acid; (C) hydrogen peroxide; (D) water and (E) water without pre-treatment.

Water hyacinth/steps	1° Water loss	2° Cellulose and hemicellulose loss	3° Lignin loss	4° Remaining Material loss	Residue
Sulfuric acid					
Mass (%)	4.70	64.73	30.64	0	0
Temperature (°C)	(149.07)	(373.80)	(575.09)	—	(807.00)
Acetic acid					
Mass (%)	9.01	48.72	20.76	1.12	20.58
Temperature (°C)	(164.39)	(379.96)	(538.30)	(668.06)	(950.00)
Hydrogen peroxide					
Mass (%)	8.15	59.12	22.38	1.73	8.71
Temperature (°C)	(187.89)	(373.83)	(510.73)	(682.86)	(950.00)
Water					
Mass (%)	8.82	56.70	23.39	1.98	9.05
Temperature (°C)	(190.96)	(370.76)	(509.71)	(674.20)	(902.01)
Water hyacinth withou pre-treatment					
Mass (%)	8.91	40.58	21.88	1.16	6.32
Temperature (°C)	(176.65)	(372.80)	(530.14)	(649.60)	(919.38)

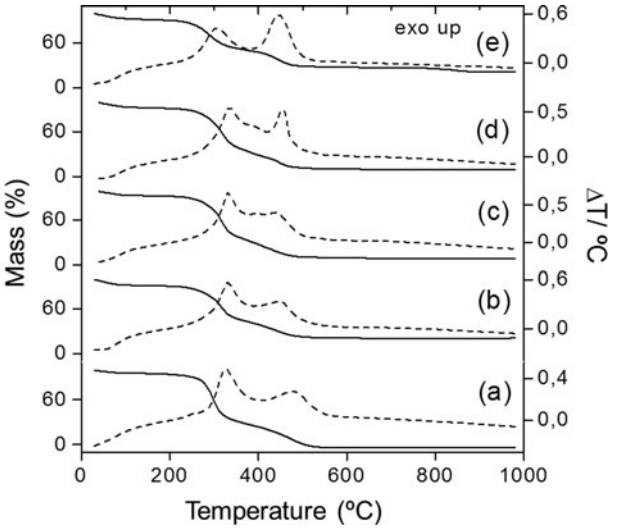

Figure 1. TG-DTA curves of the pre-treated water hyacinth with (a) sulfuric acid; (b) acetic acid; (c) hydrogen peroxide; (d) water and (e) water without pre-treatment. The continuous line refers to the TG – mass of the sample recorded as a function of temperature [m = f (T)] and the dotted line for the DTA curve – difference between sample and reference temperature (Tr – Ta = ΔT) as a function of temperature, with a linear rate heating (dT / dt = Cte) [ΔT − f (T)], the peaks are proportional to the heat of reaction per unit mass of the active substances in the sample [16].

to the dehydration of the sample. In the second step, the temperature range varies between 200°C and 500°C, where two exothermic peaks are observed, being associated to the loss of hemicellulose and cellulose. In the third step, between 500°C and 650°C, the exothermic peak refers to the decomposition of lignin. The fourth stage of decomposition at temperatures above 650°C refers to the burning of the remaining material.

Table 11 shows the results obtained with the TG-DTA curves, showing the percentage loss of mass of each pre-treatment referring to each of the four stages. The results are consistent with those found in the literature. Bergier et al. (2015) [17] and Gao et al. (2016) [15], reported that the largest loss of mass was in the second stage, related to cellulose and hemicellulose, in an approximate temperature of 400° C and that the lignin, being more stable, degraded at a higher temperature.

Table 11 shows that, in the first stage, where sample dehydration occurs, the pre-treatment with acetic acid had the largest mass loss, 9.01%. Between the phases it is possible to observe that the second one is where the water hyacinth loses more mass, followed by the phase 3. The water hyacinth pre-treated with sulfuric acid was the one that lost more mass in the phases 2 and 3, not being quantified material in the following stages, which shows that the pre-treatment was efficient in the elimination of extractives and residues. The highest amount

Table 11. Index of crystallinity of the pre-treated water hyacinth calculated by Equation 8.

Pre-treatment	IC (%)
Sulfuric acid	46.44
Acetic acid	48.54
Hydrogen peroxide	45.60
Water	58.48
Water hyacinth *in natura*	20.48

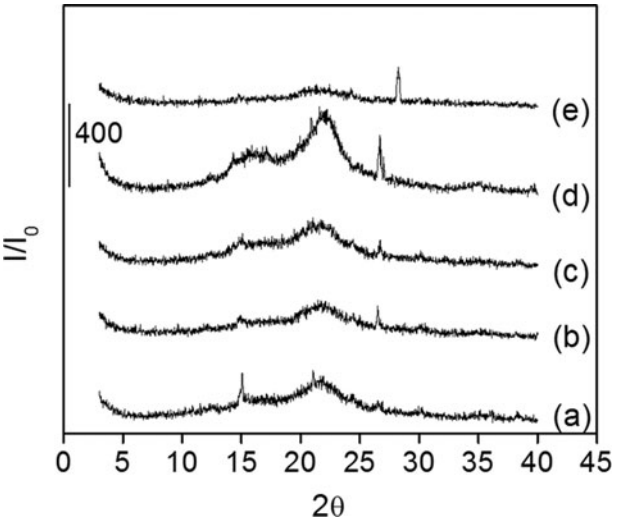

Figure 2. X-ray diffraction analysis of the pre-treated water-hole with (a) sulfuric acid; (b) acetic acid; (c) hydrogen peroxide; (d) water and (e) water without pre-treatment.

of residues was found in the water without pre-treatment. With these data it is possible to affirm that the pre-treatment processes significantly modify the characteristics of the biomass of water hyacinth.

3.1.5. X-ray diffraction analysis (XRD)

The peaks of the X-ray diffraction plot indicate the crystallinity of material. The lignin has an amorphous character, while the cellulose is mostly crystalline, according to [18]. Type I cellulose is characterized by peaks $2\theta = 23°$, 21°, 17° and 15°. Therefore, it can be assumed that an increase in the crystalline area in the XRD plot means removal of the lignin and/or the amorphous part of the cellulose, in both cases increasing the accessibility to crystalline cellulose [18, 19]. Figure 2 shows the graphs of the X-ray diffraction analysis of the pre-treated water hyacinth with (a) sulfuric acid; (b) acetic acid; (c) hydrogen peroxide; (d) water and (e) water without pre-treatment.

Figure 2 shows that the unprepared water hyacinth has only a peak around $2\theta = 27ø$. The Buschle-Diller-Zeronian equation (Equation 11) allows to calculate the degree of crystallinity of the samples. Table 13 shows these indices.

$$\mathrm{IC} = (1 - \mathrm{I1/I2}) \times 100 \tag{11}$$

where: I1 is the minimum intensity related to the amorphous material and I2 is the maximum intensity referring to the graph crystallinity peak.

In Table 12 it can be observed that the crystallinity index for the unprepared water hyacinth is 20.48%, a similar result to the 21.63% found by Satyanagalakshmi et al. (2011) [19]. However, the sulfuric acid index was lower than that found in the literature of 52.9%. All the CIs of the pre-treatments presented significant differences of the water hyacinth *in natura*, proving that the chemical processes altered the characteristics of the biomass.

3.1.6. Infrared spectroscopy with fourier transform (FTIR)

Figure 3 shows peaks in the regions of 3400 cm^{-1}, 2900 cm^{-1}, 1700 cm^{-1} and 1050 cm^{-1}. Yang et al. (2007) [20] reported that the bands pertaining to the chemical bonds of cellulose are

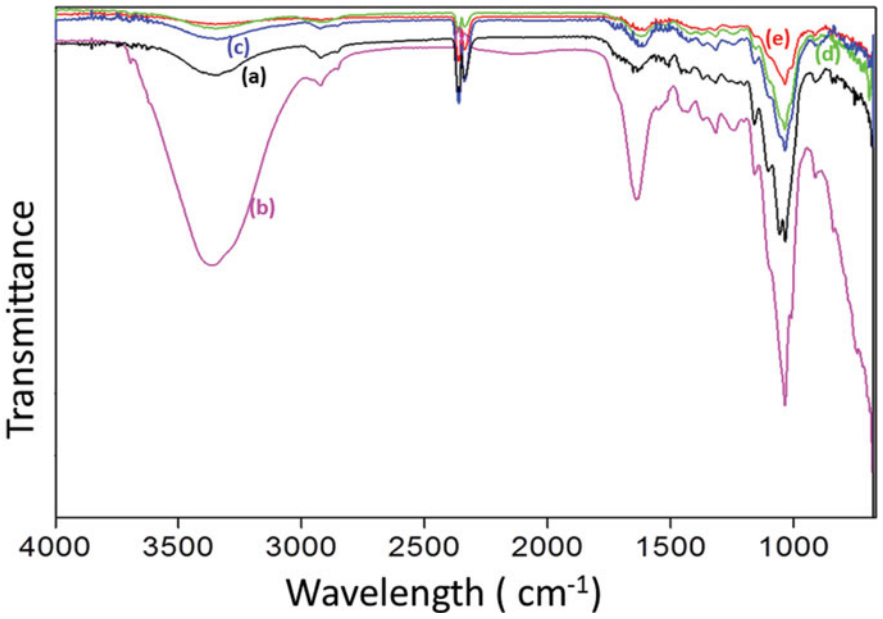

Figure 3. FTIR analysis of the pre-treated water hyacinth with (a) sulfuric acid; (b) acetic acid; (c) hydrogen peroxide; (d) water and (e) water without pre-treatment. The peak in the 3400 cm^{-1} band represents the hydroxyl (-OH); 2900 cm^{-1} is the -CH bond; 1700 cm^{-1} represents either acetyl or can also be uronic ether bonds of carboxylic groups on ferulic acids and p-coumaric acids, both acids can be found in lignin. The 1050 cm^{-1} region usually indicates biomass decrease by hemicellulose solubilization [5].

3400–3200 cm^{-1} and 1050 cm^{-1}, the hemicellulose is indicated by the band 1765–1715 cm^{-1}, and the lignin is indicated by 3 bonds referring to the bands: 1270 cm^{-1}, 1430–1470 cm^{-1} and 1450–1630 cm^{-1}. In this analysis, the pre-treatment with acetic acid showed more changes in the biomass, however the graph of the FTIR curve shows that this change is due to probable remnants of acetic acid that remained in the sample after the washing.

According to the graph of Figure 3 the pre-treatments with hydrogen peroxide and with water did not present many changes compared to the water hyacinth without pre-treatment. The curve referring to the pre-treatment with sulfuric acid is the one that presents a greater difference with respect to the curve of the water hyacinth, thus indicating that this is the best pre-treatment.

4. Conclusion

In this context, it is concluded that the best methodology found for the pre-treatment process of *Eichornia crassipes* (water hyacinth) biomass aiming the production of second generation ethanol is the hydrolysis with sulfuric acid, since it is the one that is able to degrade the highest amount of lignin and hemicellulose. This biomass pre-treated by means of acid hydrolysis can be used for the production of second generation ethanol or biobased materials such as nanocellulose.

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