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Lignin degradation efficiency of chemical pre-treatments on banana rachis for bioethanol production

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Abstract: Valuable biomass conversion processes are highly dependent on the use of effective pretreatments for lignocellulose degradation and enzymes for saccharification. Among the nowadays available treatments, chemical delignification represents a promising alternative to physical-mechanical treatments. Banana is one of the most important fruit crops around the world. After harvesting, it generates large amounts of rachis, a lignocellulosic residue, that could be used for second generation ethanol production, *via* saccharification and fermentation. In the present study, eight chemical pretreatments for lignin degradation (acid-*organosolv*, alcohol-*organosolv*, sodium hypochlorite, hypochlorous acid, hydrogen peroxide, alkaline hydrogen peroxide and some combinations thereof) have been tested on banana rachis and the effects evaluated. The delignificated samples were then saccharified with enzymes (cellulase and beta-glucosidase) and hydrolysis efficiency was evaluated in terms of final sugars recovery before fermentation. Analysis of Fourier Transform Infrared Spectra (FT-IR) has been carried out on treated samples, in order to better understand the structural effects of delignification on lignocellulose. Active chlorine oxidations, hypochlorous acid in particular, were the best effective for lignin removal obtaining also the most promising cellulose-to-glucose conversion.

Keywords: delignification, *organosolv*, oxidation, hypochlorous acid, rachis, lignocellulosic materials, FT-IR

1. Introduction

During the last years, shortages of petroleum-based energy, fast resources depletion and increasing problem of CO_2 emissions have arisen the interest for alternative fuels and more sustainable energy supply in many countries [1]. Among the so-called clean energies, ethanol is considered particularly promising because of some known advantages such as clean burning characteristics, reduction of particulate and NOx emission from combustion, and so on [2]. However, as it is well recognized, nowadays it is not sustainable to produce ethanol from bioconversion of starchy materials especially because it competes with food and feed chains [3]. Therefore, its production from non-grain feedstock as lignocellulosic biomass has been becoming a hot spot in many countries, due to their eco-friendly nature and low cost availability [4]. Lignocellulosic biomass, with a worldwide production estimated in 10^{10} MT/year is rightly so

considered as the only foreseeable, feasible and sustainable raw material for biofuel production [5] and for the ultimate consolidation of the *biorefinery* concept [6].

Various technological developments based on saccharification and fermentation have improved for the conversion of these substrates into bioethanol [7]. Agricultural residues, food processing wastes and forestry residues are all potential sources of fermentable sugars to be converted in bioethanol, but the typical recalcitrance of lignocellulosic biomass to enzyme and microorganisms' attack necessitates of pretreatment as unavoidable pre-requisite [8]. The main focus of those pretreatments is indeed to remove from the plant cell wall the barrier due to lignin, pectin, hemicellulose, glucans and their spatial interlinks, for increasing the enzymatic and microbial digestibility of cellulose [9].

Different pretreatment technologies have been already extensively described in terms of the mechanisms involved, advantages and disadvantages, and economic assessment [10]. They include biological [11], physical-mechanical [12], chemical [13] methods and various combinations thereof [14].

Even though biological approach would be in perspective the ideal solution, because it is less harmful to the environment and needs milder conditions, still necessitates of improvements in process duration and cost reduction, factors that strongly limit their actual efficient industrial application [15-16]. To date, chemical pretreatments are the best alternative to steam explosion, because they are effective and enhance the biodegradation of complex and particularly recalcitrance woody materials, recently becoming potentially sustainable in terms of costs and hazardous waste thanks to solvents recovery and recycling [17-19]. To this group belong all pretreatments that involved chemical reactions for the lignocellulosic structure disruption by means of organic or inorganic acids [20], alkali [21], organic solvents (*organosolv*) [22] and oxidative reagents as ozone [23] or active chlorine [24].

After delignification, residual biomass must be hydrolyzed to produce glucose which is then converted to bioethanol by *Saccharomices cerevisiae* or other microorganisms via alcoholic fermentation [25]. Even though concentrated acid hydrolysis process has a long history of commercial use [26], hydrolysis can be also accomplished by fungal and bacterial hydrolyzing enzymes [27]. There are several enzymes which are required for complete hydrolysis of biomass such as cellulase, xylanase, ligninase, pectinase, etc., among which cellulase is the most important one. Cellulase is a multi-enzyme complex of three different enzymes; exoglucanase, endoglucanase and beta-glucosidase which acts synergistically for complete hydrolysis of cellulose to cellobiose (an intermediate product of cellulose hydrolysis) and finally to glucose [28].

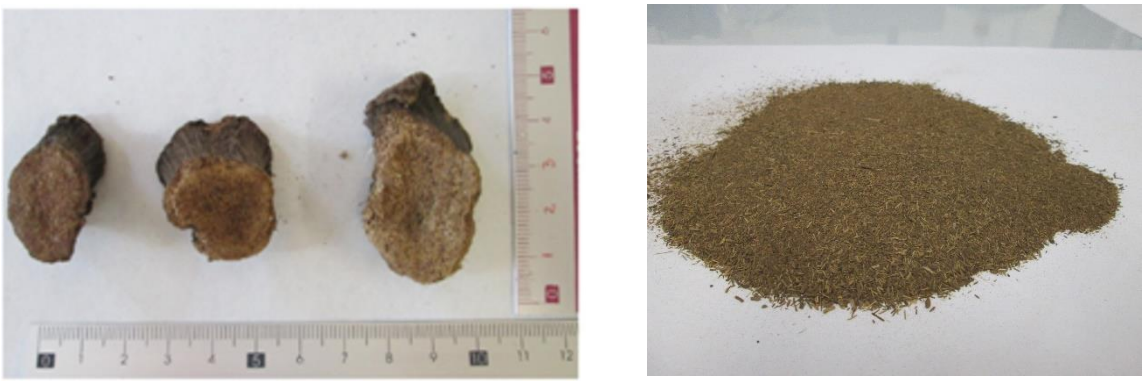
In the present study, eight chemical pretreatments for lignin degradation (acid-*organosolv*, alcohol- *organosolv*, sodium hypochlorite, hypochlorous acid, hydrogen peroxide, alkaline hydrogen peroxide and some combinations thereof) have been tested on lignocellulosic feedstock as banana rachis and the effects evaluated. The pretreated samples were then hydrolyzed with cellulase enzymes pool and the cellulosic saccharification efficiency was evaluated in terms of final sugars recovery before fermentation.

The rachis is the stalk of the inflorescence from the first fruit to the male bud. After fruit harvesting, banana lignocellulosic biomass (rachis, foliage and stems) represent a waste and in producing countries as Ecuador or India the majority of the producers prefer to leave these residues to decompose outdoors, causing environmental problems such as the spread of diseases or polluting groundwater [29]. Some attempts have been done to use those materials for cellulose fiber recovery [30] or thermovalorization [31], but, at the best Authors' knowledge, no information is still available in scientific literature about chemical delignification treatments on banana rachis as key step for bioethanol production. However, it could represent a great amount of valuable lignocellulosic materials, thus constituting an additional economical profit to farmers [32].

2. Materials and Methods

2.1 Banana rachis samples

The lignocellulosic residue was rachis from banana plants (*Musa paradisiaca* var. barraganete) cultivated and collected in the region of Guayas, Ecuador. 1000 g of samples were locally cut in small pieces and sent to our laboratory for subsequent treatments. The material was ground up, sieved using a < 0.5 mm mesh size to obtain a homogeneous powder and then dried at 60°C overnight (Figure 1). Sample was stored in and stored in a desiccator with $CaCl_2$ at room temperature.



(a) **(b)**

Figure 1. Samples of raw banana rachis (a) and rachis powder after grinding and drying (b).

2.2 Chemical pretreatments

2.2.1 Acid-organosolv pretreatment with acetic acid and acetone (AA)

A mixture of glacial acetic acid, acetone (Sigma Aldrich) and water has been prepared at a ratio 10:50:40 (v/v) and pH adjusted to 2.7. Treatment was carried out on lignocellulosic substrate (20 g) by adding 250 ml of the acid-*organosolv* mixture for 30 min at boiling temperature. After treatment, the residual biomass was filtered on paper filter, extensively washed with water and buffered to neutral pH with NaOH 1M, and dried in a hot air oven at 60°C overnight. The dried treated was weighed and stored in a desiccator with $CaCl_2$.

2.2.2 Alcohol-organosolv pretreatment with ethanol (ET)

Rachis powder (20 g) was treated with 250 ml of 96% ethanol (Sigma Aldrich) for 30 min at boiling temperature. The residual biomass was filtered on paper filter, extensively washed with water and dried in a hot air oven at 60°C overnight. The dried treated was weighed. and stored in a desiccator with $CaCl_2$.

2.2.3 Oxidative pretreatment with sodium hypochlorite (SH)

A solution of NaClO 5% (250 ml) was added to rachis powder (20 g) at room temperature for 30 min. The residual biomass was filtered on paper filter and extensively washed with water and buffered to neutral pH with HCl 1M, and dried in a hot air oven at 60°C overnight. The dried treated was weighed. and stored in a desiccator with $CaCl_2$.

2.2.4 Oxidative pretreatment with hypochlorous acid (ECA)

Rachis powder (20 g) was treated with hypochlorous acid (ECA solution) at pH 6 (250 ml) for 10 min at room temperature. According to Tamburini et al [33], ECA solution was prepared by electrolysis of a solution of NaCl (5 g/L) in a flow-through electrochemical cell at pH 6 to allow hypochlorite/hypochlorous acid conversion. As a result, an ECA solution of about 1500 ppm of oxidizing substances (determined by iodometric titration and expressed as "active chlorine") were obtained. Such solution, stored in glass containers and preferably in the dark, maintain its properties for some days. After treatment, the residual biomass was filtered on paper filter and extensively

washed with water and buffered to neutral pH with NaOH 1M, and dried in a hot air oven at 60°C overnight. The dried treated was weighed. and stored in a desiccator with $CaCl_2$.

2.2.5 Oxidative pretreatment with hydrogen peroxide and hydrogen peroxide with alkali (HP and HPA)

To rachis powder (20 g) H_2O_2 2% (v/v) (900 ml) was added (sample HP). In sample HPA NaOH solution 5% (w/w) (100 ml) was added alongside. The final volume of HP and HPA was completed up to 1000 ml with water. Both treatments were kept under stirring for 90 min at room temperature. The the residual biomass was vacuum filtered in glass microfiber, washed with water until the hydrogen peroxide was completely removed, buffered to neutral pH with HCl 1M and dried in a hot air oven at 60°C overnight. The dried treated was weighed and stored in a desiccator with $CaCl_2$.

2.2.6 Combination of ECA and ET

To rachis powder (40 g) 96% ethanol (500 ml) was added and refluxed for 30 min. The solid suspension was separated by filtration and dried at 60°C overnight. The dried powder (20 g) was thereafter mixed up with ECA solution (250 ml) for 10 min at room temperature. After filtration the powder was dried at 60°C up to constant weight, weighed and stored in a desiccator with $CaCl_2$.

2.2.7 Combination of ECA and AA

To rachis powder (40 g) a mixture of glacial acetic acid:acetone:water (10:50:40) (500 ml) was added and refluxed for 30 min. The solid suspension was separated by filtration and dried at 60°C overnight. The treated powder (20 g) was thereafter put in contact with ECA solution (250 ml) for 10 min at room temperature. After filtration the powder was dried at 60°C up to constant weight, weighed and stored in a desiccator with $CaCl_2$..

2.3 Hydrolysis of pretreated samples

Delignificated rachis samples have been submitted to enzymatic hydrolysis. Cellulase from *Trichoderma reesei* (Novozyme 188) with an activity of 6.5 FPU/g, supplemented with beta-glucosidase from *Aspergillus niger* (Novozyme 188) 250 U/g were used for saccharifying the cellulosic material. Enzymatic hydrolysis has been carried out on 1 g of sample in 50mM citrate phosphate buffer (pH 5.0). The substrate with buffer was pre-incubated at 50°C on a rotatory shaker (Innova-40, NewBrunswick Scientific, Germany) at 150 rpm for 2 h. Thereafter the slurry was added with cellulase, 0.125 g and beta-glucosidase, 0.3 g. Tween 80 (1% (v/v)) was also added to the reaction mixture and the reaction continued up to 72 h [34]. Samples of enzymatic hydrolysate were analysed for amount of monosaccharides released.

2.4 Analytical methods

The chemical composition (cellulose, lignin, emicellulose, moisture and ash) of untreated rachis sample and of all the residual solid fraction post pretreatments were determined following standard TAPPI protocols [35]. The monosaccharides (glucose, xylose and arabinose) released after hydrolysis were quantitatively estimated using HPLC with a refractive index detector (Jasco RI-4030). A Rezex ROA-Organic Acid H^+ (8%), 300 x 7.8 mm (Phenomenex) was used at 80°C. Isocratic elution was carried out with H_2SO_4 0.01M water at 0.6 ml/min. Samples were analyze in triplicate. Infrared spectroscopic analysis of residual lignin has been carried out by means of FT-IR spectrophotometer (Perkin Elmer® Spectrum 1000), using a KBr disc containing 1% finely ground samples. Through the Spectrum 10 Software, the corresponding absorbance spectra were obtained in light beam transmission measurement mode, within the range of 4400 cm^{-1} to 500 cm^{-1}, with baseline correction, noise correction (smooth) and identification of the wave numbers of the main peaks.

3. Results and Discussion

3.1 Chemical characterization of untreated banana rachis

Banana rachis pretreated materials was the substrate used in this study. The raw material was analyzed to determine the chemical composition. At the best Authors' knowledge, in literature

compositional analysis of banana rachis has been reported only from Guerrero et al. [32], otherwise data have to be compared with chemical composition of ~~date~~ palm rachis, the most similar matrix [36]. The chemical characterization of Ecuadorian banana rachis has revealed the presence of cellulose (36.5%), hemicellulose (22.3%), lignin (26.2%), and ash (15.2%). Comparing the lignin content of palm rachis has been found in the range 14-27%, and cellulose in the range 30-44%, whereas banana rachis analyzed in the cited paper has a content of lignin, cellulose and hemicellulose of 10.8%, 26.1%, and 11.2%, respectively. All data are expressed on dry weight basis. These data led us to the conclusion that the chemical composition of rachis depends more up to region of cultivation (Ecuador versus Morocco for date palm and Spain for banana) than to the cultivar or species. The high cellulose content of Ecuadorian banana rachis qualifies it as potential valuable agricultural biomass for bioenergy production.

3.2 Delignification efficiency of chemical pretreatments

To facilitate a general view of the different conditions applied during chemical treatments, a synoptic table is proposed (Table 1).

Table 1. Comparison among different chemical treatments

#	Pretreatment	Chemical agent	Temperature (°C)	Time (min)	pH
1	Acid-*organosolv* (AA)	Glacial acetic acid:acetone:water (10:50:40)	100	30	2.7
2	Alcohol-*organosolv* (ET)	96% Ethanol	100	30	-
3	Sodium Hypochlorite (SH)	NaClO 5%	25	30	11
4	Hypochlorous acid (ECA)	$HClO/ClO^-$	25	10	6
5	Hydrogen Peroxide (HP)	H_2O_2 2%	25	90	-
6	Hydrogen Peroxide Alkaline (HPA)	H_2O_2 2% + NaOH 5%	25	90	14
7	ECA+AA	Treat.#1 + Treat.#3			
8	ECA+ET	Treat.#2 + Treat.#3			

Notably, taking the amount of raw material as starting invariant, conditions were very different, and unavoidably had different impacts, in terms of costs and in terms of environmental burden. Even though a detailed description of both of those aspects are beyond the aim of this study, a balance between efficiency and energy/water consumption should be done when defining the *best treatment*.

Effects of different chemical pretreatments on rachis powder, compared to not-treated sample, are shown in Table 2. Treatments based on active chlorine (SH, ECA, ECA+AA and ECA+ET) were more effective than treatments based on hydrogen peroxide, which both (HP and HPA) leaved great amounts of residual lignin in the samples. Even though among chemical treatments the *organosolv* are the most commonly used methods to pretreat lignocellulosic biomass, they have proved not to be so successful for banana rachis.

Table 2. Residual lignin content of banana rachis after chemical treatments (NT= not treated)

Pretreatment	Residual lignin (%)
Rachis (NT)	26.2 ± 2.7
Acid-*organosolv* (AA)	6.4 ± 0.3
Alcohol-*organosolv* (ET)	7.2 ± 0.6
Sodium Hypochlorite (SH)	4.2 ± 0.7
Hypochlorous acid (ECA)	3.7 ± 0.5
Hydrogen Peroxide (HP)	14.5 ± 1.2
Hydrogen Peroxide Alkaline (HPA)	12.3 ± 0.9

Hypochlorous acid + Acid-*organosolv* (ECA+AA)	3.9 ± 0.4
Hypochlorous acid + Alcohol-*organosolv* (ECA+ET)	4.2 ± 0.4

Reporting data in terms of percentage of lignin loss, i.e. delignification capacity of various treatments, compared with 100% of not-treated sample (NT), it is even more evident that treatments based on the oxidant effect of chlorine show high and comparable efficiency (Figure 2).

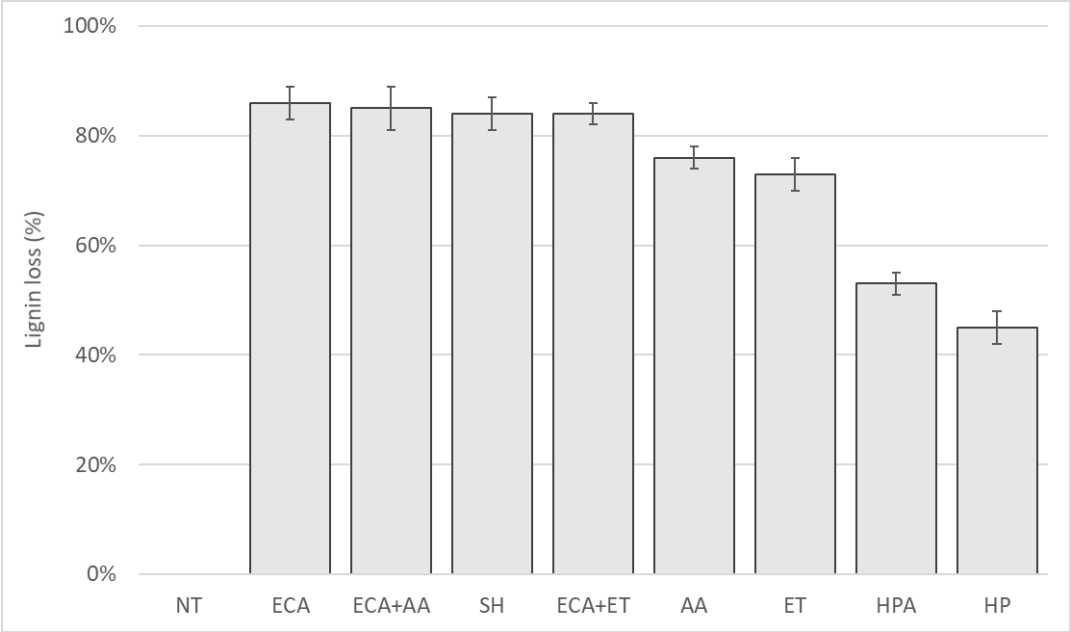


Figure 2. Lignin loss (%, w/w) expressed as percentage of lignin removal in comparison with lignin content before sample pretreatments.

The results indicate that the pretreatment of biomass with oxidizing ECA solution combined with both *organosolv* does not produce a significant advantage over the absolute yield of the dignified biomass treated only with the oxidizing ECA solution. The oxidizing treatment with the ECA solution simultaneously removes a similar amount of soluble substances and lignin.

3.3 Enzymatic saccharification

The results of enzymatic hydrolysis and quantification of monosaccharides recovery are shown in Table 3. It has been reported that a hydrolysis of pretreated biomass can be considered valuable when it gives a concentration of at least 10% of free glucose in the medium for later fermentation [37], so all treatments have given satisfying results. Besides glucose, xylose and arabinose were analyzed because they usually can be found after hydrolysis being the principal components of hemicellulose [38] fraction. Hemicellulose could be partially hydrolyzed during delignification treatments.

Table 3. Fermentable sugars recovery after enzymatic hydrolysis of pretreated banana rachis samples (NT=Not-treated).

Pretreatment	**Glucose (%, w/w)**	**Xylose (%, w/w)**	**Arabinose (%, w/w)**
Rachis (NT)	2.8 ± 0.2	9.0 ± 0.7	1.5 ± 0.0
Acid-*organosolv* (AA)	16.6 ± 0.7	12.8 ± 1.0	2.1 ± 0.0
Alcohol-*organosolv* (ET)	19.8 ± 0.6	10.8 ± 0.7	0.8 ± 0.0
Sodium Hypochlorite (SH)	44.7 ± 1.1	5.0 ± 0.3	1.8 ± 0.0
Hypochlorous acid (ECA)	51.4 ± 1.2	4.2 ± 0.2	2.3 ± 0.0
Hydrogen Peroxide (HP)	35.1 ± 1.8	5.2 ± 0.3	1.5 ± 0.0

Hydrogen Peroxide Alkaline (HPA)	36.5 ± 1.5	5.6 ± 0.3	1.6 ± 0.0
Hypochlorous acid + Acid-*organosolv* (ECA+AA)	48.3 ± 1.4	7.5 ± 0.4	1.8 ± 0.0
Hypochlorous acid + Alcohol-*organosolv* (ECA+ET)	48.7 ± 1.4	6.9 ± 0.3	1.9 ± 0.0

The presence of small amount of free monosaccharides in not-treated rachis sample could derive from natural phenomena, and they have been quantified as a benchmark. The overall amount of free pentoses (xylose and arabinose) were low, and their concentration in some cases decrease after treatments, compared with NT sample, probably due to degradation occurring in the severe conditions maintained during chemical delignification. The enzymatic saccharification of all pretreated samples showed a significant conversion of cellulose to glucose because of lignin and/or hemicellulose removal during pretreatment had permitted to cellulase and beta-glucosidase to act on their elective substrate (i.e., cellulose). It is worthwhile noting that, in general, glucose recovery followed the same trend of delignification, namely higher is the lignin loss higher is the enzymes efficiency of hydrolyze cellulose. From one side it is due to the barrier disruption which *unlocks* cellulose to enzymes, from the other side the limited enzymatic saccharification in the presence of higher lignin content can be due to the high affinity of cellulase towards lignin, which resulted in unavailability of enzyme to cellulose moieties and led to poor saccharification yields. Similar observation has by other Authors [39-40].

Among the different samples here evaluated, those treated with the active chlorine were observed to be more vulnerable to enzymatic hydrolysis and resulted in maximum glucose recovery. The higher enzymatic saccharification in SH, ECA, ECA+AA and ECA+ET pretreated samples can be attributed to the presence of higher free cellulose content with a minimum amount of residual lignin (3.7-4.2%). On the other hand, the hydrogen peroxide treatments with minimum lignin removal showed higher glucose recovery than both *organosolv* treatments. This observed reverse correspondence between percentage of delignification and glucose recovery in the case of organosolv (AA and ET) and hydrogen peroxide (HP and HPA) treatments can be right explained by different actions of various chemical agents on lignin structure [41]. It has been reported by several workers that delignification not only remove the lignin but also act as a swelling agent, which in turn enhances the surface area of the sample and make the cellulose more amenable for enzymatic action [42].

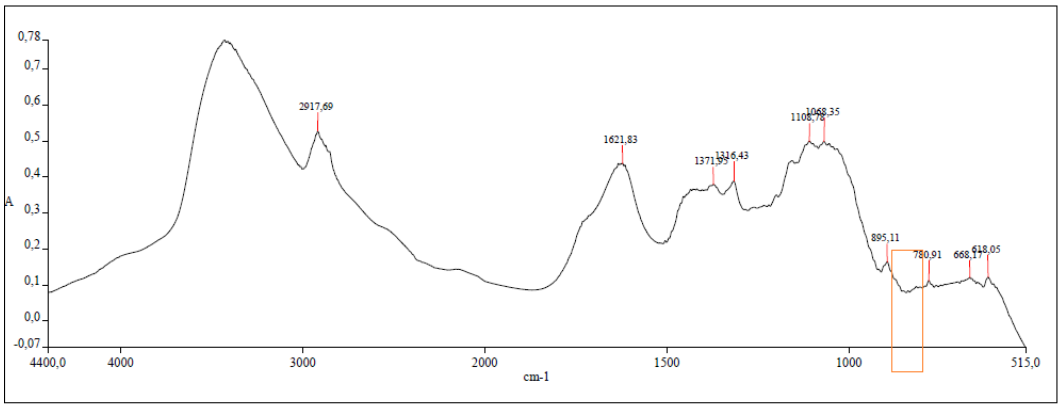
3.2 Comparison of pre-treatments effect on lignocellulose structure by FT-IR

The accessible surface area is one of the key factor affecting the hydrolysis of lignocellulosic material and the overall process efficiency. Pores dimension, bonds cleavage and structural breakage induced by the chemical agents have a great influence on enzymatic attack efficiency [43].

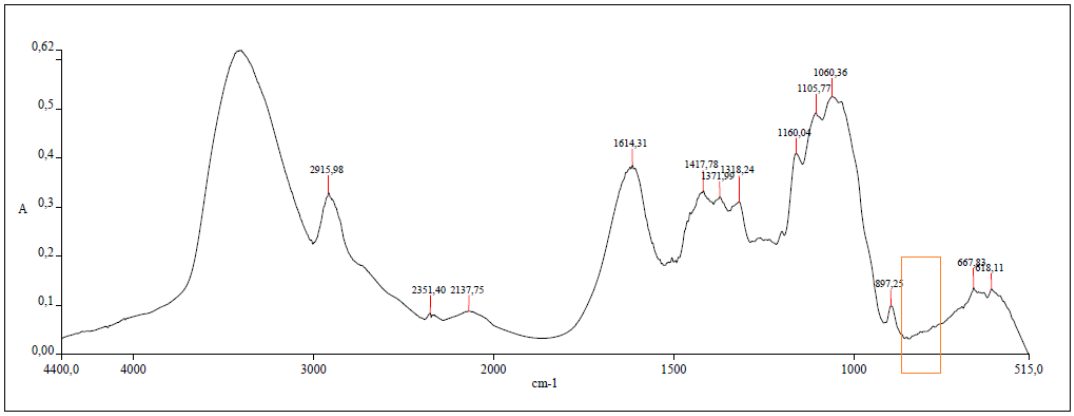
As demonstrated elsewhere [44], different chemical treatments based on ECA solution, sodium hypochlorite and *organosolv* gave rise to different residual lignocellulose structure. ECA solution was the most efficient in lignin degradation, whereas sodium hypochlorite and *organosolv* seemed to have more effect against inter-and intra-polymeric bonds of other polysaccharides of the lignocellulose structure. This finding is supported by several other authors [45-46] who suggested that the main wood component determining enzymatic hydrolyzability is not only the amount of lignin itself, but also its post-delignification structure and organization within the other wood cell wall components. In fact, beyond the quantitative aspects of the delignification yield, what differs considerably ~~in~~ the different treatments is the type of fragments that are produced during the action of the delignificant agents. During the process, the fragmenting and flaking of polymers ~~occur, consequently~~ induce a partial breakdown of ~~part of their initial~~ the chemical structure and subsequently lead to an increased ~~in~~ internal surface area and median pore volume.

Non-destructive elucidation and analysis of the lignin and hemicellulosic samples are nowadays carried out primarily by spectroscopic methods, which constitute important tools in

connection with the characterization of the polymers [47]. In this study we focused on Fourier transform infrared (FT-IR), which has enabled structural information to be derived from the intact residual lignocellulose, avoiding the possibility of degradation artifacts. Figure 3 illustrated the FT-IR spectra of not-treated rachis sample, compared with FT-IR of samples treated with active chlorine (ECA), organosolv (AA) and hydrogen peroxide (HP), as examples of the paradigm of chemical pretreatments here applied.



(a)



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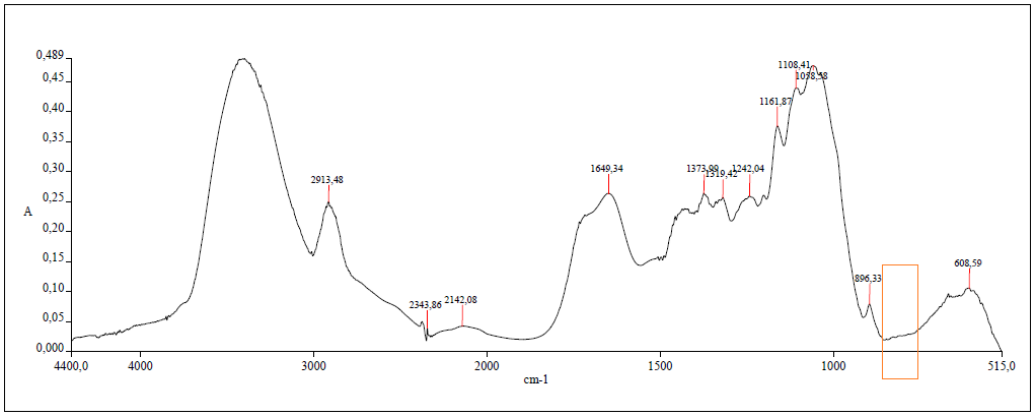

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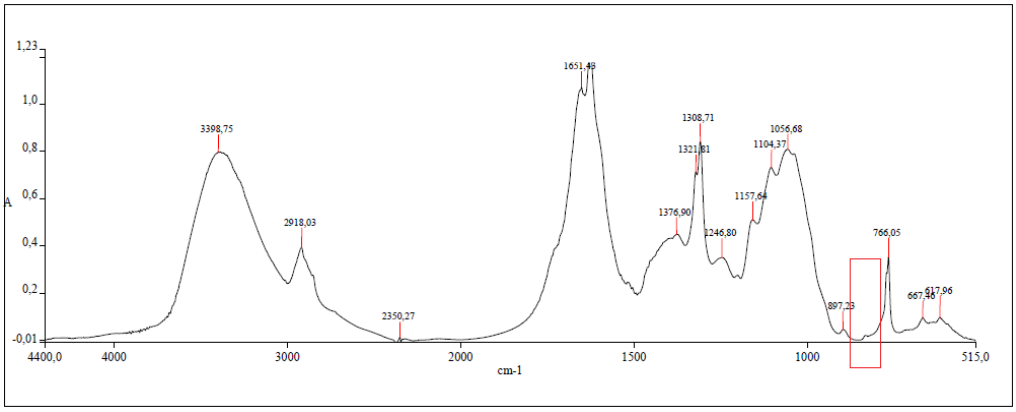

(d)

Figure 3. FT-IR spectra of rachis samples not-treated (a), treated with ECA solution (b), with organosolv AA solution (c) and with hydrogen peroxide alkaline (d), in the range 4400-515 cm^{-1}.

In the spectrum of not-treated samples (Figure 3a), typical peaks corresponding to lignin, cellulose and hemicellulose can be recognized. In particular, signals at 3600-3000 cm^{-1} corresponding to OH-stretching and at 2900-2800 cm^{-1} corresponding to CH_n stretching, together with signals at 1170-1100 and 1060 cm^{-1}, corresponding to C-O-C stretching of pyranose ring skeletal and C-OH alcoholic bonds of sugars, are found in FT-IR spectra of pure cellulose and hemicellulose [48]. Lignin is mainly responsible of signals in the finger print region (1830-700 cm^{-1}). This group of complex and superimposed peaks could indicate rachis lignin rich of metoxyl-O-CH_3, C-O-C aryl-alkyl ether linkages containing compounds and C=C bonds from aromatic rings (corresponding to 1700, 1371-1316, and 1621 cm^{-1}, respectively).

Oxidant treatment with ECA solution (Figure 3b) seems to have the most dramatic effect on lignin structure, disappearing the peak at 1730 cm^{-1} of C=O stretching of carbonyl bonds and comparing weak signals at 1500 and 1250 cm^{-1}, probably due to degradation fragments. Moreover, two new peaks are evidenced at 2351 and 2137 cm^{-1}, probably due to the production of nitrogen-based degradation products. During the oxidative delignification process, oxidation reagents release a large number of free radicals, resulting in remarkable oxidative fragmentation and removal of lignin from lignocellulosic matrix.

According to literature [49], IR-spectra analysis confirms that with the assistance of oxidation reagents, almost all lignin can be removed from lignocellulosic materials with the remaining of most cellulose and hemicelluloses, permitting a higher recovery of fermentable sugars. In the spectrum of rachis treated with *organosolv* AA (Figure 3c), on the contrary, the degrading effect on lignin appears less effective, corresponding to the low delignification yield here observed.

The oxidative action of hydrogen peroxide-derived radicals is thought to contribute to the depolymerization of lignin by attacking lignin side chains and fragmenting lignin into a number of low molecular weight compounds [50]. In fact, the fingerprint region shows a very different profile, with two spiky peaks at 1650 and 1308 cm^{-1} (Figure 3d). It is anyway worthwhile noting as peaks corresponding to cellulose and hemicellulose are here less intense, being due to a possible degradation during treatment, which reflects the low cellulose-to-glucose conversion during hydrolysis.

4. Conclusion

Delignification and saccharification of banana rachis for bioethanol production were performed and evaluated. Pretreatments have been chosen to compare solvolitic effects (*organosolv*) and oxidant effects with active chlorine in form of hypoclorite/hypochlorous acid and hydrogen peroxide, with and without alkali. Hypochlorous acid was the most performing in terms of delignification yield, and its combinations with *organosolv* did not reach a significant improvement. In general, active chlorine oxidations were the best effective for lignin removal, obtaining in the meanwhile the most promising cellulose-to-glucose conversion. Further research will be focused on optimizing the ECA pretreatment up to be applied as a valuable industrial delignification process.

Conflict of interest

The Authors declare no conflict of interest.

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