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Lignocellulosic biomass fractionation with the use of deep natural eutectic solvents

Fracionamento da biomassa lignocelulósica com o uso de solventes eutéticos naturais profundos

Fraccionamiento de biomasa lignocelulósica con el uso de disolventes eutécticos naturales profundos

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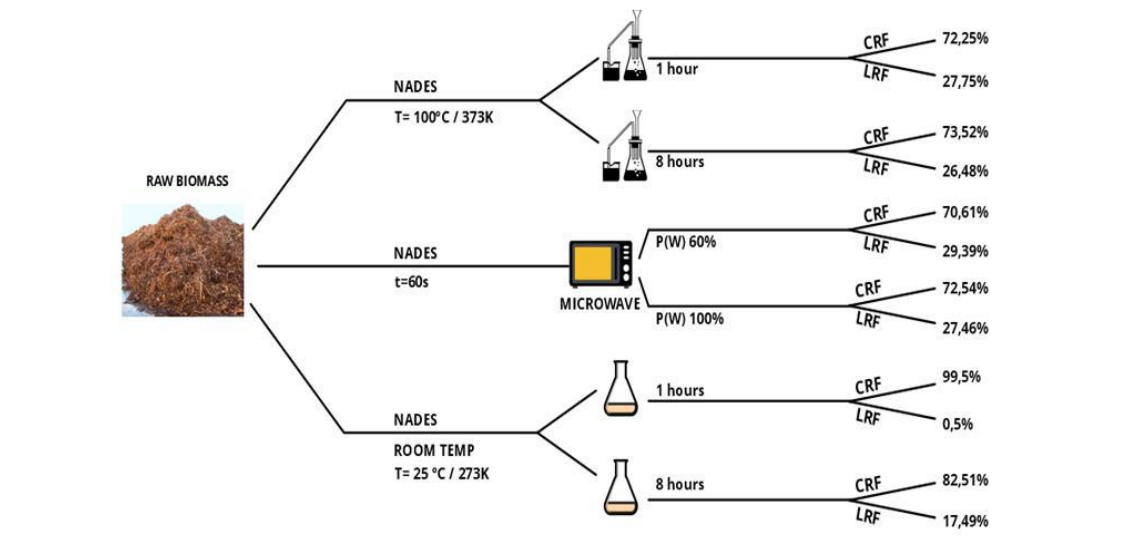
Graphic Abstract



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Abstract
Brazil has a privileged position as a leader in the integral use of vegetable biomass because it has the largest biodiversity on the planet. Adding value to a refinery is associated with the treatment and disaggregation of the biomass components. There are processes applied to biomass fractionation to remove or break the layers of lignin and hemicellulose, which allows enzymes to access cellulose more easily. In view of this a great diversity of technological routes can be used, and alternative techniques that have been developed such as the use of the natural deep eutectic solvent (*NADES*). Thus, the objective of the present work was to perform the fractionation of the bark biomass from *E. urograndis* species into two fractions: cellulose rich fraction (CRF) and lignin rich fraction (LRF) using *NADES*. In this process three conditions

were applied for biomass fractionation: conventional heating at 100ºC, microwave radiation with power of 600W and 1000 W, and room temperature at 25ºC. The percentages obtained through conventional heating were 72,25% of CRF, 27,75% of LRF; 72,54% of CRF with microwave radiation at a power of 1000W, 27,46% of LRF. The techniques used for characterization of CRF were: Fourier transformed infrared (FTIR) spectroscopy and X-ray diffractometry (XRD) for crystallinity analysis and scanning electron microscopy (SEM). For characterization of LRF the techniques were used: UV-vis and FTIR spectroscopy. The use of *NADES* a low cost, sustainable organic solvent combined with microwave radiation proved to be efficient for biomass fractionation.
Keywords: Biomass; Fractionation; *NADES.*

Resumo
O Brasil tem uma posição privilegiada como líder na utilização integral da biomassa vegetal porque possui a maior biodiversidade do planeta. A adição de valor a uma refinaria está associada ao tratamento e desagregação dos componentes da biomassa. Existem processos aplicados ao fracionamento da biomassa para remover ou quebrar as camadas de lignina e hemicelulose, o que permite às enzimas acessarem mais facilmente à celulose. Em virtude disto, pode ser utilizada uma grande diversidade de vias tecnológicas e técnicas alternativas que foram desenvolvidas, tais como a utilização do solvente natural eutético profundo (*NADES*). Assim, o objetivo do presente trabalho foi realizar o fracionamento da biomassa da casca de eucalipto da espécie *E. urograndis* em duas frações: fração rica em celulose (FRC) e fração rica em lignina (FRL) utilizando *NADES*. Neste processo foram aplicadas três condições para o fracionamento da biomassa: aquecimento convencional a 100ºC, radiação de micro-ondas com potência de 600W e 1000 W, e temperatura ambiente a 25ºC. As porcentagens obtidas através do aquecimento convencional foram 72,25% de FRC, 27,75% de FRL; 72,54% de FRC com radiação de micro-ondas a uma potência de 1000W, 27,46% de FRL. As técnicas utilizadas para a caracterização de CRF foram: espectroscopia de infravermelho transformada de Fourier (FTIR) e difratometria de raios-X (XRD) para análise de cristalinidade e microscopia eletrônica de varredura (MEV). Para a caracterização de FRL foram utilizadas as técnicas: UV-vis e FTIR. A utilização de *NADES*, um solvente orgânico sustentável e de baixo custo, combinado com radiação de micro-ondas provou ser eficiente para o fracionamento da biomassa.
Palavras-chave: Biomassa; Fracionamento; *NADES.*

Resumen
Brasil tiene una posición privilegiada como líder en el uso integral de la biomasa vegetal porque tiene la mayor biodiversidad del planeta. El valor añadido de una refinería está asociado al tratamiento y la disgregación de los componentes de la biomasa. Existen procesos aplicados al fraccionamiento de la biomasa para eliminar o romper las capas de lignina y hemicelulosa, lo que permite que las enzimas accedan más fácilmente a la celulosa. Em vista de ello, se puede utilizar una amplia diversidad de rutas tecnológicas y técnicas alternativas que se han desarrollado, como el uso del disolvente eutéctico profundo natural (*NADES*). Así, el objetivo del presente trabajo fue realizar el fraccionamiento de la biomasa de la corteza de la especie de eucalipto *E. urograndis* en dos fracciones: fracción rica en celulosa (FRC) y fracción rica en lignina (FRL) mediante *NADES*. En este proceso se aplicaron tres condiciones para el fraccionamiento de la biomasa: calentamiento convencional a 100ºC, radiación de microondas con potencias de 600W y 1000 W, y temperatura ambiente a 25ºC. Los porcentajes obtenidos mediante calentamiento convencional fueron 72,25% de FRC, 27,75% de FRL; 72,54% de FRC con radiación de microondas a una potencia de 1000W, 27,46% de FRL. Las técnicas utilizadas para la caracterización del FRC fueron: la espectroscopia infrarrojo por transformada de Fourier (FTIR) y la difractometría de rayos-X (XRD) para el análisis de la cristalinidad y la microscopía electrónica de barrido (MEB). Para la caracterización del FRL se utilizaron las seguientes técnicas: UV-vis y FTIR. El uso de *NADES*, un disolvente orgánico sostenible y de bajo coste, combinado con la radiación de microondas, demostró ser efectivo para el fraccionamiento de la biomasa.
Palabras clave: Biomasa; Fraccionamiento; *NADES.*

1. Introduction

In order to increase the accessibility to lignin there are pre-treatments that remove hemicellulose and remove or modify the structure of lignin and are important for exposing cellulose, as the research which emphasizes the efficiency of pre-treatment processes for the production of lignin (Sá & Bianchi, 2015).

Most of these Pretreatment have advantages and disadvantages such as high energy and reagents cost, formation of inhibitors that can occur in alkaline, acid, and oxidative Pretreatment (Trajano &Wyman, 2013).

As an alternative route to the fractionation of lignocellulosic biomass, natural deep eutectic solvents (*NADES*) have been considered as variation of ionic liquids, because they have similar physicochemical properties: they are liquids at room temperature. They constitute a good alternative because they have some advantages over other ionic liquids, highlighting lower

cost and ease of synthesis. Thus, they ensure a significant reduction in the cost process, demonstrating economic their relevance to overcome the problems of known methods and minimize the generation of waste and consequently the costs related to their treatment (Dai et al., 2015).

In recent years, Pretreatment with *NADES* has been studied since they are easy to synthesize, and according to a study developed by Hou et al. (2017) are biodegradable and environmentally friendly, are products of the interactions of organic acids and sugars through hydrogen bonds and Van der Waals type interactions, offer advantages such as low toxicity, biodegradability, sustainability, and simple preparation.

The application of *NADES* associated with microwave radiation characteristics evidenced in the study proposed by Chen &Wan (2018) that revealed that more than 90% of sugar production can be obtained from pretreated corn waste and corn cob using *NADES*-based choline chloride (ChCl).

The research conducted by Hou *et al.* (2017) points out that radiation directly applied on biomass generates an electromagnetic field to molecular structures of heated objects, microwave led to biomass breakdown mainly through molecular collision due to dielectric polarization. In addition, microwave irradiation can maximize ionic characteristics and increase the molecular polarity of the solvent.

In this sense the present study aims to promote the fractionation of eucalyptus bark biomass of *Eucalyptus urograndis* species into two fractions, using *NADES* composed of a mixture of organic sugars: (glucose, fructose and sucrose), a sustainable solvent of low cost, whose mixture presents melting point which is lower than that of its constituents, does not present toxicity or generation of waste as ionic liquids, organosolvents or acid and alkaline treatments, and its use associated with microwave radiation, optimize the efficiency of *NADES* for the fractionation of lignocellulosic biomass.

2. Methodology

2.1 Physical preparation of the sample

The bark of the eucalyptus species *E. urograndis* was washed with running water and dried in an oven with forced air circulation at 40 °C until constant mass. Then it was grounded in a Tecna l® Willey type TE 680 mill and passed through a 60-mesh sieve, the bark was grounded to obtain a homogeneous powder and medium granulation and stored in plastic bags under refrigeration until its use.

2.2 Characterization of the eucalyptus bark biomass

The determinations of cellulose, hemicellulose and lignin contents were performed as proposed by (Lin et al., 2004), and as described below:

For the determination of extractables from eucalyptus bark 5 g of the bark was heated at 100°C in Soxhlet extractor for 8h, using about 300 mL of hexane PA. The difference in mass corresponds to the extractable content.

In order to determine the hemicellulose and lignin content: 1 g of the bark material (free of extractables) was weighed and 10 mL of aqueous sodium hydroxide solution (0.5 M; 20 g/L) was added; the mixture was heated for 3.5 hours, filtered, and washed with distilled water; the difference in mass corresponds to the hemicellulose content; and 1 g of the extractables-free bark was treated with 30 mL of aqueous 72% sulfuric acid solution. The filtered residue corresponds to the lignin content. The percentages of the bark constituents: extractables, cellulose, hemicellulose, and lignin are described in Table 1.

2.3 Preparation of *NADES*

For the preparation of *NADES,* a solution of fructose, glucose, sucrose and water in molar ratios of 1:1:1:11 was prepared by heating until complete dissolution and reserved in refrigeration 8°C (Dai *et al.*,2015).

2.4 Fractionation of biomass using *NADES*

Portions with about 1 g mass of Eucalyptus bark free of extractables were weighed, accurately, in triplicate and transferred to a beaker, were subjected to the following conditions by applying the *NADES*: addition of 10 mL and resting for 1h at room temperature, addition of 50 mL subjected to conventional heating for 1h at 100º C, addition of 30 mL, subjected to microwave treatment interspersed 60 seconds with radiation and without radiation at a power of 1000W.

The same protocol was performed with 1g of extractables-free samples in triplicate by changing the exposure time and/or *NADES* volume: addition of 10 mL of *NADES* and resting for 8h at room temperature, addition of 50 mL subjected to conventional heating for 8h at 100º C, addition of 30 mL, subjected to microwave radiation for 60 seconds with radiation applied at a power of 600W. After fractionation the samples were filtered. The cellulose rich fraction (CRF) was washed 5 times with distilled water and characterized by scanning electron microscopy (SEM), Fourier transformed infrared (FTIR) spectroscopy and X-ray diffractometry (XRD) for crystallinity analysis. The lignin-rich liquid fraction (LRF) was mixed with 100mL of distilled water and centrifuged for 15 minutes and characterized by UV-vis and FTIR spectroscopy.

2.5 Characterization methods

The UV-visible absorption spectrophotometry of the lignin-rich fraction FRL was performed in a UV-Bel phofonics spectrophotometer, single beam, 1200 lines/mm grid, volume 1000 µL. For the application of the UV-vis technique the samples of the LRF lignin rich fractions were diluted in proportions: 100 µL of LRF to 900 µL of water, in 1000 µL capacity cuvette: the analyses were carried out in the laboratory of technological chemistry, Goiano Federal Institute Rio Verde *Campus* Brazil.

The infrared spectroscopy analyses were performed in the FTIR Spectrometer Frontier PerkinElmer (Frontier®) equipment with Total Reflectance attenuated at a frequency of 600-4000 cm^{-1} in the Analytical Center also in the Goiano Federal Institute Rio Verde *Campus*, Brazil.

The scanning electron microscopy (SEM) analyses were performed in a Tesam Amber® apparatus with an electron accelerating voltage of 5kV, operating in secondary electron detection (SED) mode. X-ray diffractometry was performed in a Bruker® diffractometer using monochromatic radiation from a tube with a copper anode coupled to a Johansson monochromator for K1 operating at 40kV and 40mA, Bragg- Brentano -2 configuration, Lynxeye one-dimensional detector, 2 range from 2º to 70º, with a 0.01º step. The samples were kept rotating at 15 rpm during the measurement. The result of each diffractogram is the average of six measurements for each sample. The analyses were performed at the Regional Center for Technological Development and Innovation of the Federal University of Goiás (UFG), Brazil.

2.6 Statistical analysis

Statistical analyses were performed using the ANOVA test. The samples were analyzed in triplicate and the average, standard deviation, confidence intervals of the means and variances were calculated with a confidence limit of 95% ($p<0.05$). Aiming to make the comparison between the average of the same treatment group and between different groups, Tukey's test was applied at 5% significance level. The minimum significant difference DMS was calculated to obtain the contrast of sample average for statistical differences between treatments.

3. Results and Discussion

3.1 Characterization of eucalyptus bark biomass

The results of the determined compositions of the constituents of the eucalyptus bark are listed in Table 1.

Table 1 - Determination of the contents in percentage of the main constituents of eucalyptus bark content.

Extractable content (%)	Hemicellulose content (%)	Cellulose Content (%)	Lignin content (%)
10,3 ± 0,6	36,5± 1,15	38,14 ± 0,1	15,06 ±0,64

Source: Authors.

The data obtained shows an average percentage value of 10.3 % found for extractables content with a coefficient of 0.6% with a similar result which was also found in a study conducted by Chen (2014). The extractives present in the bark of commercial eucalyptus species range from 5 to 20%. Most are present in the inner bark, rich in elaborate sap, practically already solubilized in water (Mika et al., 2018).

The differences found between the contents of biomass constituents depend on the way the bark was sampled, the ratio between inner and outer bark, and the physiological situation of the tree (Trugilho et al., 2003).

The hemicellulose content obtained was 36.5% with standard deviation of 1.15%, close to values found by Chen (2014), whose percentage found is 20 to 40% for hemicellulose.

The chemical composition of lignocellulosic biomass generally contains 35-50% cellulose, followed by 20-35% hemicellulose, 10-25% lignin and a small amount of ash and extractives. This chemical composition varies depending on the type of biomass (Trugilho *et al*., 2003).

3.2 Results obtained for FRC and FRL after fractionation of biomass with *NADES*

Table 2 presents the contents of FRC and FRL obtained after fractionation with *NADES*: for biomass exposure time of 1 and 8 hours (h)

Table 2 - Data obtained for CRF and LRF after 1 and 8 h treatments with *NADES:*

Treatment	Room temperature 25ºC		Conventional Heating 100ºC		Micro-wave radiation	
					1000W	600W
Weather in (h)	1[c]	8 [b]	1 [a]	8 [a]	1 [a]	1 [a]
CRF (%)	99,5 ± 0,10	82,51±0,03	72,25±0,53	73,52 ±0,06	72,54± 1,3	70,61±0,08
LRF (%)	0,5 ± 0,01	17,49±0,17	27,75 ±0,7	26,48 ±0,25	27,46 ±1,3	29,39±0,23

Source: Authors.

The results obtained in Table 2 shows that: treatments that have the same letter (a) are statistically equal do not differ by Tukey's test at 5% significance. For the 1h treatment: Fcal>Ftab.and p-value <0.01.

The fractionations that were statistically similar were: conventional heating (1 and 8h) and microwave when applied power of 1000W and 600W.The fractionations (b) and (c) were statistically different: room temperature at 25°C and applied time of 1 and 8h.

3.3 Application of microwave radiation combined with *NADES*

The microwave extraction method is promising for the pretreatment of lignocellulosic biomass (Mood et al., 2013).

According to a study by Hou *et al*. (2017) this pretreatment combined with the use of eutectic solvents can be done in a very short time but leads to high digestibility of cellulose.

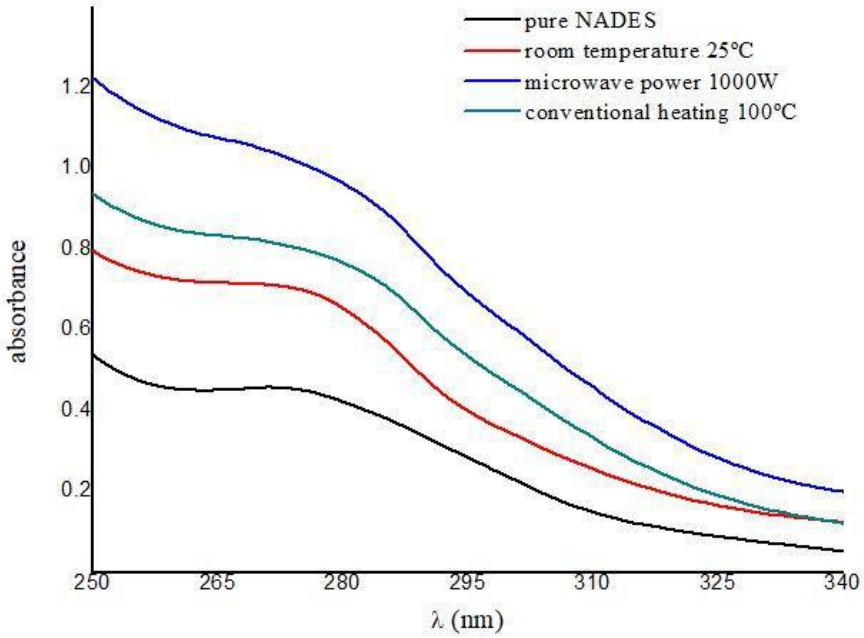
By directly applying an electromagnetic field to molecular structures of heated objects, microwave leads to biomass breakdown mainly through molecular collision due to dielectric polarization according to a study by Chen (2014). In addition, microwave irradiation can maximize ionic characteristics and increase molecular polarity of solvent which influence digestibility result also observed by Liu et al. (2017).

From the point of view of energy and economics, the treatment performed with *NADES* for 1 hour via conventional heating at 100°C and microwave with power of 1000 and 6000W were more effective for the fractionation of biomass Table 2. Aditionally the treatment with *NADES* at room temperature with 8h seems to be statistically significant when compared to the others that were heated, so this treatment presents itself as a promising alternative for the fractionation of lignocellulosic biomass, which even without heating obtained the fractionation under condition.

3.4 Results obtained by UV-vis analysis

All the treatments performed using *NADES* for 1h, submitted to the following conditions: room temperature 25ºC, conventional heating 100ºC and microwave radiation with 1000W power were analyzed by absorption spectroscopy in the UV-Vis region. The spectra obtained are presented in Figure 1.

Figure 1 - UV-vis. Spectra obtained for the different treatments with *NADES*.



Source: Authors.

It is observed in figure 1 that for the same heating time of 1h the conventional fractionation at 100°C and microwave applied at 1000W power there were higher absorbance bands at the wavelength of 280 nm when compared to pure *NADES*. In the fractions with higher absorbance it is suggestive the presence of other constituents which are different from the carbohydrates of *NADES* (Kumar et al., 2016).

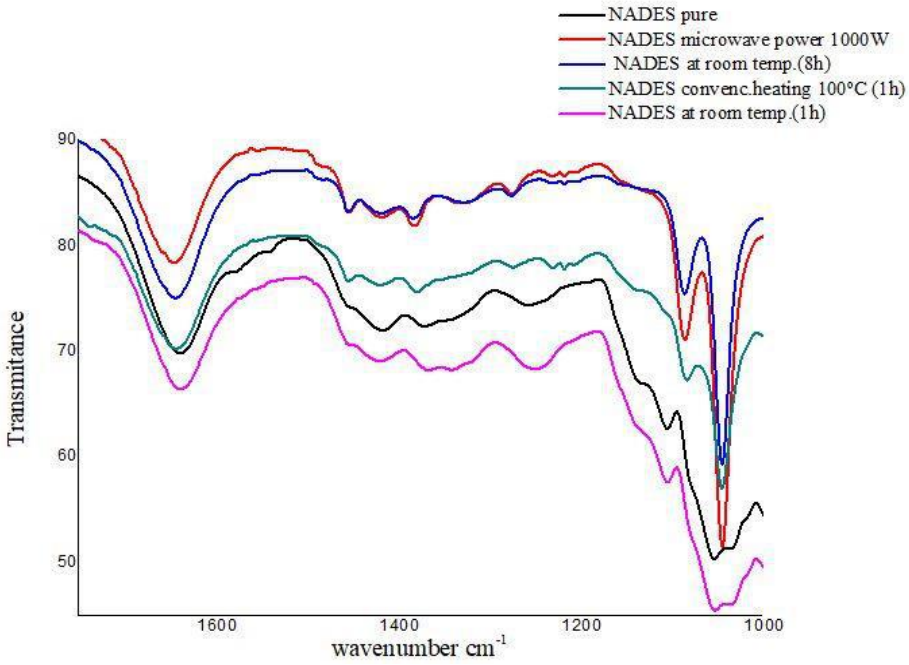
In a research conducted by Skulcova et al. (2017) lignin was identified in absorbance values at 205 or 280 nm, and similar range was observed in the spectrum of *NADES* solution Figure 1 on conventional heating for 1h.

3.5 FTIR spectroscopy analysis

From the FTIR spectra it is possible to notice the appearance of bands related to functional groups characteristic of the substances present in LRF. The spectrum of pure *NADES* was performed to compare bands of possible functional groups of LRF.

The FTIR spectroscopic analyses of the following samples were done: pure *NADES* solution, *NADES* solution of LRF via conventional heating at 100°C, 1h and 8h, *NADES* solution of FRL with application of radiation at 1000W power and *NADES* solution at room temperature 25°C for 1h. The spectra are presented in Figure 2.

Figure 2 - FT-IR spectra of biomass samples fractionated with *NADES*.



Source: Authors.

In Figure 2 a peak is observed at approximately 1720 cm^{-1} that can be attributed to the stretching of the carboxylic group (C=O) observed in the *NADES* solution with 1h conventional and microwave heating. In a study developed by Arafat et al. (2019) related to lignin characterization, found regions at 1703 cm^{-1} C=O vibration and at 1154 cm^{-1} C-O-C vibration typical of lignin groups.

The band at 1680 cm^{-1} can be attributed to the carbohydrates of *NADES* being a common band to the other spectra.

The band between 1640-1600cm^{-1} in Figure 2 is related to aromatic C=C ring vibrations that is observed in almost all spectra. In the study of with biocarbons reports the presence of the aromatic ring in the region of 1600-1500 cm^{-1} through the absorption band of the C=C bond of the ring (Pinto, 2018).

The band at 1160 cm^{-1} is related to the vibration of C-O-C, in Figure 2 found quite intensely in the sample subjected to microwave heating at 1000W power which can be attributed to the vibration of typical lignin groups.

The band at 1430 cm^{-1} is characteristic of crystalline cellulose, due to the aromatic rings combined with stretching and deformation in the C-H plane. The band at approximately 900 cm^{-1}, is related to cellulose, a reduction of the band is observed in the *NADES* treated material under heating, indicating a structural change of the sample (Dunn et al., 2019; Huang et al., 2011).

The stretching at 870 cm^{-1} is related to a C-O angular deformation of the methoxyl group, observed in the *NADES* samples submitted to microwave treatment with 1000W power and conventional heating for 1h, such deformation is not observed in the spectrum of the pure *NADES* sample or at room temperature submitted for 1h.

Total crystallinity index (TCI), hydrogen bonding intensity (HBI) and lateral order index (LOI) were calculated by FTIR spectroscopy to associate the changes in cellulose crystallinity. The calculation of the TCI index can be found by the ratio of the

absorption between the bending of O-H groups and the elongation of C-H groups, the TCI is proportional to the degree of crystallinity of cellulose can be calculated by Equation 1 (Nelson & O'Connor, 1964).

$$(TCI = A1375\ cm^{-1}/A2900\ cm^{-1}) \quad \text{Eq. (1)}$$

The lateral order crystallinity LOI is the absorption ratio between the bending of the C-H2 groups and the β-glycosidic bonds Equation 2 (Nelson & O'Connor, 1964).

$$(RIGHT = A1420\ cm^{-1}/A893\ cm^{-1}) \quad \text{Eq. (2)}$$

Table 3 presents the results obtained from the TCI, LOI and HBI indices calculated on the biomasses after treatment.

Table 3: HBI index, TCI, LOI of crude and fractionated biomass with *NADES.*

	FTIR Index		
Biomass	HBI	TCI	LAW
	3338/1334 (cm·cm^{-1})	**1375/2900 (cm·cm^{-1})**	**1420/893 (cm·cm^{-1})**
1 Gross	1,031	0,94	1,032
2 with 1h heating	1,0097	0,95	1,042
3 with 8h heating	1,0201	0,94	1,034

Source: Authors.

The Hydrogen Bonding Intensity HBI, is related to the degree of intermolecular regularity as well as the amount of water. HBI was used to observe the hydrogen bonding changes between certain hydroxyl groups in cellulose. It can be determined by Equation 3 (Nelson & O'Connor, 1964).

$$(HBI = A3338\ cm^{-1}/A1334\ cm^{-1}) \quad \text{Eq. (3)}$$

The inter- and intramolecular hydrogen bonds are responsible for maintaining the crystalline regions and make cellulose highly resistant to acid, alkaline or enzymatic hydrolysis. The HBI result denotes the decrease in the HBI ratio of the *NADES* treated samples which infers in modifications and/or disruption of the hydrogen bonds that maintained the regularity of the crystalline region which after the treatment were modified (Oh et al., 2005).

For the crude biomass and treated for 1h there was an increase in the total crystallinity index, TCI and there was also an increase in the degree of lateral order, LOI in the biomasses treated with *NADES* which relates to increase in the crystalline fraction as a result of the exit of part of the amorphous fraction associated with the use of *NADES* (Nelson & O'Connor, 1964).

The higher values found for overall degree of order in LOI cellulose denotes an increase in the crystallinity index of the treated biomass. The ratio of amorphous to crystalline regions of cellulose decreased after pretreatment due to partial removal of hemicellulose and lignin from the material and not by an increase in crystalline regions. As a result, an increase in crystallinity of the material occurs after pretreatment (Brienzo et al., 2015).

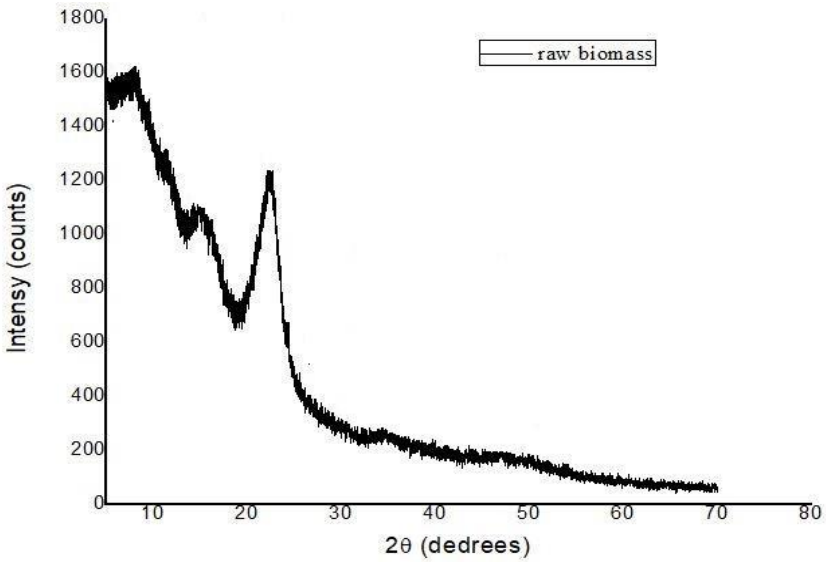
3.6 X-ray diffractometry (XRD)

The crystallinity of biomass is an important characteristic affecting the enzymatic hydrolysis activity. The crystallinity index shown in Table 3 was calculated from the maximum intensity obtained in the main crystallinity peak of the diffractogram and the minimum intensity located between the two crystalline peaks by Equation 4 (Segal et al., 1959).

$$Cr\ I\ (\%) = [(I002\text{-}Iam)/I002] \times 100 \qquad \text{(Eq 4)}$$

Figure 3 depicts the XRD spectrum of the crude biomass that denotes lower intensities for same 2θ angle when compared to Figure 4 that presents the biomass fractionated with *NADES*.

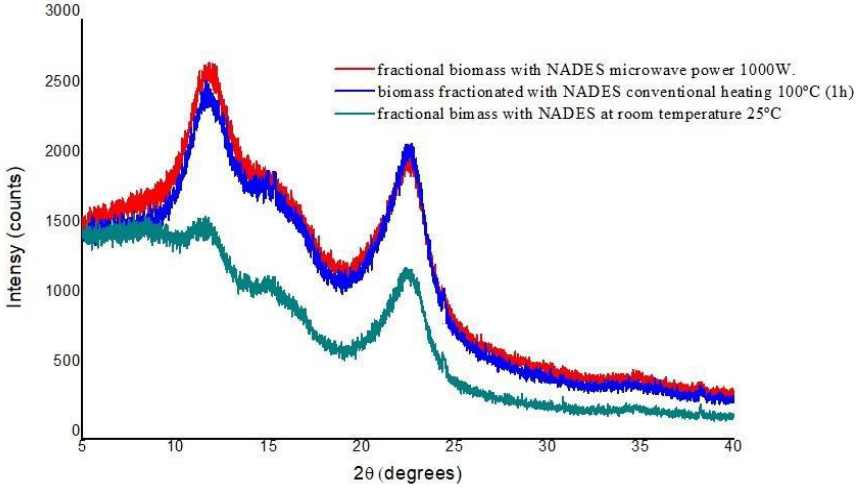
Figure 3: XRD spectrum of crude biomass.



Source: Authors.

Figure 4 depicts the XRD spectra of eucalyptus bark fractionated with *NADES*.

Figure 4: Xrd spectra of crude and fractionated biomass with *NADES*.



Source: Authors.

The intensities of the crystalline peaks and the amorphous halo were obtained from the XRD plots. The crystallinity index along with the intensity of the main crystalline plane (2θ) and the amorphous fraction was calculated using the intensity between 18° and 19° for pulp I and the maximum intensity between 18° and 22° for pulp II, for the crystalline fraction and the results obtained are presented in Table 4.

Table 4 presents the crystallinity index calculated for the crude sample, the sample fractionated with *NADES* at room temperature 25°C, and conventional heating 100°C.

Table 4 - Crystallinity index of biomass after fractionation of lignocellulosic biomass fractionated with *NADES* for 1 h:

	Gross Biomass	room temperature 25ºC	Conventional heating 100ºC	Potence 1000W Microwave
18°	749	1221	1184	666
22°	1099	1873	1918	1068
Crystalline index fraction Crystalline cellulose I CI% 18º≤2θ≤22º	31,8	34,3	35,6	37,6

Source: Authors.

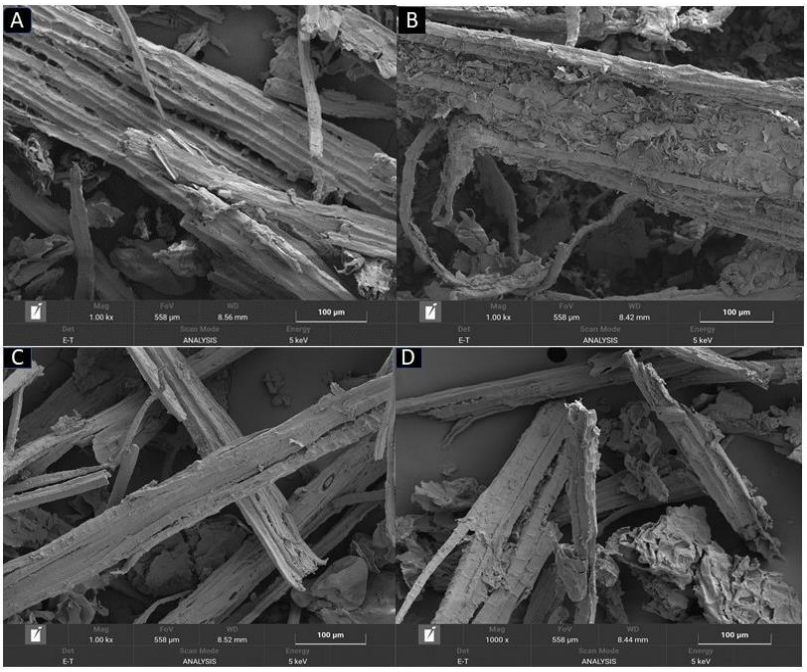
According to Table 4 an increase in CI is observed for biomass in the 1h conventional and microwave heating applied 1000W power. According to a study by Karimi & Taherzadeh (2016) the increase in CI is suggestive to the attack of the solvent on the amorphous regions of cellulose and hemicelluloses which are more susceptible, also causing the swelling of the crystalline region which leads to the increase of the internal surface area and decreases the degree of polymerization.

A similar study with alkali treatment by Oh *et al.* (2005) describes that the likely initial decrease in the degree of polymerization by the breakdown of the amorphous regions of cellulose happens. The increase in the CI of the amorphous regions after pretreatment with *NADES*, is done by removing the amorphous regions of the cellulose, increasing the crystallinity of the samples.

3.7 Scanning electron microscopy

The structural changes of the crude and fractionated biomass with *NADES* can be observed in the images obtained by scanning electron microscopy (SEM). The crude sample Figure 5(A) showed a rigid and smooth surface, as the fibrillates were all intact. However, after biomass fractionation Figure 5(C) and (D), the samples showed cell deformation and fiber loosening suggestive to the effect of *NADES*. Through the micrographs obtained it is possible to observe the structural changes by the appearance of cracks, with probable appearance motivated by the dissolution of hemicelluloses and destructuring of the lignocellulosic material, changes such as increased surface area between the raw biomass (A) and the one treated with microwave radiation (D) (Singh et al., 2020).

Figure 5 - Images of biomass scanning electron microscopy after fractionation with *NADES*. A = Crude biomass; B= Fractionated biomass at room temperature 1h; C= Fractionated biomass with conventional heating 1h; D= fractional biomass with 1000W microwave radiation of power ,1h. The Images of The SEM were enlarged 1000 times.



Source: Authors.

4. Conclusion

The exposure of lignocellulosic biomass to *NADES* under heating of 1h and microwaves with applied power of 1000W and 600W, resulted in the fractionation of about 70% of CRF and 30% of LRF, and for the condition submitted to room temperature results in fractionation around 80% of CRF and about 20% of LRF, which denotes the effect of *NADES* on biomass even without the use of heating. This allows us to infer that this process in the fractionation chain can contribute to reduce the recalcitrance of the lignocellulosic material for further enzymatic process.

The set of analyzed results denotes the positive effect of *NADES* on lignocellulosic biomass: contributing to its morphological modification, the preservation of CRF that can be used for a subsequent process such as paper production among others. The non-toxicity of *NADES*, energy saving, no waste generation applied to the process are effects that can be attributed to the use of this sustainable solvent, besides the low cost of the process.

The use of eutectic solvents for the fractionation of eucalyptus bark biomass may contribute to further comparative studies with other solvents such as ionic liquids and acids, for example. It is also possible to expand the use of *Eucalyptus spp* bark biomass to the production of sugars, cellulose and hemicellulose derivatives, bioethanol, nanocellulose particles and resins.

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