Lignin Valorisation from Side-Streams Produced during Agriculture Waste Pulping and TCF Bleaching

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The lignocellulosic raw materials used for cellulose production contain other interesting components such lignin. In this work, an approach for the valorisation of side-streams from cellulose extraction and purification processes was carried out. The working procedure began with a sulfur-free pulping process for the separation of lignin. It consisted in an organosolv process using ethanol-water (70:30 v/v) as white liquor during 90 min at 180 ºC and a solid to liquid ratio of 1:15. After the separation of the solid fraction from the black liquor, two total chlorine free bleaching stages were effectuated to obtain high purity cellulose for further nanocellulose production. The bleaching stages consisted in a double oxygen treatment with sodium hydroxide (1.5 % by weight). Finally, lignin from the black liquor as well as from the spent bleaching liquors was isolated by selective precipitation in order to be valorised as a source of phenolic compounds. The comparison between the obtained lignins from the different stages in term of extraction yield and physico-chemical properties was carried. Higher yields in lignin extraction were achieved from agave bagasse than from leaves (around 90 % for bagasse and more than 70 % for leaves). As it was expected, the lignin extraction yields were reduced in each stage. The purity of the lignins referred to the acid insoluble and soluble lignin, was also lower after the bleaching stages, but a level of purity higher than 75 % remained in every sample. The molecular weight distribution of all the samples and the functional chemical groups were evaluated by means of gel permeation chromatography and Fourier transform infrared spectroscopy, demonstrating the potential of the valorisation of lignin as a part of an integrated biorefinery process.

1. Introduction

Generally the use of biomass valorisation is reduced to energy generation; however, the biomass presents a chemical composition that has the potential to be transformed into chemicals and bioproducts that are currently obtained from fossil resources by means of the conversion of its main macrocomponents: cellulose, hemicelluloses and lignin (Nunes et al., 2016).
Blue agave bagasse is one of the most abundant agricultural waste products in western Mexico since it represents up to 40 % (on a wet weight basis) of the blue agave (*Agave tequilana*) plant, a traditional crop cultivated for the production of the distilled spirit called *tequila* after extracting sugars from its stem, which is shredded and milled after being cooked, thus being an undervalued product (Kestur et al., 2013). Recent works were carried out with the aim to use blue agave bagasse as a source for high value-added materials such as nanocellulose (Robles et al., 2015). These studies have demonstrated the possibility to valorise a residual stream into high value-added products with properties similar to those obtained from neat raw material, but with lower production costs.
Nevertheless, the works focused on the nanocellulose production have generally omitted the valorisation of the whole biomass fractions, forgetting for instance the side-streams produced during the cellulose purification that are rich in lignin.

Lignin is mainly extracted during the pulping process, although a smaller fraction still remains in the cellulose pulp. For this reason bleaching sequences have to be applied to achieve purified fibres with high cellulose content (> 98 %) for their further defibrillation or hydrolysis. Lignin can be removed from the lignocellulosic raw material by several methods. The most widely used processes in the industry have been the sulphate (Kraft) and the sulphite process. However, environmental aspects and the difficulty of the valorisation of the lignin extracted by these methods due to its non-uniform structure or the presence of organic and inorganic impurities; have made sulphur free delignification processes emerge as novel possibilities (Vishtal and Kraslawski, 2011). Organosolv process is one of the most relevant methods, which is based on the solubilisation of lignin using a mixture of different organic solvents and water as cooking liquor, extracting highly homogeneous lignin, which enables its further valorisation into value-added products. As a result, high lignin enriched liquor is obtained. In addition, this process enables the solvent recovery by distillation, allowing its reutilization as fresh liquor (González-Alriols et al., 2010). Therefore, the production of high-value added materials from these streams can be carried out, reducing the cost of the used feedstock and generating more than just one final product from the same feedstock, approaching a multiproduct integrated biorefinery (Fernández-Rodríguez et al., 2015).

Total Chlorine-Free (TCF) sequences constitute environmentally friendly alternatives to remove phenolic chromophores originated from precipitated lignin, since chlorine has been eliminated from industrial bleaching of most pulp types because of its negative environmental effects. Thus, the industry has to focus in getting the highest yield of final products as a consequence of the higher production costs of TCF bleaching sequences (Ibarra et al. 2006).

Lignin is considered the second more plentiful natural polymer after cellulose. Its complex structure, consisting of a dimensionally randomized net built by three main phenilpropane units, namely coniferyl alcohol (G), sinapyl alcohol (S) and *p*-coumaryl alcohol (H). In spite of their recalcitrant behavior and its traditional use in the industry for energy generation as a byproduct from pulp production, it can be converted into value-added products (Weinwurm et al., 2014). The obtaining and conversion of lignin to value-added products is an essential part of the integrated biorefinery concept and together with nanocellulose extraction, it can offer good cost/benefit balances (Modahl et al., 2015). Therefore, by the isolation not only of the cellulose, but also of the lignin contained in the blue agave, the valorisation of all the fractions that compound this whole biomass waste can be considered integrally, boosting the concept of the "Integrated Waste Biomass Biorefinery".

In this work, the extraction and characterization of lignin obtained as a byproduct from the global process of purification of cellulose from blue agave bagasse and leaves is carried out. In this way, the possibility of the complete valorisation of this lignocellulosic waste is studied, taking into account the principles of the integrated biorefinery, treating also the valorisation of other fractions such as lignin, by means of environmental friendly processes.

2. Materials and methods

2.1 Raw material and equipment

Blue agave (*agave tequilana*) bagasse and leaves were provided by Eng. D. R., a local cropper from Amatitán, Jalisco, Mexico. Bagasse is obtained from tequila industry, where it is subjected to an autohydrolysis stage; leaves (or stalk) are trimmed from the core and depithed with an industrial depither. The rest of reagents were provided by Sigma-Aldrich. All reactions were carried out in a 4 L batch stainless steel reactor (EL0723 Iberfluid) with PC-controlled stirring, pressure and temperature.

2.2 Experimental set up

The stages of the whole process (pulping and bleaching procedures) were based on the approach of developing an environmentally friendly process to obtain different biomaterials from this agricultural waste stream. Organosolv process was accomplished as a sulphur-free delignification method in the pulping stage. After that, the remaining cellulose was subjected to a bleaching stage in order to obtain a high purity material for their further conversion into nanocellulose entities. The selected process for this purpose was a Total Chlorine Free Bleaching (TFC) sequence, which was based on two identical oxygen bleaching stages, consisting in the oxidation of the fibers to solubilize lignin in alkaline liquor.

2.2.1. Organosolv

Organosolv delignification process was conducted using a mixture of ethanol/water (70:30 v/v) as white liquor, at 200 ºC during 90 min and a fibre to liquid ratio of 1:15 (w/v); conditions used by Gordobil et al. (2014). Liquid fraction was separated via filtration and the solid was washed several times until driving the remaining fibres until neutral pH level. Fibbers were dried and separated for further treatment.

2.2.2. Total Chlorine Free Bleaching

An industrial type of total chlorine free TCF bleaching process was performed on the pre-treated fibres using an O-O-P_Q-P_O sequence. This sequence from which black liquors were extracted, were those corresponding to the double oxygen stage in which water was set at pH 11 using NaOH and 0.2 wt% $MnSO_4$ to neutralize remaining metals. This stage was performed under a 6 bar oxygen atmosphere at 98 ºC during 60 min each time. After these stages, pulp followed peroxy stages from which no relevant liquor was obtained, thus it was discarded for this work (Ibarra et al. 2006).

2.2.3. Lignin precipitation

The lignin precipitation method used in this study is different according to the process in which lignin was extracted. In this sense, lignin from organosolv pulping was isolated adding 2 volumes of acidified water (pH=2, water adjusted with H_2SO_4). In the case of bleaching liquors, due to the alkalinity of these streams, the precipitation of lignin was reached by acidifying the liquors with concentrated H_2SO_4 until pH=2. After that, precipitated lignin is filtrated with polyamide filters (0.2 μm pore size), washed with acidified water and dried at 50 ºC during 24 h.

2.3 Analytical methods

The initial characterization of the blue agave bagasse and leaves was conducted according to their macromolecular components. TAPPI standard methods (Tappi 2007) were used for this purpose. Therefore, moisture (TAPPI T264-97), inorganic content (TAPPI T211 cm-93), ethanol-toluene extractives (TAPPI T204 cm-97), Klason lignin (TAPPI T222 cm-98), holocellulose (Wise et al., 1946), cellulose (Rowell, 1984) and hemicelluloses, as difference between hollocellulose and cellulose were quantified for evaluating the initial potential of these waste streams.

After the lignin precipitation in the different stages of the global process, a physico-chemical characterization was carried out in order to determine the purity and composition of each obtained sample.

Acid Insoluble Lignin (AIL) as well as Acid Soluble Lignin (ASL) were measured following the TAPPI UM250 um-83 standard for quantifying the purity of lignin samples. This step was carried out firstly adding 3.75 mL of sulfuric acid 72 wt. % to 0.375 g of precipitated lignin and leaving during 1 h at 30 ºC. Secondly, the mixture was diluted with 36.25 mL of deionized water for 3 h at 100 ºC. Then, the solution was cooled and filtered with filters over G4 glass filter crucible. The remaining solid was the AIL whereas ASL was obtained diluting the filtrate sample with H_2SO_4 1 M. Solutions were analysed by UV spectrophotometry at 205 nm and absorptions between 0.1 and 0.8 were taken into account., A Jasco V-630 UV equipment with UV quartz cells with 10 mm light path, was used for these analyses.

The sugar content represented as impurities, was determined by means of High Performance Liquid Chromatography (HPLC), using a Jasco LC-Net II/ADC equipped with a Rezex ROA-Organic Acid H^+ (8 %) column, photodiade array detector and refractive index detector over the liquid fraction obtained during the AIL experiment. Samples were analysed with a 0.005 N H_2SO_4 solution with 100 % deionized and degassed HPLC water at 30 ºC, 0.35 mL/min flow and 40 μL as injection volume. High purity standards of arabinose, xylose, and glucose (≥ 99 %) were used for its calibration.

The inorganic content of the lignin samples were measured by thermogravimetric analysis (TGA) using a TGA/SDTA RSI analyser from Mettler Toledo). Around 6-7 mg of the lignin samples were heated from 25 to 800 ºC at a heating rate of 10 ºC/min using air atmosphere with constant flow.

The comparison of the molecular size of the different samples collected along the process from both agave sources was developed determining the average molecular weight (M_w), and polydispersity index (M_w/M_n) by Gel Permeation Chromatography (GPC). A Jasco LC-Net II/ADC device was used for this purpose, equipped with a reflex index detector, PolarGel-M column (300 mm 7.5 mm) and PolarGel-M guard (50 mm 7.5 mm). Samples were analysed with dimethylformamide as the mobile phase with 0.1 % of lithium bromide, with a 0.7 mL/min flow at 40 ºC. Calibration was carried out using polystyrene standards ranging from 266 to 70,000 g/mol.

The chemical composition of the lignin samples, regarding their functional groups, was analysed by Fourier Transform Infrared Spectroscopy (FT-IR), using a Perkin Elmer (Spectrum Two Model) equipment. The spectra were recorded between 4,000 and 600 cm^{-1}, performing 8 scans with a resolution of 4 cm^{-1}.

3. Results and discussion

3.1 Feedstock characterization

The composition of the used feedstock (bagasse and leaves), according to their macromolecular composition, is detailed in Table 1.

Table 1: Macromolecular composition of the raw materials used in this work

Sample	Cellulose (%)	Hemicelluloses (%)	Lignin (%)	Ashes (%)	Extractives (g/mol)
AB	54.60 ± 1.70	13.95 ± 0.28	16.20 ± 0.48	4.50 ± 0.33	5.42 ± 0.23
AL	63.10 ± 0.75	15.50 ± 1.71	12.40 ± 0.36	1.20 ± 0.56	7.30 ± 0.98

Cellulose content is higher than 50 % for both materials, demonstrating their use for the obtaining of high purity cellulose. For AB, the amount of lignin is higher than hemicellulose content because the AB was subjected to the tequila production process, in which a considerable part of the sugars, mainly form hemicelluloses, were solubilized from the raw material. Considering these compositions, blue agave is a suitable biomass source to obtain cellulose and lignin by using the same extraction process, thus generating an integrated biorefinery process.

3.2 Lignin extraction yields

The precipitation of the lignin from the spent liquors resulting after each stage (pulping and bleaching) was accomplished. Afterwards, the precipitated lignin was filtered, washed and dried. The assessment of the performance for each stage was carried out by gravimetric analysis. The average yield after each stage and overall yield (Oy) are presented in Table 2. This last parameter is the sum of the total lignin yields for each raw material with regard to the starting mass. The relative yield (Ry) is the relation between the overall yield and the amount of lignin presents in the raw material according to TAPPI standards.

Table 2: Lignin yield (Y) after each treatment

Raw material	Y_O (%)	Y_{B1} (%)	Y_{B2} (%)	Oy^1 (%)	Ry^2 (%)
AB	9.49 ± 1.37	3.64 ± 0.84	1.95 ± 0.53	14.48	89.40
AL	7.46 ± 1.29	1.74 ± 0.77	-	9.07	73.12

[1] Oy corresponds to overall yield, the amount of lignin obtained related to the biomass used.
[2] Ry corresponds to relative yield, the obtained lignin compared to the lignin content as obtained from TAPPI methods.

The largest amount of lining was extracted during organosolv treatments for both materials (ABO and ALO). The recovery of lignin after pulping and bleaching for cellulose products production, resulted in a high amount of this byproduct, obtaining total yield values (Ry) referred to initial lignin existing in the raw biomass, of ~90 % for AB and ~70 % for AL. In case of AL, after the second oxygenated bleaching (ALB2) no significant amount of lignin was precipitated from the liquor, so this sample was neglected. This situation may occur because during the process, while the fractionation of the main components went on, it became more complex to eliminate impurities, which in case of pulping and bleaching were represented by lignin and hemicelluloses. This situation lowered the yield in the following stages. This was also consistent during the bagasse bleaching, as the percent of lignin recovered from the black liquors after the oxygen bleaching maintained a decreasing trend, but in the end relative yield resulted to be higher than the one of agave leaves. The minor yields obtained for leaves could be due to the higher difficulty of extraction when less quantity of a component existed in the initial mass, highlighting the concept of cascade process.

3.3 Lignin characterization

Results of chemical composition of isolated lignin samples together with molecular weight properties are shown in Table 3. The lignin isolation process had an impact on the purity of lignin samples referred to the acid insoluble and soluble lignin. All lignins presented purity levels above 75 %, a high level taking into account the purity of lignin from other feedstock (García et al., 2011). It could be observed that lignins obtained from the bleaching stages presented lower purity (75-85 %) than those from organosolv stages (85-90 %). Lignin is also linked to hemicelluloses forming lignin carbohydrate complexes, which can be quantified by measuring the sugar content of the samples. The lowest values of sugar content were reached for organsolv lignin, both bagasse and leaves (less than 3 % for bagasse and around 6 % for leaves). This higher quantity of sugar impurities in leaves lignin samples could be justified due to the higher amount of carbohydrates that the leaves as raw material presented. Lignin from bleaching stages showed greatest quantities of sugar content (around 10 % or more), indicating that the most purified lignin was extracted in the first step i.e., during the pulping process. However, the approach of a further valorisation of lignin from the bleaching liquors could be different due to its variation in composition when compared to organsolv lignin. Regarding the inorganic matter of lignins, referred as the ash content, no difference was found among all the studied samples (4-5 %), demonstrating the suitability of washing the lignin samples after being precipitated.

Table 3: Composition of the lignin samples collected along the different stages of the experimental procedure

Sample	AIL (%)	ASL (%)	Sugars (%)	Ashes (%)	M_w (g/mol)	M_w/M_n
AB O	89.63 ± 2.73	2.85 ± 0.92	2.67 ± 1.32	4.95 ± 0.48	2,933	3.61
AB B1	84.79 ± 2.68	1.87 ± 0.07	8.97 ± 2.75	4.25 ± 0.78	7,414	5.79
AB B2	79.99 ± 1.87	2.16 ± 0.29	12.32 ± 2.34	5.86 ± 1.29	6,741	5.26
AL O	85.19 ± 0.88	2.51 ±0.51	6.03 ± 0.32	4.13 ± 0.39	4,904	4.09
AL B1	74.29 ± 1.34	2.83 ± 0.74	11.67 ± 2.11	5.36 ± 0.97	3,942	3.39

Regarding the molecular weight of the different obtained lignins, higher Mw and polydispersity values were observed for bagasse isolated lignins during bleaching stages than by organosolv process. This could be a consequence of the higher content of sugars in these samples. Wen et al. (2013 proposed that the carbohydrate chains linked to lignin can increase the hydrodynamic volume of lignin and therefore increase itsapparent molecular weight when measured using GPC. Nevertheless, in case of lignins from leaves the opposite happened, presenting higher Mw and polydispersity than lignin obtained from organosolv process.

The chemical structure of the different lignin samples was observed by FTIR analysis. The spectra of isolated lignin samples are shown in Figure 1.
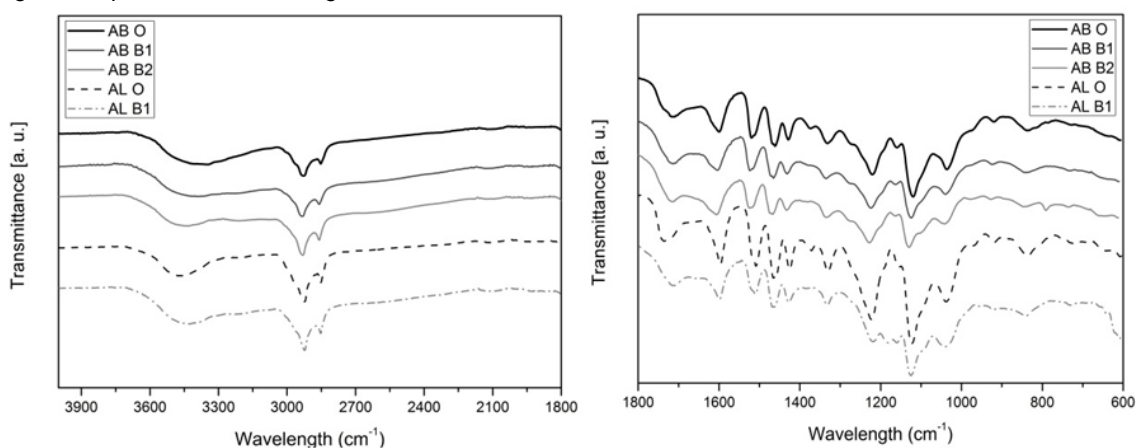

Figure 1: FT-IR spectra of the precipitated lignin samples

All lignin samples showed a wide absorption band at 3,400 cm^{-1}, which indicated the presence of O-H stretching vibrations in aromatic and aliphatic O-H groups. For AL this band is more acute, fact that may occur due to a higher availability of these groups in AL than in AB lignins. Bands around 2,930 and 2,840 cm^{-1} can be assigned to C-H stretching in -CH$_2$- and -CH$_3$ groups (Cachet et al., 2014). On the other hand, the small band al 1,710 cm^{-1} indicated the presence of non-conjugated carboxylic acids in the lignins (Cachet et al., 2014). Signals between 1,400 and 1,700 cm^{-1} were attributed to the aromatic skeletal vibrations of lignins. The peaks at 1,595 and 1,510 cm^{-1} were due to C-C of aromatic skeletal vibrations. The bands found at 1,460 cm^{-1} and 1,420 cm^{-1} were attributed to the C-H deformation in -CH2-and -CH$_3$ groups and C-H aromatic ring vibrations, respectively. The band at 1,325 cm^{-1} can be attributed to the presence of syringyl units (C-O stretch). Moreover, at 1,265 cm^{-1} it can be observed a small shoulder with very low intensity which corresponds to guaiacyl (C-O stretch) ring. Some characteristic bands associated with syringyl and guaiacyl units in lignin were detected at 1,220, 1,125 and 1,030 cm^{-1}, corresponding to C-C, C-O and C=O stretching (G), aromatic C-H in-plane deformation (S) andaromatic C-H in-plane deformation (G > S) (García et al., 2009).

4. Conclusions

The extraction of lignin from side-streams of pulping industry is a suitable option for further obtention of high value added products. In addition, the more interest emerges if it is possible to transform an agricultural waste (blue agave bagasse and leaves) in such products. In this sense, the extraction of lignin by means of an environmentally friendly global process as an innovative green process is proposed. This process consists in an organsolv pulping and an oxygen-alkaline bleaching; whose aim is the manufacturing of high-purity cellulose. The yield of pulping process is much higher than for the bleaching stages as it was expected and

being lower for the last step of the bleaching. The extraction is more complex when less lignin exists in the feedstock as in a cascade process. Even no significant lignin was obtained from the second oxygenated bleaching for leaves, being neglected this stream. However, the whole yield of lignin extraction for the global process reached high values (around 90 % for bagasse and more than 70 % for leaves). Hence, great amount of this byproduct, as it is considered lignin, could be valorisation by these precipitation processes, allowing a huge level of integration degree. According to the chemical composition of lignin samples, organsolv lignins presented higher purity level, since lignin from bleaching stages showed higher level of carbohydrate impurities. Different effect of M_w was experimented for lignin from bagasse and leaves. Bagasse lignin presented higher M_w and polydispersity for bleaching lignin while the opposite occurred for leave lignins.

Acknowledgements

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