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## Lignin as co-product of second generation bioethanol production from ligno-cellulosic biomass

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### Abstract

To improve the economic viability of the biofuel production from biomass, it is of increasing importance to add value to the lignin produced as a bio-residue. Moreover, to meet the goal to replace 30% of fossil fuel by biofuels by 2030, a huge amount of lignin will soon be produced. The first major step involved to add value to the unconverted lignin is its separation from other biomass constituents to give high purity lignin. In this current work, extraction of lignin from a bio-residue (containing ca. 40% lignin) from second generation bioethanol production is presented. The biomass chosen is *Arundo donax* L. (or giant reed), which is non-food plant, can tolerate a wide variety of ecological conditions with all types of soils, and has increasingly importance as raw material for industrial purposes as a source of fibers alternative to wood, which availability is decreasing. Slightly different extraction procedures are investigated. Methods used are simple, mild, safe, and avoid destruction of fiber content in the bio-residue, with the final aim to valorize all fractions of the bio-residue, which is an essential step to make biofuel production to be cost effective. Lignins extracted are characterized by morphological analysis, using Scanning Electron Microscopy, SEM, and in terms of thermal behavior -using thermo gravimetric analysis TGA- which is critical for determining suitability of the lignin for polymer composite preparation with improved thermomechanical performance. The method judged as the best of the three leads rapidly to extraction of lignin free from fibers and ash, with thermal behavior suitable for composite preparation.

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## 1. Introduction

Lignocellulosic biorefinery for production of biofuel requires valorization of all fractions to be cost effective [1]. Various strategies are under development, including research for fermentation of hemicellulose fraction [2] and research for methods to isolate the high value-added cellulose nanocrystals [2, 3]. However, a key point is the valorization of residual lignin, and to economically utilize it as value-added co-product at commercial scale is considered a challenge [4]. Lignin is nature's most abundant aromatic polymer, and the second most abundant renewable natural resource (after cellulose) [5], and it is traditionally viewed as waste material or low value by-product to fire. This is true also in implants for conversion of lignocellulosic biomass to ethanol, where the residual lignin is often used just for power generation to drive the fermentation [1, 6].

Moreover, in biorefinery processes, lignin will be produced in large quantities, in addition to lignin production in the paper and pulp industry. Between 40 and 50 million tons (Mtons) per annum are produced worldwide from pulp and paper industry as a mostly non commercialized waste product [7], and to meet the goal to replace 30% of fossil fuels by biofuels by 2030, about 227 hm<sup>3</sup> of bioethanol will be needed, which would generate around 225 Mtons of lignin [8]. Therefore, development of value-added polymers and bioproducts from lignin is considered of utmost importance in order to achieve sustainable economy and to reduce carbon footprint [8].

The outlined perspective shows how the investigation about the efficient and cost effective utilization of biomass into fuels, chemicals and materials represents an essential research issue for the transformation from an oil-based to a bio-based society. To this regard, it is worth recalling that the technologies used to convert coal and oil (non renewable materials, no less complex than lignin) to products required 50-100 years of development [9].

The future use of lignin (except as fuel) will depend on the possibilities of either degrading it into low molecular weight aromatics or to take advantage of lignin as a multi-functional macromolecule [10]. As a consequence of its polyaromatic nature and large availability, lignin is the obvious candidate to serve as future "green" source for aromatic chemicals, especially phenols [11]. Aromatic building blocks derived from lignin are particularly attractive for use in polymerization, as substituent of oil-derived phenols, e.g. phenol formaldehyde resins [12, 13] or epoxy resins [14]. Moreover various possible value-added applications are under study for use of lignin without conversion to molecules of lower molecular mass, such as, e.g., blending with polyolefins [15, 16] giving UV stabilization, or blending with PLA giving 100% renewable resins [17], or implementing uses which take advantage of its antimicrobial and antioxidant action [4, 18, 19].

Applications of both lignin-derived aromatics and lignin as macromolecule are at lab stage, and seem to work optimally with sulfur-free lignins which have low levels of impurities too [8, 20]. Actually, at present, technical lignins, coming from steam explosion processes and others, present large heterogeneity in terms of purity and chemical structures [21]. Therefore, the first major step involved to add value to the unconverted lignin is its separation from other biomass constituents, such as hemicelluloses, cellulose, ashes to give high purity lignin. The research of novel methods for the efficient and cost effective extraction of lignin is active, and for instance, recent work focusing on bamboo biomass makes use of microwave assisted extraction [22] or sequential processes with ethanol and soda [23], or steam explosion followed by methanol treatment [14]; other methods reported in the literature include use of innovative "green" solvents such as Ionic liquids, IL [24] and use of newer ones such as deep eutectic solvents – second generation of IL - could be suggested [25].

Here we present our work about attempts to use simple, mild and economic methods for extraction of lignin from a bio-residue, obtained from second generation bioethanol production. Our methods avoid destruction of fiber content in the bio-residue, with the final aim to valorize all fractions of the bio-residue, which is an essential step to make biofuel production to be cost effective. Slightly different extraction procedure were used, and extracts were characterized by SEM and in terms of thermal behavior which is critical for determining suitability of the lignin for composite preparation with improved thermomechanical performance [8]. The biomass chosen is *Arundo donax* L. (or giant reed). Nowadays, due to decrease of wood availability, the interest in alternative sources of fibers from annual or biennial crops is increasing. Such non-woody fibers include bamboo, cereal straws, sugar-cane bagasse, and *Arundo donax* L. [26]. This plant has high biomass productivity, can tolerate a wide variety of ecological conditions with all types of soils, and grows very fast.

## 2. Experimental

### 2.1 Materials

Lignin used is the bio-residue obtained after biomass-to-ethanol process. Biomass used is *Arundo donax* L. Reagents such as NaOH, ethanol, H<sub>2</sub>SO<sub>4</sub> are of commercial grade from Sigma Aldrich, used without further purification. Deionized water used is from ELGA, of lab quality.

### 2.2 Analysis

TGA analysis is carried out under oxygen with LECO 701 instrument with the following temperature ramp rates: from room temperature to 300°C: 5°C/min; from 300 to 900°C: 25°C/min; 30 min stay at 900°C. SEM instrument is FEG LEO 1525, with Inlens detector. Samples were metalized with Chromium. Water content is evaluated by thermobalance Mettler Toledo HB43-S according to NERL laboratory analytical procedures, NERL/TP-510-42621. The ash content is determined after burning the lignin under oxygen using TGA equipment at a temperature range from room temperature to 900°C and then maintaining the sample at 900°C for 30 minutes. The content of extractives, hemicellulose, and lignin is carried out according to NERL laboratory analytical procedures, NERL/TP-510-42619 and NERL/TP-510-42618 respectively.

## 3. Results and discussion

### 3.1 The bio-residue from bioethanol production

A bio-residue is used as starting material in this work, obtained in our laboratory after conversion of *Arundo donax* L. biomass to bioethanol with the following processes: milling, steam explosion pretreatment, hemicellulose separation, enzymatic reactions, filtration, as schematically shown in Figure 1.

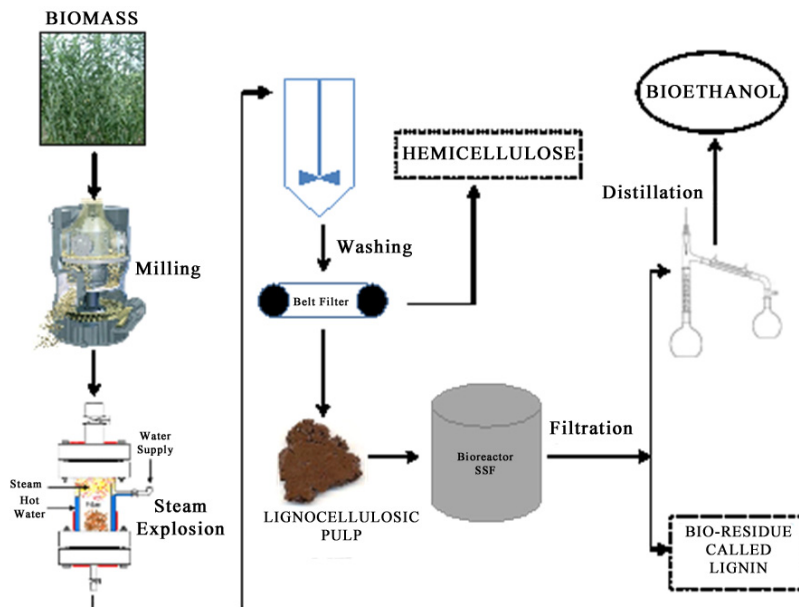


Fig. 1: Scheme of the processes used to produce bioethanol; the bio-residue called lignin is a co-product, as is hemicellulose.

The bio-residue called lignin is filtered to remove water, and dried at 40°C, until constant weight. Its appearance is a brown powder, as shown in Figure 2.



Fig. 2: The bio-residue obtained after the conversion of *Arundo donax* L. biomass into bioethanol.

In Table 1 the composition of *Arundo donax* L. biomass and of the bio-residue used are reported. Data for the *Arundo donax* L. are consistent with the literature [26, 27]. Data for the bio-residue clearly show how the composition is rather different from the initial one, with significantly increased amounts of lignin and ashes, increased extractives content, and decreased holocellulose. The bio-residue can not be properly called lignin, its content being only around 40%, and it will be called only bio-residue in the following part of this paper.

**Table 1:** Composition of raw *Arundo donax* L. biomass and of bio-residue<sup>a</sup>

Component	<i>Arundo donax</i> L.	Bio-residue
Klaison lignin	20.2	38.0
Extractives in hot water	7.0	5.4
Extractives in ethanol	5.3	13.6
Holocellulose <sup>b</sup>	60.6	26.0
ash	5	14
other	2	3

<sup>a</sup> weight % on a dry mass basis.

SEM images of the bio-residue are shown in Figure 3. The appearance of lignin is round shaped. Moreover, the presence of fibers is evident, in both images, and especially in the zoomed one. Such fibers are clearly completely defibrillated, and fibers with diameters around 50 nm are evident.

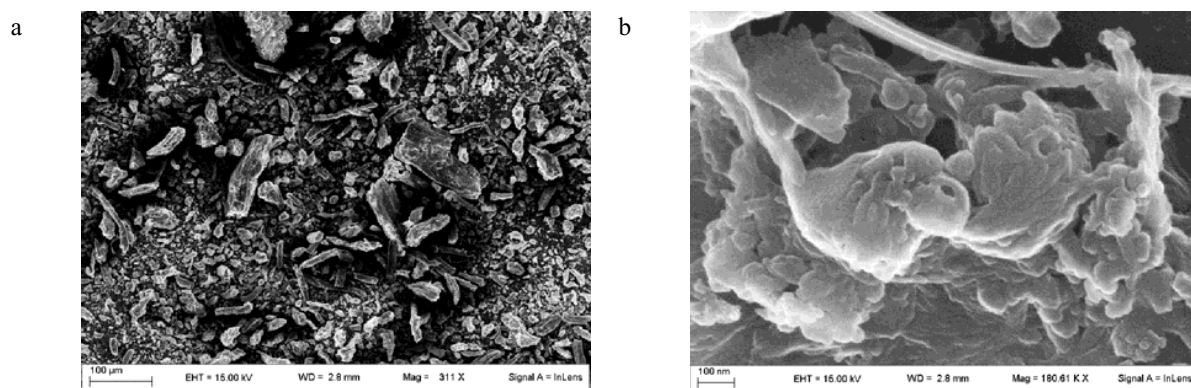


Fig. 3: SEM of the bio-residue at smaller (a) and higher (b) magnification.

### 3.2 Lignins extracted from the bio-residue

Methods used to isolate lignin from the bio-residue are selected for being simple, mild and safe, and particular attention is paid not to destroy the fiber content. Various steps are shown schematically in Figure 4. First steps of extractions are carried out according to the method proposed by Bolanos et al.: lignin is washed with warm water, and then an alkaline extraction follows [28]. It is extracted with NaOH 2% for 15 minutes; the procedure is repeated twice. The solid and the liquid filtrate are processed separately, as follows. The residual solid is washed with warm water until all traces of alkali are removed, obtaining a brown solid, which has to be further processed for recovery of fibers. Combined filtrates are precipitated by acidification with sulfuric acid until pH 3.

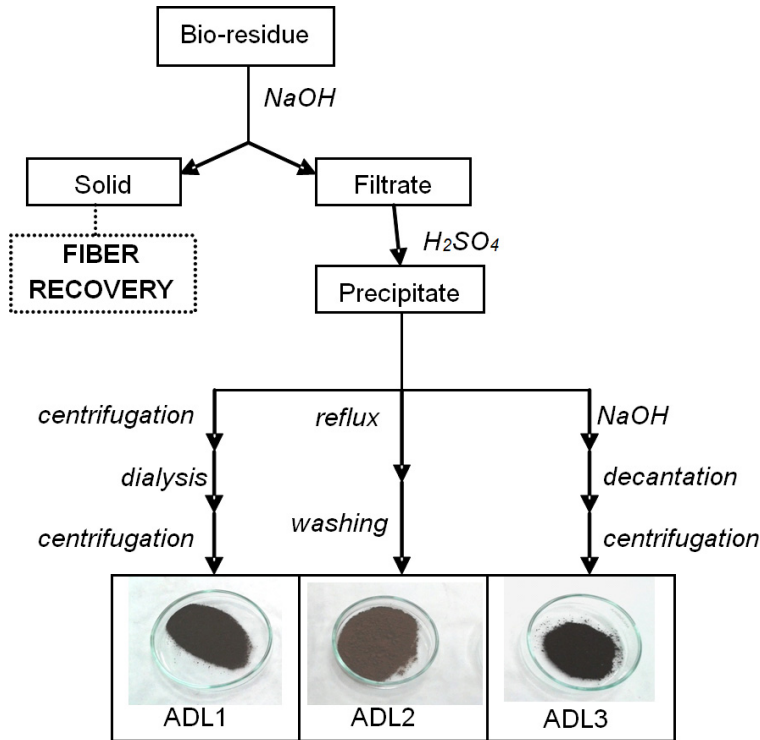


Fig. 4: Scheme of the steps used for lignin extraction from the bio-residue; dot lines are for future work aimed at recovering also fibers.

Subsequent different treatments are developed in our lab, and in particular:

- *Arundo donax extracted lignin 1 - ADL1*. The acid suspension is centrifugated to remove most of the excess acid; the recovered precipitate is suspended in deionized water and dialyzed against deionized water until neutral pH. The solid is thereafter centrifugated, and dried at 40°C.
- *Arundo donax extracted lignin 2 - ADL2*. The acid suspension is treated with water at reflux for 1h. The solid is easily filtered by gravity, and washed until neutrality. Then it is dried at 40°C.
- *Arundo donax extracted lignin 3 - ADL3*. The acid suspension is neutralized with NaOH, added dropwise. After decantation and elimination of supernatant, the suspension is centrifugated, and dried at 40°C.

Final lignins recovered are all with similar appearance of a brown powder, with ADL2 being of a slightly lighter brown (Figure 4). SEM images of different extracts are shown in Figure 5-7 which clearly show different morphologies. Particles of the sample ADL1 show a polyhedral shape, with granules of various dimensions, whereas

particles of ADL2 show a rather round shape, as the original bio-residue. Also in this case granules of various dimensions are present. Sample ADL3 is quite different, and shows two kinds of particles, and probably the white one in Figure 7 may be attributed to salts. In all the three samples there is no evidence of fiber presence, and the powder surface of lignin is rather smooth.

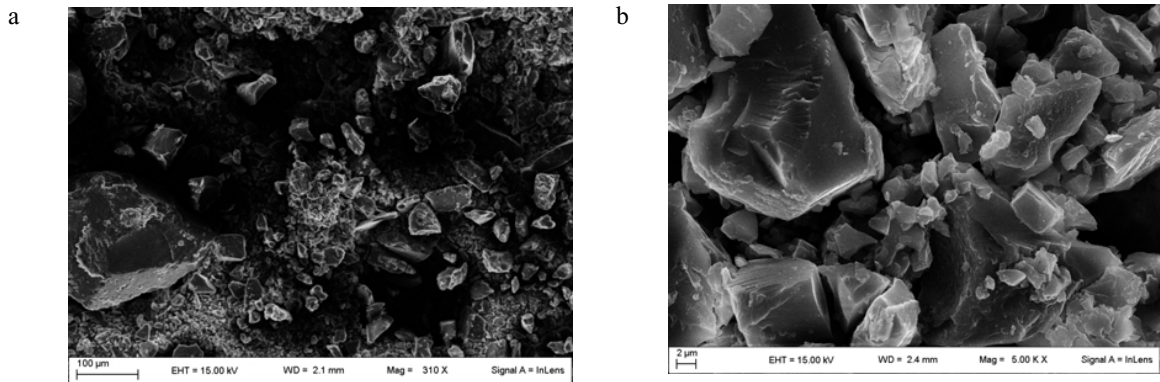


Fig. 5: SEM of the ADL1 sample at smaller (a) and higher (b) magnification.

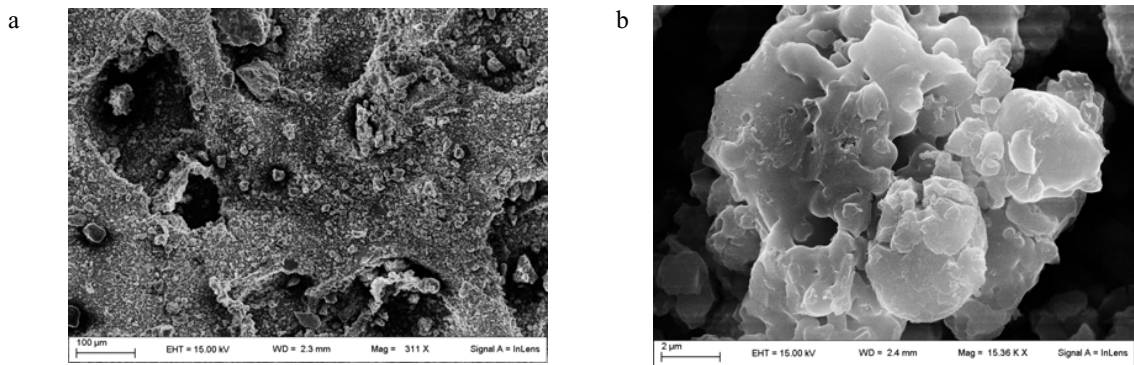


Fig. 6: SEM of the ADL2 sample at smaller (a) and higher (b) magnification.

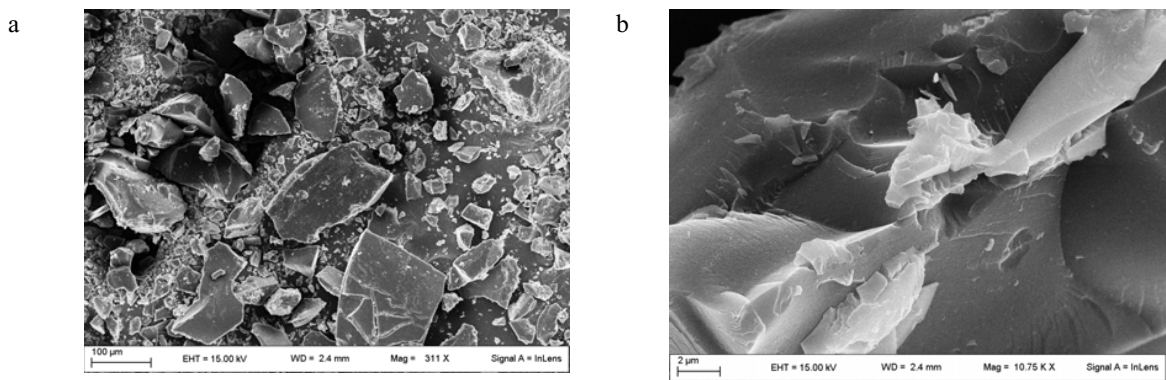


Fig. 7: SEM of the ADL3 sample at smaller (a) and higher (b) magnification.

### 3.3 Thermo gravimetric analysis (TGA)

The thermal stability of fillers is a very crucial factor for the polymer processing, especially through melt processing techniques [8]. During polymer processing, melting temperature of the polymers should always be lower than the degradation temperature of the filler, and biomass materials are often used to improve the mechanical resistance of polymers that are processed close to 200°C [29]. Therefore thermo gravimetric analysis, TGA, is used in this work to help understand the thermal stability of the extracted lignins, and also of the bio-residue, to gain information about possible use as fillers in polymer processing. The plots of the thermo gravimetric analysis for the three extracted lignins and also for the bio-residue are shown in Figures 8a and 8b. Values of some important parameters from these analyses are reported in Table 2. Weight loss within 100°C can be attributed to the loss of moisture from lignin. Accordingly the values of weight loss within 100°C (reported in Table 2) are consistent with values of moisture content carried out with the standard method NERL /TP-510-42621 which are 6.93, 6.18, 10.3, 3.42, wt %, for ADL1, ADL2, ADL3 and bio-residue respectively. Differences are consistent with the procedures used; in fact the NERL method stops the temperature at 105°C until a constant weight is reached, whereas TGA ramp rate is 5°C/min: TGA leads to underestimate the moisture content. As clear from Figure 8a the thermal decomposition extends to the whole temperature range for all the samples investigated, persisting even above 800°C. This is consistent with already observed thermal decomposition of lignins and explained with the different activities of the chemical bonds present on its structure [29, 30].

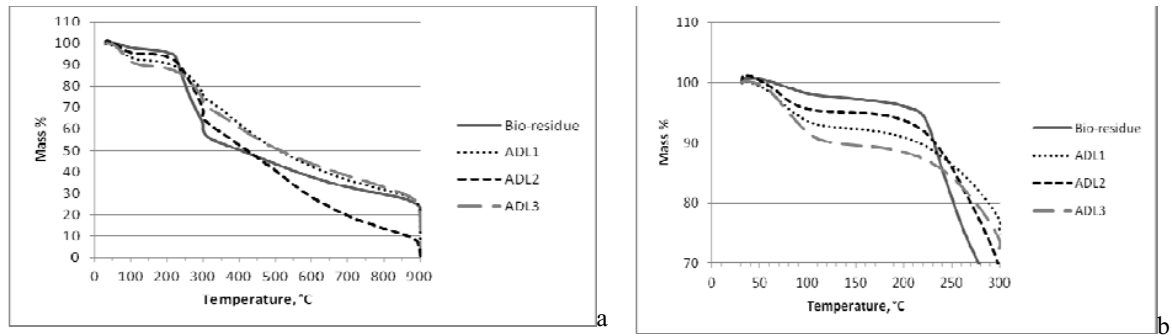


Fig. 8: TGA curves of samples ADL1, ADL2, ADL3 and bio-residue under oxygen atmosphere in the whole temperature range investigated (a) and zoomed in the range from room temperature to 300°C (b)..

**Table 2** : Data from thermo gravimetric analysis

	ADL1	ADL2	ADL3	Bio-residue
Weight loss within 100°C, wt %	6	4	8	2
Temperature at 10% degradation, °C	216	230	120	230
degradation at 200-600 °C, wt%	45	56	42	56
Ash, wt%	2	0.5	5	14

Important parameters reported in Table 2 are: (i) the temperature at 10% degradation, (ii) the % degradation at 200-600 °C, and (iii) ash content. Significant differences are clear between the various samples. As regards the temperature at 10% degradation the sample ADL2 and bio-residue show the best performance, with a value of 230°C, whereas ADL1 has a slightly lower value, 216°C (Table 2). These values are rather good because according to Kim et al. [31], the degradation of lignin starts at about 250°C. On the other hand, the temperature at 10% degradation for sample ADL3 is quite worse, down to 120°C, which excludes the possibility of its use in polymer

composites. As regards the % degradation at 200-600 °C, the values for the three samples and the bio-residue are similar, and consistent with values reported for other lignins, which are in the range 40-60 % [8]. Also in this case the sample ADL2 and bio-residue show a similar behavior. On the other hand, sample ADL1 is quite similar to ADL3, with TGA curve almost superimposable in this temperature range (Figura 8a). As regards the ash content, the variability between the samples is high. The bio-residue has quite a high value of ash content, up to 14%, whereas the lowest value is reported for sample ADL2, with only 0.5%. ADL3 show a relatively high ash content, as consistent with the extraction method which implies formation of sodium sulfate salt.

#### 4. Conclusions and future developments

Extraction of lignin from a bio-residue from second generation bioethanol production is investigated. The biomass chosen is *Arundo donax* L. (or giant reed), which has high biomass productivity, can tolerate a wide variety of ecological conditions with all types of soils, and has increasingly importance as raw material for industrial purposes as a source of fiber alternative to wood, which availability is decreasing. Methods developed are simple, mild, safe, and avoid destruction of fiber content in the bio-residue, with the final aim to valorize all fractions of the bio-residue, which is an essential step to make biofuel production to be cost effective. The search for simple and mild methods for recovery of fibers in the solid portion are in due course (see Figure 4).

Three extraction methods lead to three samples of lignins, which show some differences in terms of the properties investigated. All the samples do not have fibers, which were present in the bio-residue, as evident from SEM images. Sample ADL3 has poor thermal properties, sample ADL1 has rather good thermal properties but its preparation is time requiring (dialysis is rather a time-consuming step). Therefore the conclusion is that the sample ADL2 is the best one, which extraction is rapid and work up is very easy; this sample shows the best performance in terms of thermal properties. Its composition was determined and it is lignin 73% pure, with traces of fibers (<2%) and traces of ashes (0.5%), whereas extractive content was 25%.

Further developments of this work include applications of the lignins extracted in polymer composites and in niche application, such as cosmetics or composites for medical applications. In the field of mechanical resistance improvement, sample ADL2 will be used, which has shown the best thermal behavior, and its performance will be compared with the bio-residue which is by far less pure (as shown in Table 1), but has a comparable thermal behavior and quite a minor cost. On the other hand, for niche applications, where purity is an important issue, ADL2 sample can be fruitfully used, which is relatively pure. Moreover, modification in the extraction procedure are under development to remove extractives without use of time-consuming Soxhelt extraction, so that an even better lignin can be obtained in terms of purity, whereas scale-up of the process is in due course.

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