EXTRACTION AND FRACTIONATION OF A LIGNOCELLULOSIC BIOMASS AND ITS USE AS A BIO-FILLER IN POLY(3-HYDROXYBUTYRATE)

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A lignocellulosic biomass, biowaste of second generation bioethanol production, was treated with a toluene/ethanol mixture and with water in order to leach organic and water soluble extractives. Moreover, the parent lignocellulosic biomass was fractionated in order to isolate its main components: acid-insoluble lignin and holocellulose (hemicelluloses and cellulose). The extractive-free solid residues and the isolated fractions were studied through scanning electron microscopy, vibrational spectroscopy and thermogravimetry. Subsequently, the lignocellulosic biomass and its derivatives were used as bio-fillers in poly(3-hydroxybutyrate) (PHB), a microbial biodegradable polyester. The obtained blends were compression-molded to produce films. The unprocessed blends were characterized by means of thermogravimetric analysis, while the dumbbell-shaped films were analyzed through tensile tests. A slight increase of thermal stability and a modest stiffening of the PHB matrix were detected.

Keywords: biorefinery, lignocellulosic biomass, acid-insoluble lignin, holocellulose, poly(3-hydroxybutyrate)

INTRODUCTION

The implementation of sustainable approaches in a forward-looking bio-based economy has become a major research topic over the last years. The rising awareness amongst the scientific community about the progressive depletion of fossil carbon sources, as petroleum and natural gas, has contributed to developing new technologies designed to produce bio-fuels and bio-chemicals from renewable biomasses.[1] Biomass is defined as any organic matter that is naturally available and renewable, including dedicated energy crops, agricultural food and feed crop residues, aquatic plants, wood, animal wastes.[2] Processing and conversion of biomass into bio-fuels and a palette of bio-chemicals are performed in biorefineries.[3] Three types of biorefineries can be distinguished according to the feedstock used: whole crop, green, and lignocellulosic feedstock biorefineries. The first uses dry grain or maize, the second wet biomass as grass, lucerne, clover and the third lignocellulosic-based materials as forest residues, straw, reed, paper wastes.[4] Lignocellulosic materials are the most abundant biopolymers on earth and consist of hemicelluloses, cellulose and lignin along with small amounts of proteins, waxes and minerals. Hemicelluloses are branched polymers composed of several sugar residues with five and six carbon atoms connected to each other through β and α bonds.[5] Hemicelluloses are linked to lignin and cellulose through covalent and hydrogen bonds, respectively.[6] Cellulose is a linear homopolymer composed of glucose units linked through β-1,4 bonds. Cellulose chains are arranged in parallel arrays to form an ordered fibril macrostructure linked through hydrogen bonds.[7] Lignin is an amorphous branched polymer composed of phenylpropane units linked by β-aryl ether, di-aryl propane and C-C bonds. Lignin derives from the polymerization of three monolignols: coniferyl, sinapyl and p-coumaryl alcohols.[8] Its composition differs according to the kind and part of the plant. In all biorefineries, the whole process is pursued with an approach aimed to add value to the waste streams, and to involve minimal energy and environmental impact. In particular, in the lignocellulosic feedstock biorefinery, this objective is pursued through a "no-waste philosophy", aiming to find an application even to the by-products of low economical interest. The second generation bioethanol production process represents a typical

example of lignocellulosic feedstock biorefinery.[9] The lignocellulosic biomass is usually first pretreated through several possible approaches as physical,[10] chemical,[11] physical-chemical[12] and biological pretreatments[13] in order to separate the biomass components and to make crystalline cellulose accessible to enzymatic hydrolysis. After the hydrolysis, the sugars are fermented to ethanol by yeasts and the product is distilled. In spite of the pretreatments performed on the starting biomass, only a part of the cellulose is converted to ethanol, due to the presence of crystalline regions that are recalcitrant to hydrolysis, and to the stable lignin/cellulose/hemicelluloses framework. Therefore, a bio-residue, mainly made up of lignin and a non-negligible portion of polysaccharides, is recovered in the black liquor at the bottom of the distillation column. This bio-residue is a promising source of energy, fuels, organic acids, solvents, polymers and bio-chemicals. Moreover, it can be viewed as a low-cost reinforcing bio-filler to be incorporated in biopolymers. Following this approach, the first step involved to add value to the lignocellulosic bio-residue, consists in processing it in order to remove the extractives and to separate its main macromolecular components. Our previous work[14] dealt with the use of a lignocellulosic bio-residue (LC) from the second generation bioethanol production as bio-filler in a microbial biodegradable polyester, namely poly(3-hydroxybutyrate) (PHB). PHB is an attractive bio-polymer with properties similar to those of polypropylene, however it is stiff and brittle, and with a narrow processing window. Commercial production of PHB goods is limited due to its high costs.[15] Blending lignocellulosic bio-residue in PHB was purposed to balance and modify the polymer production costs and properties. An increase of PHB storage modulus at temperatures above its melting point and a promotion of crystallization at a high cooling rate were recorded. Therefore, due to the positive effects obtained on PHB after adding this bio-filler, in the present work LC was treated with a toluene/ethanol mixture and with water, in order to obtain two extractive-free solid residues, referred to as TE and W, respectively. Moreover, LC was fractionated to isolate its two macromolecular components, namely acid-insoluble lignin (IL) and holocellulose (hemicelluloses and cellulose) (HC). All the obtained materials were characterized through Scanning Electron Microscopy (SEM) in order to reveal the morphological differences between LC and its derivatives. Then, chemical and thermal characterizations were performed through ATR-FTIR and TGA. In a second step, these bio-fillers and the parent LC were blended to PHB. The obtained blends were compression-molded to produce films. Finally, the thermal stability of the blends and the mechanical properties of the films were evaluated.

EXPERIMENTAL

Materials

Poly(3-hydroxybutyrate) (PHB), T19 grade, was provided by Biomer (Germany). A lignocellulosic biomass (LC) was kindly supplied by the Biomass Research Centre (CRB) of the University of Perugia as a bio-waste of the second generation bioethanol production process. LC was obtained from humid and fine-cut *Arundo donax* biomass that underwent the following treatments: steam explosion, enzymatic hydrolysis and sugar fermentation by yeasts. The bioethanol was purified through azeotropic distillation and black liquor containing LC was recovered at the bottom of the distillation column. LC was filtrated and dried to constant weight.

Extractions performed on the lignocellulosic biomass

An extraction procedure with a toluene/ethanol mixture was applied to LC in order to remove the organic-soluble extractives. In this procedure, 2.42 g of LC was treated by a mixture of toluene/ethanol (2:1 v/v) in a Soxhlet extractor for 6 h.[16] The extractive-free solid-residue was air-dried for 24 h and dried to constant weight in an oven at 60 °C under vacuum. Subsequently, 1.41 g of the solid-residue was further extracted with absolute ethanol in a Soxhlet apparatus for 4 h and dried to constant weight.[17]

An extraction procedure with water was applied to LC in order to remove water-soluble extractives. Briefly, 2.04 g of LC was treated with 100 mL of Millipore water at room temperature under intense magnetic stirring for 5 h. The sludge was filtrated on paper and washed with water. The extractive-free solid residue was dried to constant weight in an oven at 60 °C under vacuum. The solid residues from toluene/ethanol and water are referred to as TE and W, respectively.

The yield of the extractives was calculated from the mass loss of the raw starting biomass. In particular, extractives in toluene/ethanol and water amounted to 12.4%, and 2.9%, respectively.

Fractionations performed on the lignocellulosic biomass

LC was processed through acid hydrolysis in order to isolate acid-insoluble lignin (IL), and by sodium

chlorite in an acid environment (CH_3COOH) to recover holocellulose (HC). IL isolation was performed according to the following procedure. Briefly, 5 g of LC were treated with 150 mL of 72 wt% H_2SO_4 under magnetic stirring overnight. Then, the H_2SO_4 concentration was reduced to 3 wt% by adding distilled water and the suspension was kept under mechanical stirring for 4 h at 100 °C.[18] The acid-insoluble lignin residue was allowed to settle until the supernatant solution was clear, then the mixture was filtrated on a Buchner funnel and washed with distilled water several times until neutral pH.[19] HC was prepared by adapting the protocol used by Yeh *et al.*,[20] using six $NaClO_2/CH_3COOH$ oxidation cycles. Once isolated, IL and HC were dried to constant weight in an oven at 80 °C under vacuum, and sorted through a 140 mesh sieve. The amount of the two fractions, calculated on the dry weight of the parent LC biomass was 53 ± 1% of IL and 46 ± 2% of HC.

Preparation of PHB-based blends and films

LC, TE, W, IL and HC were introduced as bio-fillers at 30 wt% in the PHB matrix. The blends were obtained by a solvent-assisted method.[21] Briefly, 3 g of each filler was dispersed in 35 mL of methanol. 7 g of PHB was added to the dispersions under mechanical stirring. The resulting dispersions were kept under continuous stirring at 190 rpm for 30 min. The blends were air-dried and then desiccated in an oven under vacuum at 60 °C until constant weight. The blends are referred to as PHB/LC, PHB/TE, PHB/W, PHB/IL and PHB/HC. Prior to measurements, all the samples were stored at 60 °C under vacuum for 48 h. Compression-molded films were obtained by pressing 1.0 g of the blends at 190 °C and 4 tons for 5 min by means of a Carver Laboratory Press Model C. Dumbbell-shaped specimens (average thickness = 0.080 mm, width = 4 mm, length = 50 mm) were cut from the PHB-based films and characterized through tensile tests.

Characterization methods

Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) analyses were performed by means of a FEI Quanta 200 FEG device. The morphological characterization of LC, TE, W, IL and HC was performed by means of SEM. The samples were mounted onto stubs by means of carbon adhesive tape and coated with a 20 nm thick gold/palladium layer by means of a modular high-vacuum coating system Emitech K575X. The elemental composition of the water-soluble extractive was determined by EDS.

ATR-FTIR spectroscopy was carried out on LC, TE, W, IL and HC by means of a Perkin Elmer Spectrum 100 spectrometer, equipped with a Universal ATR diamond crystal sampling accessory. Infrared spectra were recorded by accumulating up to 32 scans with a resolution of 4 cm^{-1}, in the range of 4000-400 cm^{-1}.

Thermogravimetric investigations (TGA) were carried out by means of a Perkin Elmer Pyris Diamond TG/SDTA thermobalance with alumina pans using about 5 ± 0.5 mg of each sample. Measurements were carried out in nitrogen atmosphere under a flow rate of 200 mL min^{-1}. Each sample was analysed according to the following thermal program: heating from 30 to 90 °C at 20 °C min^{-1}; isotherm at 90 °C for 30 min and heating from 90 to 800 °C at 10 °C min^{-1}. The measurements were carried out in duplicate.

Tensile tests were performed by means of an Instron model 4505 dynamometer, equipped with a 1 kN load cell. Dumbbell-shaped films were conditioned in an environmental chamber at 25 °C and 50% RH for 72 h prior to testing. The tests were performed with a 2 mm min^{-1} clamp separation rate and the reported mechanical parameters were the average values of 9±2 determinations.

RESULTS AND DISCUSSION

Morphological, structural and thermal properties of LC, TE, W, IL and HC

SEM micrographs of the parent lignocellulosic feedstock (LC) and its derivatives (TE, W, IL and HC) are presented in Figure 1. As already observed in a previous paper[14] and confirmed in Figure 1a, LC resulted to be a complex material, made up of lignin irregular particles and polysaccharide fibrous structures of micrometre length. The removal of the water-soluble extractives from LC did not lead to remarkable morphological changes (Figure 1c). Similarly, the extraction with the toluene/ethanol mixture caused only slight deterioration of the biomass along with the reduction of lignin particle size (Figure 1b). Conversely, SEM analysis of IL and HC evidenced that the main components of the feedstock were isolated successfully. Indeed, the chemical treatments to isolate acid-insoluble lignin and holocellulose led to noticeable morphological changes with respect to the parent biomass. The picture of IL (Figure 1d) only shows irregularly shaped particles, while the micrograph relative to HC (Figure 1e) displays compact bundles of polysaccharide microfibers along with a small number of residual lignin particles.

ATR-FTIR spectra of LC, TE, W, IL and HC are illustrated in Figure 2a. The spectroscopic investigation evidenced the complex nature of LC and provided further evidence that the fractionation process performed to isolate IL and HC was effective. More specifically, the LC spectrum evidenced the presence of absorption bands at around 3300 and 2900 cm^{-1}, due to hydroxyl and hydrocarbon moieties. Moreover,

lignin related peaks were found in the 1650-1400 cm^{-1} region, while signals between 1100 and 1000 cm^{-1} were mainly ascribed to the polysaccharide fraction. The LC spectrum also showed a broad absorption at 1720±10 cm^{-1} related to the C=O stretching in unconjugated ketones, carboxylic acid and ester groups.

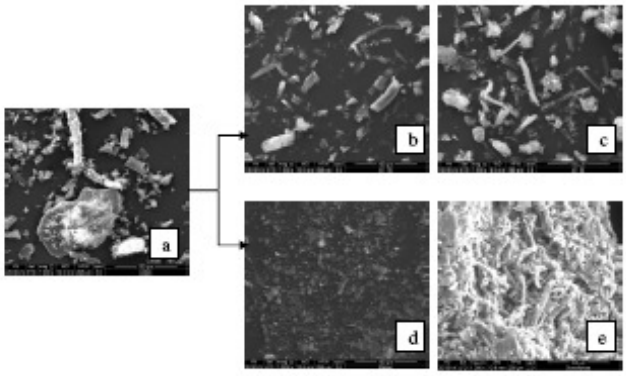

Figure 1: SEM micrographs of a) LC, b) TE, c) W, d) IL and e) HC

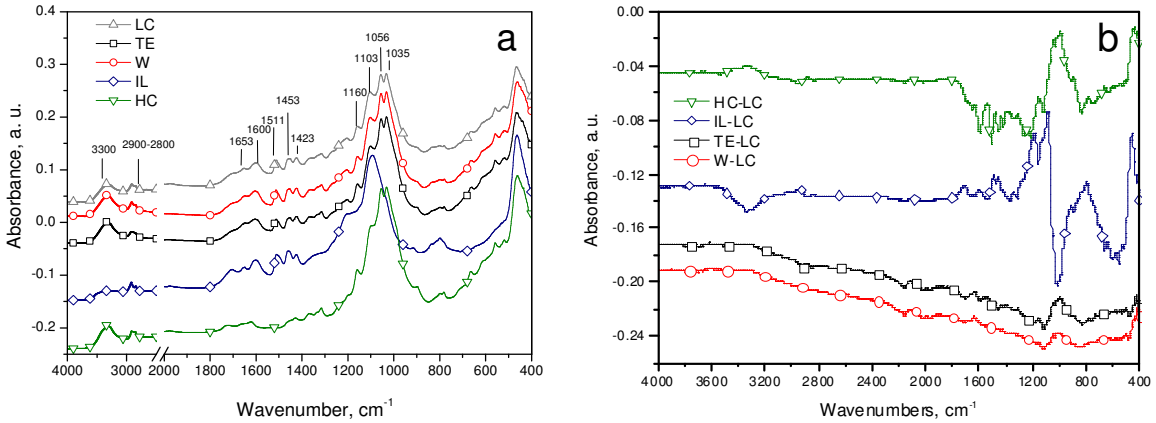

Figure 2: a) ATR-FTIR spectra of LC, TE, W, IL and HC, and b) Difference spectra between the derivatives and LC. The curves were vertically translated

The spectra relative to the IL and HC fractions showed several changes in the absorption bands, which can be better evidenced if spectral subtraction is applied to the as-acquired FTIR spectra.

In Figure 2b, IL-LC and HC-LC difference spectra are exhibited. In the IL-LC plot, the band at around 3300 cm^{-1}, due to O-H stretching, was found to decrease, since the sulfuric acid treatment removed most of the polysaccharide fractions present in the parent LC. Small peaks in the frequency range around 2900-2800 cm^{-1}, attributed to C-H bonds of lignin and carbohydrate moieties, were also recorded.[22] The spectral profile, in this region, evidenced that the acid treatment did not cause major changes in the shape or position of the bands. At lower frequencies, an absorption increase in the carbonyl region was observed with a maximum at 1715 cm^{-1}. This band was attributed to the presence of carboxylic acids, with the minor contribution of ketones, aldehydes and ester groups. In the same frequency range, a small peak at 1653 cm^{-1} was also detected and attributed to conjugated carbonyls. The presence of these carbonyl functionalities can be ascribed to the harsh acid treatment adopted to isolate the acid-insoluble lignin fraction, which resulted in oxidation of carbon atoms and hydrolysis of ester bonds. An increase in the absorptions generated from the skeleton vibrations of aromatic moieties in LC and IL was detected at about 1600 and 1500 cm^{-1}, whereas two peaks at 1453 and 1423 cm^{-1} accounted for the methoxyl groups of guaiacyl

and syringyl units.[17] Finally, a marked increase at 1185 and 1100 cm^{-1}, attributed to C-O-C and C-O stretching vibrations in aromatic rings, secondary alcohols and aliphatic ethers, was also detected. These absorptions resulted to be the major changes observed in the IL-LC spectrum. Their occurrence confirmed that the IL was mainly constituted by a condensed aromatic structure with side chains typical of lignin. It is also worth noting that the IL-LC spectrum displays a marked decrease in the 1050-1010 cm^{-1} absorption region, as a confirmation of the effective removal of the polysaccharide fraction.

On the other hand, the HC-LC spectrum (Figure 2b) evidenced a remarkable increase of two absorption peaks at 1050 and 1000 cm^{-1}, related to C-O deformation in secondary alcohols and aliphatic ethers, and C-O valence vibration from C_3-OH and β-(1→4) linkages between cellulose monomers.[23] Moreover, the content of O-H groups (3300 cm^{-1}) increased with respect to the parent biomass. Furthermore, a dramatic decrease in the 1750-1100 cm^{-1} region confirmed that most of lignin was removed. Nevertheless, a close examination of the HC spectrum (Fig. 2a), revealed the presence of weak bands at 1453 (shoulder) and 1423 cm^{-1}. These absorptions were tentatively attributed to lignin traces remaining after the sodium chlorite treatment, as already evidenced by SEM analysis (Figure 1e). In literature, several authors reported the contamination of holocellulose samples by lignin, for ex., Sannigrahi *et al.*,[24] who observed pseudo-lignin droplets on the surface of neat acid-treated holocellulose through SEM.

A comparison between the spectra of LC and the extractive-free solid residues recovered after organic and water extractions (TE and W) evidenced very small differences. The TE-LC spectrum (Figure 2b) showed a slight decrease in the aliphatic and aromatic C-H vibration regions at around 2900 cm^{-1} and 1600-1500 cm^{-1} due to the extraction of non-polar constituents, such as lipids, waxes and soluble lignin derivatives. The removal of this fraction was responsible for a relative increase of the polysaccharide fraction, as evidenced by the increase of the absorption band in the 1050-1000 cm^{-1} spectral region. As for the extractive-free solid residue recovered after water extraction, no significant differences were observed between the LC and W spectra.

TGA and DTG curves relative to LC, TE, W, IL and HC are presented in Figures 3(a,b), while the average thermal data are listed in Table 1. All the samples were subjected to an isothermal step at 90 °C, which allowed removing the absorbed moisture. The parent feedstock showed a rapid mass decrease which started at about 220 °C. More specifically, the onset temperature ($T_{5\%}$) and the temperature of the maximum decomposition rate (T_{max}) of LC were 253 °C and 344 °C, respectively. Toluene/ethanol extraction scarcely affected the thermal behaviour of LC and only modest variations of the thermal parameters were detected. Water extraction, on the contrary, caused a noticeable enhancement of the thermal performances of the extractive-free solid residue, W, which showed a considerable increase of $T_{5\%}$ and T_{max} compared to LC. The char yields, conversely, showed no relevant differences between LC and its extractive-free solid residues. These outcomes indicate that the water extraction was able to remove, from the biomass, a small quantity of a thermally unstable material, which also acted as a pro-degrading agent towards the lignocellulosic feedstock.[25]

The IL fraction that was obtained through the chemical removal of the whole polysaccharide component by acid treatment showed major variations with respect to LC. This sample exhibited a $T_{5\%}$ value of 297 °C and a T_{max} of 374 °C. These values were much higher than those recorded both for LC and the extractive-free solid residues. Moreover, the decomposition process appeared to be slower and spread over a broader temperature range. In addition, the char yield at 800 °C reached a much higher value equal to 52%. These findings could be related to the chemical structure of the acid-insoluble lignin that was mainly made up of thermally stable, condensed aromatic moieties, since the more thermolabile polysaccharide fraction was removed. The HC sample, which was obtained through an oxidative treatment aimed at removing lignin, showed a $T_{5\%}$ similar to W.

The T_{max} parameter, conversely, was close to that of the parent LC, while the char yield was the lowest among the examined samples. This can be attributed to the oxidative treatment and water washing the sample underwent.

Such treatments selectively removed the lignin component and the pro-degrading products, leaving a polysaccharidic cycloaliphatic material. In fact, HC showed a thermal behaviour comparable to LC, but it was more stable at the start of the thermodegradation process.[26] Finally, the occurrence of a low char yield can be

explained considering the complete absence of the lignin fraction.

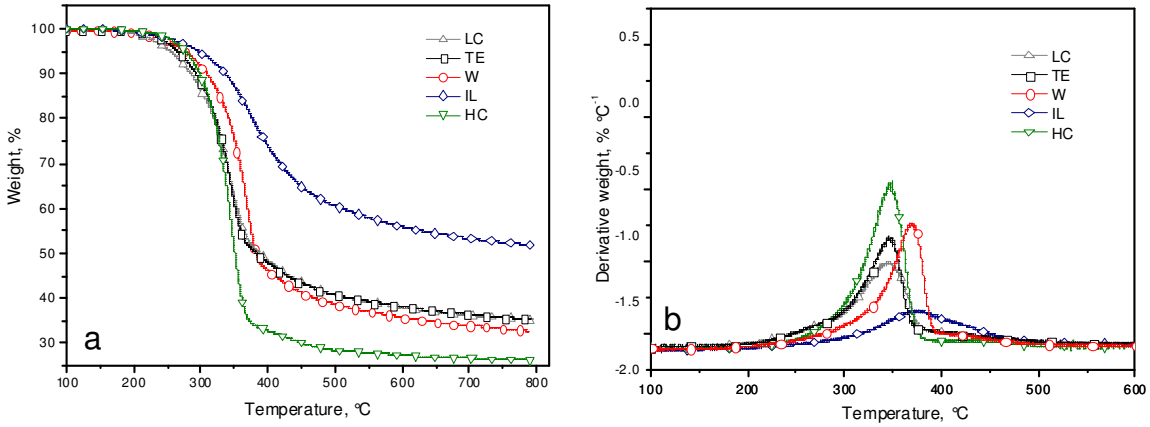

Figure 3: a) TGA and b) DTG curves under inert atmosphere of LC, TE, W, IL and HC. The DTG curves were vertically translated

Table 1
Average thermal data of LC, TE, W, IL and HC measured through TGA

Sample	$T_{5\%}$	T_{max}	$Char_{800\ °C}$ (%)
LC	253±0.9	344±1.1	35±0.7
TE	263±2.8	346±0.7	33±2.1
W	276±2.1	369±1.4	33±0.1
IL	297±1.8	374±2.1	52±0.5
HC	275±0.8	346±2.2	28±3.5

Thermal and mechanical properties of the PHB-based blends and films

In Figure 4, TGA thermograms of the PHB-based blends under nitrogen are shown. Neat PHB degraded through a single weight loss step beginning at about 250 °C. The polymer decomposition process was very fast, as complete volatilization was achieved within 300 °C. All the blends showed a second weight loss step, ascribed to the thermal degradation of the respective bio-fillers. It is worth noting that the thermal stability of PHB was significantly affected by the presence of the bio-fillers, as well as the kind of treatment the biomass was subjected to. In particular, LC acted as a pro-degrading agent, since the PHB weight loss step was anticipated by more than 10 °C, as evidenced in the inset of Figure 4 and in Table 2. A similar outcome was evidenced for PHB/TE, whereas the bio-filler W had no significant effect on PHB, the onset temperature being very similar. It was also noticed that none of the bio-fillers caused significant changes in the thermogram slope, suggesting that the degradation mechanism was not affected, although the curves were shifted in temperature.
The different behaviour between PHB/LC and PHB/W, reported above, suggests that LC contained a water-soluble fraction able to trigger the depolymerisation of PHB. The enhancement of the thermal parameters relative to the PHB/W blend confirmed that its removal restored the thermal degradation behaviour of the polymer. In order to get an insight into the nature of this pro-degrading component, the supernatant recovered after the water extraction of LC was analysed by means of EDS spectroscopy.

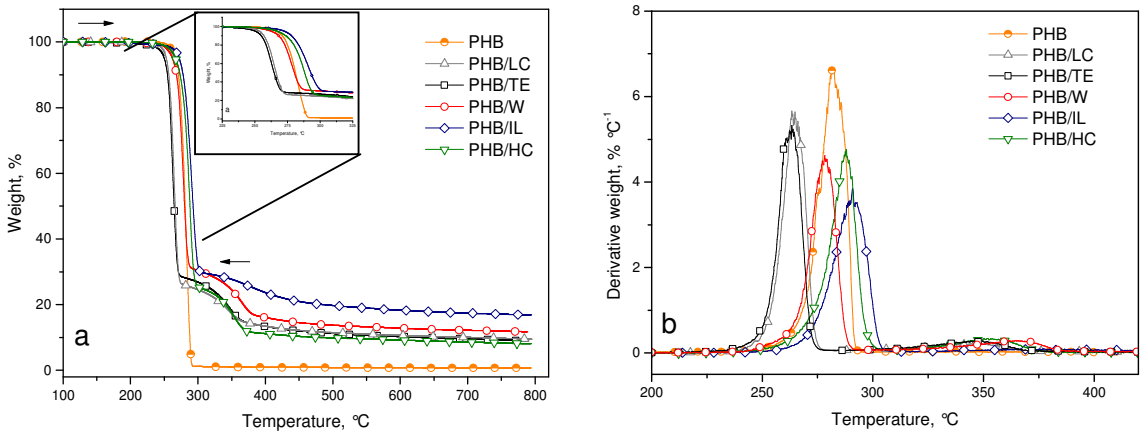

Figure 4: a) TGA and b) DTG curves under inert atmosphere of PHB-based blends and neat PHB. The DTG curves were vertically translated

Table 2
Average thermal data of PHB-based blends and neat PHB measured through TGA

Sample	$T_{5\%}$ (°C)	T_{max1} (°C)	T_{max2} (°C)	$Char_{800\ °C}$ (%)
PHB	266±0.3	282±0.2	-	0.6±0.2
PHB/LC	253±0.1	265±1.2	347±0.5	8.7±0.4
PHB/TE	253±4.0	265±2.3	348±1.4	9.9±1.2
PHB/W	265±3.0	279±2.7	365±0.9	11.8±0.2
PHB/IL	272±0.1	291±0.1	378±0.5	16.0±1.2
PHB/HC	267±2.1	289±2.7	354±0.3	9.7±2.4

It was found that the extraction liquor contained approximately 3 mol% potassium ions, which were deliberately added to the *Arundo donax* biomass to adjust pH in the first stage of the bioethanol production process. It has been recently reported that alkaline treatments can have a detrimental effect on the thermal stability of PHB,[27,28] since the presence of the cations at carboxylate chain-ends accelerate polymer β-elimination. Therefore, it is likely that the presence of potassium was responsible for the accelerated degradation. Finally, it is noteworthy to observe that both HC and IL slightly improved the thermal stability of PHB. In particular, IL was able to increase the degradation onset temperature by about 6 °C.

The PHB-based blends, in the form of dumbbell-shaped films, were then characterized through tensile tests in order to get an insight into their mechanical performance. Table 3 lists the mechanical parameters measured from the stress-strain curves. Plain PHB exhibited a stress-strain behaviour typical of a brittle polymer, characterized by failure occurring at low strain values.[29] Compared to PHB, all the biocomposites displayed an overall worsening of mechanical properties, whatever the bio-filler used. In particular, an abrupt drop in tensile strength (σ_b) and strain (ε_b) at break was evident for PHB/LC. On the other hand, the decrease in ductility was mitigated for the remaining biocomposites, while the elastic modulus (E) was even higher than that of PHB. In particular, the E value of PHB/TE was almost 40% higher than that of PHB. The decay of the mechanical performance of the biocomposites, with respect to the plain PHB, can be due to poor interfacial adhesion between bio-filler and matrix, which causes the formation of voids acting as stress concentrators and starting points for fracture propagation under tensile stress.[14]

It is worth noting, however, that no detrimental effect was observed on modulus values. This is due to the higher stiffness of the lignocellulosic-

based bio-fillers with respect to the polyester matrix. In fact, PHB/TE, which was prepared with the extractive-free solid residue from toluene/ethanol extraction, exhibited the highest modulus value.

Table 3
Average mechanical data of PHB-based blends and neat PHB as films measured through tensile tests

Sample	Tensile properties		
	E, MPa	σ_b, MPa	ε_b, %
PHB	3028±165	18.8±3.7	2.2±1.1
PHB/LC	2987±437	6.5±1.7	0.3±0.1
PHB/TE	4187±141	14.6±1.7	0.6±0.1
PHB/W	3599±123	14.0±1.3	0.6±0.1
PHB/IL	3326±193	14.0±1.2	0.6±0.1
PHB/HC	3606±391	12.0±3.5	0.5±0.1

It is likely that the solvents mixture removed a non-negligible amount of low molecular weight products, which were able to plasticize the thermoplastic matrix. Moreover, the comparison between PHB/LC and the other biocomposites also suggest that the pro-degrading effect ascribed to the untreated biomass can lead to polymer degradation during film compression moulding. Overall, these results demonstrate that the final properties of the biocomposites depend both on the type of filling biomass[30] and the kind of pretreatment carried out.

CONCLUSION

A lignocellulosic biowaste (LC) of the second generation bioethanol production was processed through different kinds of pretreatments. Extractions with water and a toluene/ethanol mixture and a chemical fractionation were performed on LC. The extractions allowed to leach organic and water-soluble extractives, and to recover two solid residues, TE and W, while the fractionation methods yielded acid-insoluble lignin (IL) and holocellulose (HC). SEM and ATR-FTIR analysis performed on LC and its derivatives showed that the removal of organic and water-soluble extractives from the biomass slightly affected the morphology and chemical structure. On the contrary, the IL and HC morphology differed significantly from that of LC, and the analysis of the chemical structure confirmed that the fractionation processes were carried out successfully. TGA analysis evidenced that the removal of a thermally unstable component by water extraction caused a noticeable enhancement of W thermal stability. IL showed the highest thermal parameters due to its highly aromatic and condensed structure. LC and all the derivatives were blended to PHB and compression-molded films were prepared. The thermal stability and mechanical properties of the resulting blends and films were evaluated. LC acted as a pro-degrading agent, since the PHB weight loss step was remarkably anticipated, whereas W, the solid residue free of water extractives, had no significant effect on PHB. This result was related to the presence in LC of potassium atoms able to accelerate the thermal degradation of the polymer. In fact, their removal restored the thermal degradation behaviour of the polymer. As for IL and HC, they produced only a slight improvement in the thermal stability of PHB. Compared to PHB, all the biocomposites displayed an overall worsening of mechanical properties in terms of ductility due to poor interfacial adhesion between the bio-fillers and the matrix. However, in the case of the PHB/TE film an increase in elastic modulus (E) up to 40% was measured. Overall, the mechanical properties of the biocomposites depended both on the type of filling biomass and the kind of pretreatment method carried out.

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