ARTICLE

Enzymatic synthesis of bio-based polyesters containing levoglucosan units

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Levoglucosan (LGA) is an anhydrous pyrolytic sugar which has gained increasing attention in recent years. The highest focus on conversion of LGA and other anhydro carbohydrates obtained from cellulose aims at the valorisation of these molecules and their application in multiple fields such as chemical and pharmaceutical industries. LGA presents a rigid structure with three hydroxyl groups on a pyranose ring, making it a promising building block for the synthesis of bio-based thermoplastic polymers with pending OH groups as soon as a regioselective polymerization is reached. In this work, a lipase-catalyzed series of LGA-containing terpolymers was synthesized for the first time *via* regioselective transesterification in two-stage polymerization. LGA was reacted with diethyl adipate/sebacate and α,ω-diols to give terpolymers with up to 35% of LGA content (related to the α,ω-diol content), number-average molecular weights (M_n) of up to 7,900 g.mol$^{-1,}$ and up to 94% yield. It was observed that the amount of LGA in the polymer structure affects the crystallinity, hydrophilicity and melting point of the polymers. We also evaluated the effect of the LGA loading and the co-monomers size on yields, molecular weight and the incorporation of LGA in the polymer structure. High loads of LGA lead to a decrease of the yield, but higher molecular weight and higher extent of LGA incorporation in the polymer can be obtained.

Introduction

Lignocellulosic biomass has emerged as an important renewable source for the production of green chemicals and materials[1–6]. Among the wide range of biomass-derived compounds, bio-based polymers are currently one of the most attractive applications, once they represent a more environmentally friendly alternative to petroleum-based polymers, presenting similar properties to their oil-based counterparts. In addition to renewability, some of these polymers also have several advantages such as biodegradability and biocompatibility, with a number of potential applications in food, cosmetic and pharmaceutical industries[7–10]. Consequently, new polyesters and polyamides have been synthesized from biomass-derived monomers, such as itaconic acid[11–13], levulinic acid[14–16], furan-2,5-dicarboxylic acid[17–21], bio-olefins[22], terpenes[23,24], and so on.

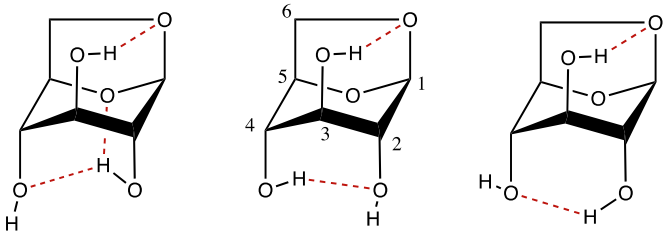

Figure 1. Levoglucosan displayed as its three conformers, showing the intramolecular hydrogen bonds (red).

Nowadays, these bio-based polymers are mainly produced using (organo-) metallic and organic catalysts. However, these reactions often require harsh conditions, which can limit the monomers scope and frequently lead to unwanted side reactions. In order to overcome these drawbacks, a variety of enzymes is alternatively employed as catalysts, since they are non-toxic, biodegradable and capable of working under mild conditions. Oxidoreductases, transferases, and hydrolases - particularly lipases - are the main enzymes classes reported in polymerization reactions, being the latter one the most widely applied, due to the high tolerance to organic solvents[25,26]. Lipases have already been applied into a number of polymerization reactions involving lignocellulosic biomass-derived monomers[27,28]. In spite of their natural role as hydrolytic enzymes converting triglycerides into fatty acids and glycerol, in organic media these biocatalysts also catalyze esterification and transesterification reactions with a broad range of unnatural substrates and high enantio- and regioselectivity[26,29,30].

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Electronic Supplementary Information (ESI) available: Tables showing absolute values for number average molecular weights, dispersity, yield, and LGA content; ^{1}H, COSY, and DOSY NMR; TGA and WAXS graphs. See DOI: 10.1039/x0xx00000x

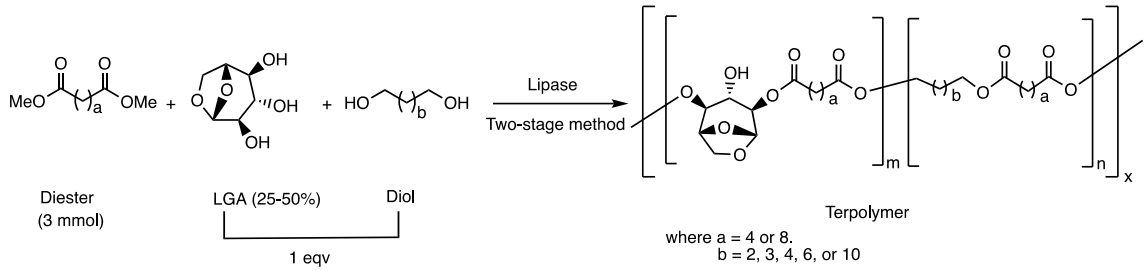

Figure 2. Simplified scheme for the production of terpolymers containing LGA via lipase-catalyzed two-stage polymerization reaction.

In this context, levoglucosan (1,6-anhydro-β-D-glucopyranose; LGA) has been identified as an attractive chemical platform[31–33]. LGA is an anhydrous sugar obtained from the fast pyrolysis of cellulose – the major component of lignocellulosic biomass – and has a rigid structure, with three hydroxyl groups disposed in *trans*-positions on a pyranose ring. It was already submitted to different reactions in order to produce diverse high-added value products, such as levoglucosenone, HMF, furfural, glucose-6-phosphate, gluconic acid, sorbitol and it was even tested for the production of biosurfactants and in antibacterial assays [34–37].

Yet, despite long-standing interest, no levoglucosan-based polymer was ever reported in literature, other than synthetic polysaccharides[38,39]. As pointed out in 1993 by researchers from the Canadian company BC Research, LGA could confer hydrophilicity, biodegradability and optical activity (chirality) to the polymeric structure[32]. These advantages are nevertheless still hampered by the low reactivity of LGA. As extensively described in literature, the spatial arrangement of the hydroxyl groups and consequent stabilization by intramolecular hydrogen bonds (Figure 1) hinders chemical and enzymatic transformations of LGA, leading to time-consuming reactions[40,41]. Enzymatically, the high polarity of LGA is also a disadvantage, restricting the scope of solvents suitable for use to a handful of polar solvents, which in general inhibit enzyme activity[42]. Our research group has recently reported the high regioselectivity of Novozym 435 (N435, *Candida antarctica* lipase B immobilized on acrylic resin) towards C2 and C4 hydroxyl moieties during the acetylation using long-chain aliphatic esters, while other lipases showed an increased preference for the OH at C4 at higher temperatures[37,43]. These findings have led to the possibility of synthesizing LGA-based linear polymers, possessing the rigid structure from LGA with pending hydroxyls moieties available for post-polymerization modifications.

Herein, we report the production of polyesters containing levoglucosan units *via* lipase-catalyzed polycondensation reactions and the effects of the LGA content on the polymer properties.

Results and discussion

We started our studies by evaluating the lipase-catalyzed polymerization between LGA and diesters with different chemical structures such as diethyl adipate, diethyl sebacate, dimethyl itaconate and dimethyl furan-2,5-carboxylate. During the preliminary tests, no polymer was obtained by using only LGA and the aforementioned monomers, despite the variety of polymerization methods evaluated, such as azeotropic polymerization, one-stage and two-stage polymerization methods (data not shown). The low reactivity, high

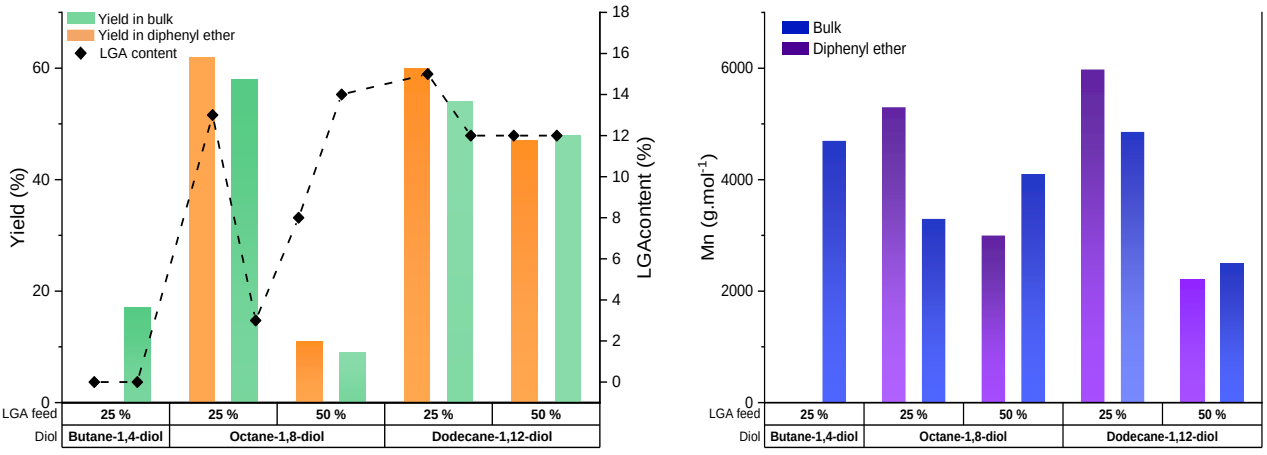

Figure 3. Enzymatic synthesis of LCPs using diethyl adipate, LGA, and three different α,ω-diols in bulk and solvent. **(A)** Yields and LGA content in the polymeric structure as a function of diol alkylene chain length and LGA load. **(B)** Number average molecular weight (M_n) related to diol alkylene chain length and LGA load; LGA feed is given in relation to the amount of diester; LGA content was calculated by ^{1}H NMR; Isolation yield obtained by weight after precipitation in MeOH and overnight drying; M_n was obtained by SEC in THF (PS Standards).

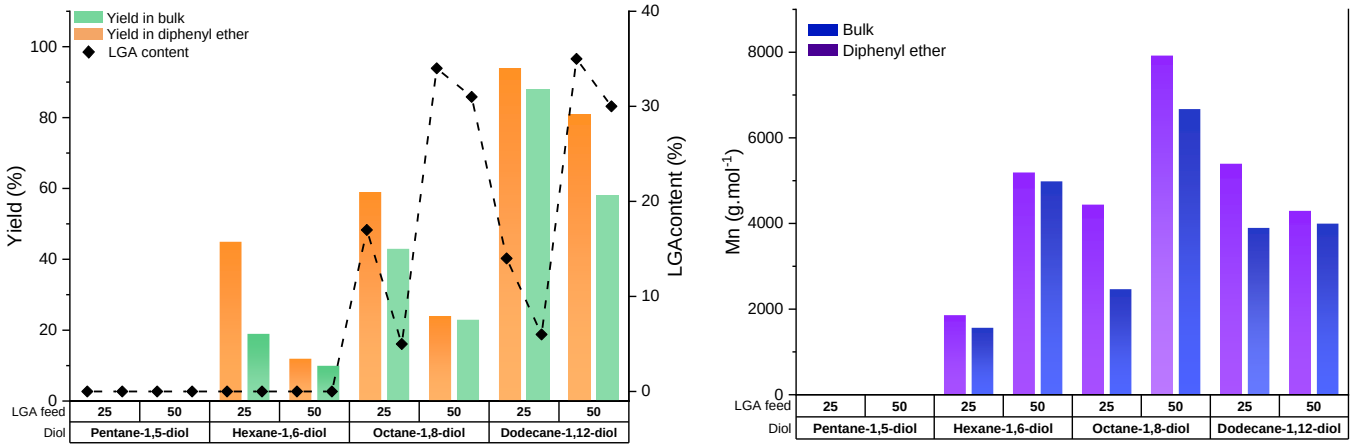

Figure 4. Enzymatic synthesis of LCPs using diethyl sebacate, LGA, and three different α,ω-diols in bulk and solvent. **(A)** Yields and LGA content in the polymeric structure as a function of diol alkylene chain length and LGA load. **(B)** Number average molecular weight (M_n) related to diol alkylene chain length and LGA load; LGA feed is given in relation to the amount of diester; LGA content was calculated by ^{1}H NMR; Isolation yield obtained by weight after precipitation in MeOH and overnight drying; M_n was obtained by SEC in THF (PS Standards).

hydrophilicity and low solubility of LGA seemed to be the main drawbacks to the reaction. Usually, long times are required to achieve good conversions. Galetti and co-workers reported that it took at least 5 days of reaction to achieve good conversions of LGA in lipase-catalyzed transesterification reactions in acetonitrile and ionic liquids[36]. In our best result, only diethyl adipate was consumed and 18% of conversion was achieved after 24 h, but no polymer was collected. Accordingly, we started to investigate the production of terpolymers containing LGA *via* the two-stage lipase-catalyzed polymerization reaction. The two-stage polymerization method usually consists of a first stage, where mainly oligomers are formed under atmospheric pressure, and a second stage, where vacuum is applied into the system in order to remove by-products and allow the polymer chain growth. It was suspected that oligomers formed during the oligomerization step could react more easily with LGA than with a single monomer[44,45]. Temperature was kept at 100 °C to improve the solubility of levoglucosan. Based on results reported by some of us and as it was herein observed, this temperature did not lead to loss of enzymatic activity, even after 24h of reaction[21,28]. A thermostability assay was also carried out to ensure that LGA would not degrade at this temperature, and after 24h, no degradation was observed.

Notwithstanding the LGA reactivity, recent works in literature reported reactivity issues by using dimethyl itaconate in polymerization reactions [44,45]. On the other hand, dimethyl furan-2,5-carboxylate (DMFDC) is already widely studied as an alternative to terephthalic acid-based polymers. However, terpolymers containing LGA and DMFDC were not obtained, probably due to rigid structures of both molecules, which can alter reactivity due to the fit in the active site of the enzyme[46]. So, the aliphatic diesters diethyl adipate and diethyl sebacate were selected to react along levoglucosan and different diols to investigate the production of LGA-containing terpolymers (LCP).

Production of LCPs catalyzed by Novozyme 435: The effect of the medium, the chain-length of monomers and LGA molar loading.

In a next step, the influence of medium, diol chain-length, and LGA loading were investigated on LCP formation (Figure 2). In order to circumvent solubility issues, LGA was added in lower amounts (25% and 50% related to diester molar concentration), considering that an equimolar ratio was kept between diester and the sum of the molar concentration of LGA and the diol used.

In our first tests using diethyl adipate, three diols were selected in combination with LGA: butane-1,4-diol, octane-1,8-diol, and dodecane-1,12-diol.

When diethyl adipate was employed (Figure 3), yields in solvent were slightly higher than in bulk. However, LGA content in the structure of the obtained polymers was drastically affected by changing the medium. The variation of LGA content is partially explained by the low solubility of levoglucosan in diphenyl ether. Even so, for most cases, diphenyl ether showed the best results.

LGA content in the polymers structure was limited to 15% (related to diol amount), even when the amount of LGA in the feed was increase from 25% to 50%. In fact, increasing LGA load even led to lower incorporation of LGA in the polymer.

Regarding the yields, it is reasonable to expect that enriching the medium with a poorly reactive and poorly soluble compound, such as LGA, could possibly lead to lower yields. However, this effect was less significant when a long chain-length diol such as dodecane-1,12-diol was used.

Under the studied conditions, yields and LGA content were higher the longer the diol length. When butane-1,4-diol was used, no polymer was obtained in diphenyl ether. On the other hand, in bulk, 17% yield was achieved, but no LGA was incorporated. Best results were reached using dodecane-1,12-diol or octane-1,8-diol in diphenyl ether, when the LGA feed ratio was 25%. M_n was limited to 6,000 g mol^{-1}.

Considering these results, we investigated the effect of the diester chain-length by using diethyl sebacate (DES). Here, pentane-1,5-diol, hexane-1,6-diol, octane-1,8-diol, and

Table 1. Thermal stability and crystalline ratio of selected terpolymers.

Entry	Polymer	M_n[a] (g mol^{-1})	LGA content[b] (%)	Thermal stability[c] (°C)		χ_c[d] (%)
				$T_{d\text{-}5\%}$	$T_{d\text{-}max}$	
1	Poly(dodecamethylene sebacate)	3,600	0	359	478	57
2	Poly(dodecamethylene sebacate–*co*–levoglucosan sebacate)	5,400	14	362	483	52
3	Poly(dodecamethylene sebacate–*co*–levoglucosan sebacate)	4,300	35	358	482	44
4	Poly(dodecamethylene adipate–*co*–levoglucosan adipate)	2,200	12	357	475	47
5	Poly(octamethylene sebacate–*co*–levoglucosan sebacate)	7,900	34	326	475	38

[a]Obtained by SEC in THF (PS standards); [b]Calculated from ^{1}H NMR; [c]Determined by thermogravimetric analysis; [d]Determined by WAXS.

dodecane-1,12-diol were employed. Results are shown in Figure 4.

Firstly, when compared to DEA, DES gave higher yields, higher M_n values, and higher LGA content in the polymer structure. Up to 94% yield was achieved (dodecane-1,12-diol, 25% LGA), but best results were obtained while using dodecane--1,12-diol and LGA feed of 50%, where 35% levoglucosan was successfully incorporated into the polymer structure and 85% yield was reached.

It is worth noting that no polymer was obtained using pentane-1,5-diol under any of the conditions tested. Despite yields of up to 45% using hexane-1,6-diol, reaction took place between DES and hexane-1,6-diol with no incorporation of LGA in the structure of the polymer. The overall results show a strong influence of the diol alkylene chain-length, since only when long diols (> C8) such as octane-1,8-diol were employed, LGA participated in the reaction. This effect of monomers bearing long hydrophobic alkylene chains in reactions catalyzed by lipases is expected due to higher solubility of these monomers and the well-known interfacial activation of lipases[47]. Indeed, *Candida antarctica* lipase B (CalB), the lipase used throughout this work, does not have a lid, leading one to think that it will not undergo interfacial activation. However, an interesting study has recently reported that CalB can suffer interfacial activation when in contact with large, bulky substrates[48]. The authors state that CalB may act as an esterase for short substrates and as lipase with the long ones. Hence, this effect is not clearly observed when short monomers are employed[49]. These results are in accordance with previous results reported in literature[50,51]. However, the low or no polymer formation here was also likely to be caused by the change in stoichiometry due to the presence of LGA, considering that polymers between DES/DEA and short chain α,ω-diols are described elsewhere[28,52]. Octane-1,8-diol have given excellent results regarding M_n (up to 7,900 g mol^{-1}) and incorporation of LGA in the structure of the polymers (up to 31%), the yields were quite low in both solvent and bulk.

Despite the correlation observed between the length of monomers and the yield, this correlation was not reflected on M_n values. When 25% LGA was used, highest molecular weights were indeed achieved the longer the diol length, but with 50% LGA loading, no correlation was noticed.

LGA loading had a negative impact on the yields, although polymers with high LGA content and high molecular weights have been obtained with 50% LGA load. Medium nature also affected the results, showing decreased yields and M_n in bulk conditions. This effect was still more prominent with high levoglucosan load, most likely caused by less efficient mixing in bulk compared to diphenyl ether.

Effect of the levoglucosan content on crystallinity and thermal properties of the obtained terpolymers.

Thermal stability of the selected polymer samples was determined by thermogravimetric analysis (TGA). In order to evaluate if the LGA amount in the polymeric structure could affect the thermal stability, a poly(dodecamethylene sebacate) was synthesized, under the same conditions, by using only dodecane-1,12-diol and diethyl sebacate as monomers.

As one can observe in Table 1, the polymers tested possess similar decomposition profiles, presenting high thermostability. Poly(octamethylene sebacate – *co* – levoglucosan sebacate) (Table 1, entry 5) – even having the highest M_n and 34% of LGA content - was the less stable polymer, showing a decomposition temperature range at 5% weight loss ($T_{d\text{-}5\%}$) of 326°C and decomposition temperature range at 10% weight loss ($T_{d\text{-}10\%}$) of 358°C (supplementary information, figure S4). So, with the exception of poly(octamethylene sebacate – *co* – levoglucosan sebacate), the samples tested showed a $T_{d\text{-}5\%}$ range from 357 to 362°C, a $T_{d\text{-}10\%}$ range from 373 to 381°C. For all samples tested, the decomposition temperature range at a maximum rate of decomposition ($T_{d\text{-}max}$) was between 478-483 °C. It is also worth to note that the increase of LGA content did not significantly affect the thermal decomposition profile of the polymers.

On the other hand, DSC analysis suggests that the amount of LGA in the polymer strongly affected crystallinity (Figure 5). As observed, the incorporation of only 14% levoglucosan into the polymeric structure led to a decrease in the melting point by around 8 °C and a drop in crystallinity from 152 J/g down to 120 J/g. Similarly, a further increase in levoglucosan to 35% led to a further decrease in the melting endotherm and crystallinity to 57 °C and 86 J/g, respectively. Unfortunately, the glass transition temperatures (T_g) were not clearly identified for the evaluated polymers. The T_g (= -31 °C) was only detected for the sample with the lowest crystallinity (35% LGA; Table 1, entry 3, see figure S6). For the other two samples, either the glass transition amplitude is too low to be detected, due to the higher degree of crystallinity, or the high LGA content leads to a stiffening of the macromolecules and consequently increases the T_g amplitude.

In the latter case, it means that the lower the LGA content, the lower the T_g amplitude will be, probably below the minimum detectable[53].

The effect of the amount of LGA in the polymer structure on crystallinity was later confirmed by wide-angle X-ray scattering (WAXS). All polymers evaluated showed similar WAXS patterns with two main diffraction peaks at 21° and 24° and low intensity peak at 30° (supplementary information, figure S5). Nevertheless, as one can observe in table 1, when comparing similar polymers with different LGA content (Entries 1-3), the degree of crystallinity decreased with increasing LGA content. Modulating the crystallinity degree is an interesting feature, since high degree of crystallinity is associated with high brittleness of some polymers, whereas low crystalline polymers are found to be more ductile[54]. In addition, polymers with extended crystalline regions have reduced water permeability, being less prone to enzymatic degradation, an important factor in terms of recyclability[55–57].

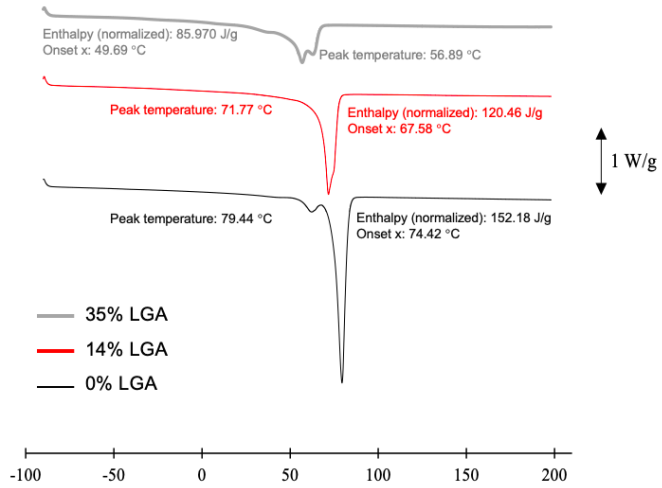

Figure 6. Variations in melting endotherms (°C) and crystallization enthalpies (J/g) as a function of increasing levoglucosan incorporation into the polymer structure.

Contact angle

Thin polymeric films were prepared to investigate the effect of LGA content on the polymers hydrophilicity by determining the contact angle of a water droplet. Besides poly(levoglucosan sebacate), poly(levoglucosan sebacate-*co*-dodecamethylene sebacate) with 14%, 28%, and 35% of LGA content were employed. As can be seen in **Erreur ! Source du renvoi introuvable.**, the increase of levoglucosan content in the polymer structure led to an increase of hydrophilicity. This effect is mainly explained by the higher density of hydroxyl groups from levoglucosan. These results follow a logical pattern, suggesting that the hydrophilicity of the polymer can be controlled by controlling levoglucosan content, which is a key feature in biomedical and textile applications[58–60]. Hydrophilicity is also a critical parameter to control degradation rates[61,62].

Assays using levoglucosan bio-oil.

The production of LCPs was also carried out using the LGA bio-oil (LGA_{bio}) obtained from fast pyrolysis of cellulose. This assay was carried out employing the monomers that gave the best results — diethyl sebacate and dodecanediol — under the same conditions.

The bio-oil was employed without previous purification or drying steps. Despite the high content of water in the oil, which

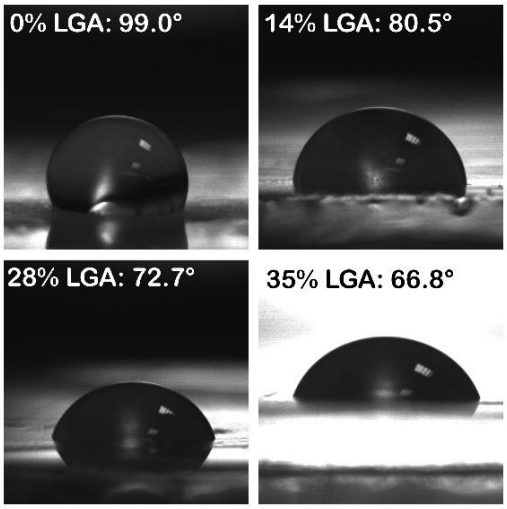

Figure 5. Images from the water contact angle tests showing relation between LGA content and hydrophilicity of the polymers. Polymers were synthesized from diethyl sebacate, dodecane-1,12-diol, and levoglucosan (if applicable).

could lead to lower rates and final conversion, good yields were achieved. Results are show in table 2.

As one can note, the incorporation of levoglucosan into the polymeric structure was limited to 8%, even with at higher LGA_{bio} loading. Notwithstanding the water content in the bio-oil — which is partially circumvented at high temperature under vacuum — the main hindrance for the incorporation of LGA is probably the poor solubility of LGA_{bio} in diphenyl ether. In fact, during the reaction, most of the LGA_{bio} remained insoluble.

Table 2. Enzymatic synthesis of LCPs using LGA bio-oil, diethyl sebacate and dodecane-1,12-diol.

Entry	LGA_{bio} Loading[a]	M_n[b] (g mol^{-1})	LGA content[c] (%)	Yield[d] (%)
1	25%	2,600	7.1	66
2	50%	2,600	8.0	43

[a]Related to the amount of diester. [b]Number-average molecular weight obtained from size-exclusion chromatography (SEC, THF, PS standards). [c]Calculated from ^{1}H NMR. [d]By weight.

Currently, some assays are being carried out by our research group in order to optimize the reaction conditions and improve LGA_{bio} content in the polymeric structure as well as the polymer properties.

Experimental

Materials

Levoglucosan (LGA, 99%) was acquired from Biosynth. Diethyl adipate (DEA, 99%), butane-1,4-diol (99%), pentane-1,5-diol (99%), hexane-1,6-diol (99%), octane-1,8-diol (98%),

dodecane-1,12-diol (98%), diphenyl ether (99%) and *Candida antarctica* lipase B (N435, CalB, 5,000 U g^{-1}) immobilized on macroporous acrylic resin were purchased from Sigma-Aldrich. Diethyl sebacate was purchased from Tokyo Chemical Industry. Analytical grade methanol and chloroform (99%) were purchased from VWR. ChloroformD ($CDCl_3$) (99.8%) and deuterated dimethyl sulfoxide (DMSO-d_6) were purchased from Euriso-Top. All the reagents and solvents were used without further purification.

Methods

Synthesis of LGA-containing terpolymers *via* lipase-catalyzed two-stage polymerization. The appropriate amount of diester (DEA or DES; 3 mmol), LGA (0.75-1.5 mmol), diol (butane-1,4-diol, pentane-1,5-diol, hexane-1,6-diol, octane-1,8-diol, dodecane-1,12-diol; 1.5-2.25 mmol), and CalB (20%wt of total monomers) were added into schlenk tubes. For reactions in solvent, 1.5 mL of diphenyl ether was added. Mixture was stirred at 100°C under atmospheric pressure during 2 hours. Afterwards, vacuum was applied into the system, reducing pressure to 10-50 mbar. Then, stirring was kept for 24 hours. Finally, reaction was stopped by the addition of 5 ml of $CHCl_3$ and the catalyst removed by filtration. Solution was concentrated and added drop-wise to an excess of cold methanol under stirring to precipitate the polymers. The precipitate was then removed by filtration and left to dry overnight.

Analytical methods

^{1}H and 2D-COSY Nuclear Magnetic Resonance. ^{1}H NMR, 2D-COSY spectra were recorded at room temperature on a Bruker Avance 300 instrument (delay time = 3 s, number of scans = 32) at 300.13 MHz using either $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts (ppm) are given in δ-units and were calibrated using the residual signal of $CDCl_3$ and DMSO-d_6 at 7.26 ppm and 2.5 ppm, respectively. DOSY spectra was recorded on Avance II 400 Bruker spectrometer (9.4 T) regulated at 298 K respectively in $CDCl_3$. Additionally, ^{1}H NMR was used to confirm conversion and determine its rate. Data acquisition and analysis were performed using the Bruker TopSpin 3.2 and MestReLab 6.0. The percentage amount of LGA was calculated from the ^{1}H NMR spectra as

$$\boldsymbol{X_{LGA}}(\%) = \frac{\boldsymbol{I_{LGA-ester}}}{(\boldsymbol{I_{LGA-ester}}+\boldsymbol{I_{diol-ester}})},$$

where $I_{LGA\text{-}ester}$ is the integration value of the peak assigned to the levoglucosan-ester bond at ~2.38 ppm, while $I_{diol\text{-}ester}$ is the integration value of the peak assigned to the diol-ester bond at ~2.28 ppm. An example of ^{1}H NMR peak assignments is given in figure 7. Additional spectra are given in supplementary information.

Poly(octamethylene adipate–*co*–levoglucosan adipate). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.44 (s, 1H), 4.77 (s, 1H), 4.66 (s, 1H), 4.57 (s, 1H), 4.28 – 4.12 (m, 1H), 4.04 (t, *J* = 6.7 Hz, 6H), 3.84 – 3.72 (m, 1H), 2.44 – 2.32 (m, 4H), 2.28 (t, *J* = 7.5 Hz, 8H), 1.69 – 1.54 (m, 18H), 1.30 (s, 39H), 1.26 (s, 8H).

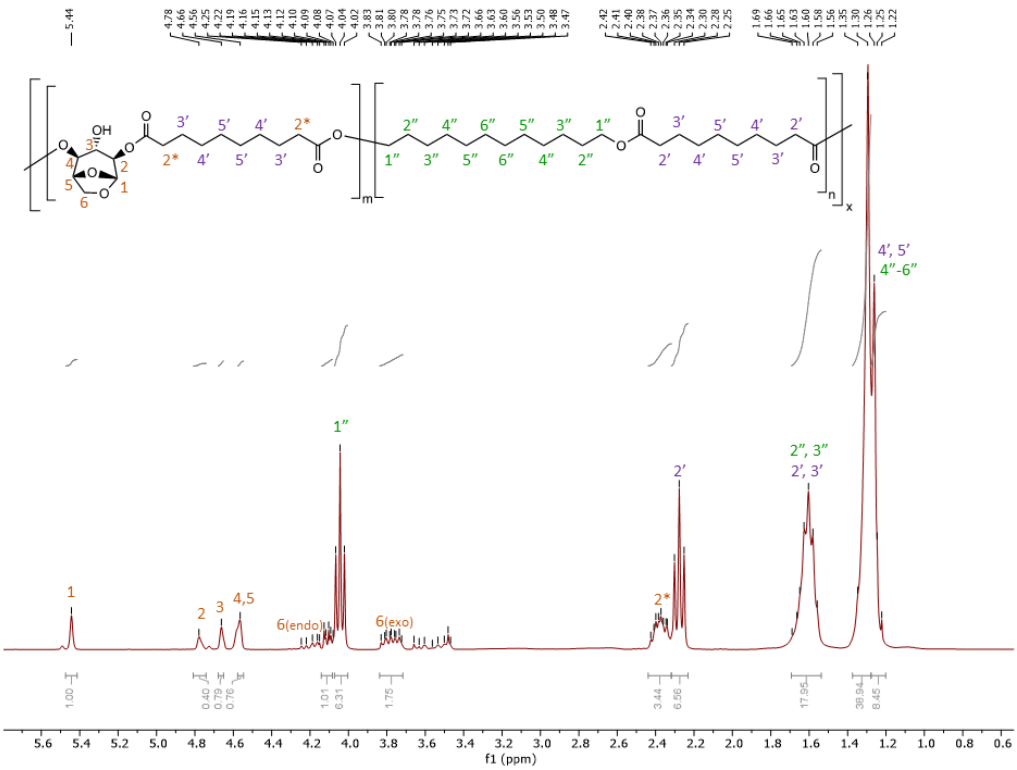

Figure 7. ^{1}H NMR spectrum (300MHz, $CDCl_3$) and peak assignment for poly(dodecamethylene sebacate–*co*–levoglucosan sebacate) (35% LGA), where R can represent an ester or a hydrogen atom. *The regioselectivity towards C2 in levoglucosan was chosen arbitrarily and might occur at C3 and C4.*

Poly(dodecamethylene adipate–*co*–levoglucosan adipate). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.45 (s, 1H), 4.78 (s, 1H), 4.67 (s, 1H), 4.57 (s, 1H), 4.30 – 4.08 (m, 1H), 4.05 (t, J = 6.7 Hz, 6H), 3.84 – 3.73 (m, 1H), 2.28 (t, J = 7.5 Hz, 6H), 1.60 (q, J = 7.0 Hz, 21H), 1.33 – 1.19 (m, 41H).

Poly(octamethylene sebacate–*co*–levoglucosan sebacate). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38 (s, 1H), 4.71 (s, 1H), 4.50 (s, 2H), 4.19 – 3.99 (m, 4H), 3.98 (t, J = 6.7 Hz, 7H), 3.79 – 3.62 (m, 2H), 3.59 (s, 1H), 2.39 – 2.25 (m, 4H), 2.21 (t, J = 7.5 Hz, 8H), 1.57 – 1.47 (m, 36H), 1.34 – 1.15 (m, 38H).

Poly(dodecamethylene sebacate–*co*–levoglucosan sebacate). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.45 (s, 1H), 4.78 (s, 1H), 4.66 (s, 1H), 4.57 (s, 1H), 4.15 (dd, *J* = 16.9, 7.3 Hz, 1H), 4.05 (t, 4H), 3.78 (dd, *J* = 5.9 Hz, 1H), 2.38 (s, 4H), 2.28 (t, 4H), 1.74 – 1.49 (m, 16H), 1.28 (d, *J* = 9.7 Hz, 26H).

Size Exclusion Chromatography (SEC). Analysis were performed on a Waters apparatus equipped with Waters Styragel columns HR2, HR3, HR5 and HR5E. Samples were dissolved in tetrahydrofuran (2 $mg.mL^{-1}$) and analyzed at 40 °C at a flow rate of 1 ml min^{-1}. M_n and Đ were determined from the Refractive Index (RI) signal using a calibration curve based on polystyrene (PS) standards from Polymer Standards Service.

Differential Scanning Calorimetry (DSC). Thermal transition was recorded on a TA Discovery DSC 25 using a cooling-heating-cooling-heating method. First, samples of ~10 mg were sealed in aluminum pans, the temperature was equilibrated at -90 °C, followed by a heating ramp of 10 °C.min^{-1} to 200 °C, then a cooling ramp of 10 °C.min^{-1} to - 90 °C, and a second heating ramp of 10 °C.min^{-1} to 200 °C. Thermograms were analyzed using TA-Instruments TRIOS software.

Thermogravimetric analysis (TGA). Thermal stability measurements were performed on a TA-Instruments Q600 SDT under nitrogen environment. Samples were heated from 30 °C to 600 °C at a scan rate of 10 °C.min^{-1}.

Wide-angle X-ray scattering (WAXS). Data were recorded on a Xeuss 2.0 apparatus (Xenocs) equipped with a micro source using a Cu Kα radiation (λ= 1.54 Å) and point collimation (beam size: 500×500μm²). The distance between the sample and the detector (~15 cm) was calibrated using silver behenate as standard. Through 2D view diffraction patterns are recorded on a Pilatus 200k detector (Dectris). Integrated intensity profiles were computed from the 2D patterns using the Foxtrot® software. Exposure time was 15 min.

Conclusions

In the present work, we report for the first time the lipase-catalyzed synthesis of levoglucosan-containing terpolymers as part of our efforts towards biomass valorization. Despite the low reactivity of levoglucosan, polyesters of up to 7,900 $g.mol^{-1}$ were obtained with up to 35% of levoglucosan content.

Results showed strong influence of monomers alkylene chain-length on the incorporation of LGA into the polymeric structure. When butane-1,4-diol and pentane-1,5-diol were used, poor results were obtained and levoglucosan was not incorporated in the polymer structure. Thus, best results were achieved by employing longer monomers (C>8). Medium nature had a minor impact on yields and LGA content. Reactions in diphenyl ether led to slightly higher yields and LGA content than in bulk when diethyl sebacate was used, but no difference was observed for diethyl adipate. LGA load had a negative effect on the yields, but led to higher incorporation of LGA.

It was also observed that the LGA content has enhanced hydrophilicity and decreased the crystallinity and melting points of the obtained polyesters. However, the LGA content does not seem to affect the thermostability.

Finally, we also performed reactions using LGA bio-oil obtained directly from the cellulose pyrolysis without any prior purification steps. In this case, only 8% of LGA was incorporated in the polymeric structure. These results show that performing the polymerization reaction using biocrude LGA is feasible, but still needs further investigation.

Author Contributions

J.B. and K.N. performed all the experiments, analysis, and conceptualization. J.B. was responsible for the writing of the original draft. G.S. provided WAXS and crystalinity data. M.B. contributed with structural elucidation and additional NMR analysis. J.-M.R., A.F.-H., and P.Z. were in charged of project supervision; R.W. and P.Z. were in charge of project administration; I.I.J. was the general research coordinator in charge of project administration and supervision.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This study was supported by the French government through the Programme Investissement d'Avenir (I-SITE ULNE/ANR-16-IDEX-0004 ULNE) managed by the Agence Nationale de la Recherche. LIA CNRS France-Brazil “Energy & Environment” and Métropole Européen de Lille (MEL) for “CatBioInnov” project are also acknowledged. This work was funded by the FWV ALPO Interreg Grant and the authors thank the European Regional Development Fund (FEDER) and the University of Lille. Chevreul Institute (FR 2638), Ministère de l’Enseignement Supérieur, de la Recherche et de l’Innovation, Région Hauts de France are also acknowledged for supporting and partially funding this work. JMR is a FNRS research fellow at University of Mons. The project ARCHI-CM, Chevreul Institute (FR 2638), Ministère de l’Enseignement Supérieur et de la Recherche, Région Nord-Pas de Calais and European Regional Development Fund (FEDER) are acknowledged for supporting and funding the SAXS-WAXS laboratory equipment.

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