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Elucidating enzymatic polymerisations: chain-length selectivity of *Candida antarctica* lipase B towards various aliphatic diols and dicarboxylic acid diesters

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Abstract

The sustainable synthesis of polymers is a field with growing interest due to the need of modern society to preserve the environment whilst making used products and food sustainable for the future generations. In this work we investigate the possibility of synthesizing aliphatic polyesters derived from various dicarboxylic acid diesters and diols in a solvent-free reaction system. *Candida antarctica* lipase B was selected as biocatalyst and its selectivity towards the carbon and ester chain length were elucidated. The selected enzyme was able to synthesize various polyesters combining C_4-C_{10} diesters and C_4-C_8 diols. All combinations led to monomer conversions above 90% in 24 h with the best number average molecular weights (M_n) being obtained through the combination of dimethyl adipate and 1,8-octanediol leading to a M_n of 7141 Da. Differential scanning calorimetry analysis shows a clear trend with an increase in melting temperature of the polymers that correlates with both the increase of the M_n or of the polymer's constitutional repeat unit carbon chain length. Thermogravimetric analysis and rheology measurements performed on selected samples also confirm the trend showing a variation of the polymer's degradation temperatures and viscosity profiles.

Introduction

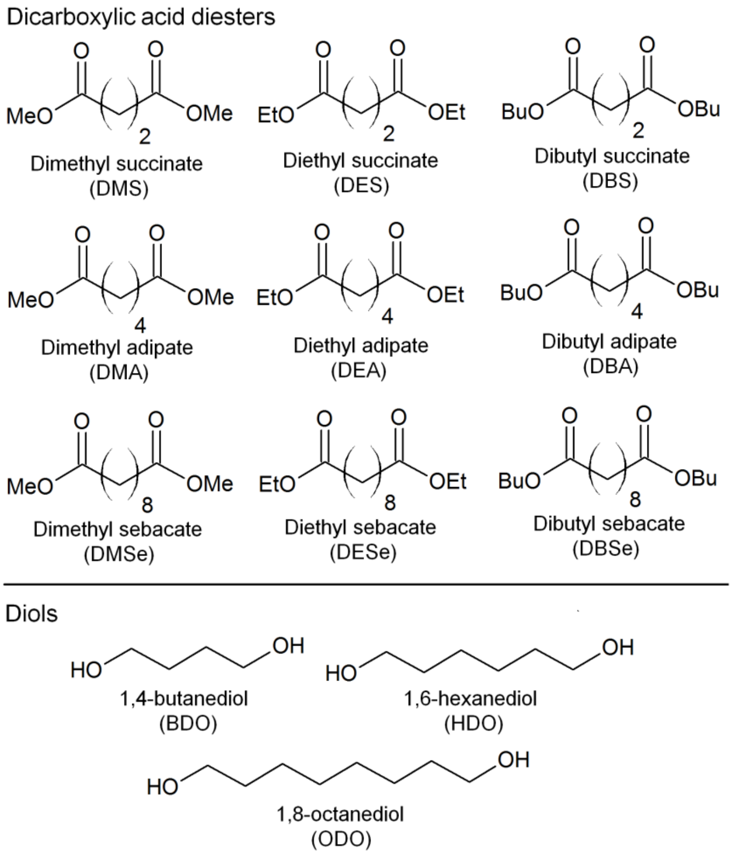
The application of biocatalysts in organic synthesis offers several advantages compared with traditional chemo-catalysts such as milder reaction conditions with regards to temperature (usually T<100 ºC), pressure and pH (normally 3-8). Such conditions often lead to remarkable energy efficiency, high enantio-, regio- and chemo-selectivities as well as controlled stereochemistry. These features allow the development of new functional compounds for pharmaceuticals, agrochemicals and polymers using nontoxic natural catalysts with a significant "green" appeal having commercial benefits and satisfying ecological requirements [1].

Despite studies focused on the use of glycosidases for the synthesis of natural and unnatural polysaccharides [2], as well as oxidoreductases for the polymerisation of phenol derivatives [3] and vinyl monomers [1] (mainly using laccases and peroxidases), the most investigated area of enzymatic synthesis is the production of polyesters via both polycondensation (transesterification) and ring opening polymerisations (ROPs) [4,5]. These areas have predominantly emerged thanks to the discovery and commercial availability of *Candida antarctica* lipase B (CaLB). Over recent years the extraordinary properties of this enzyme were brought to light from several research teams in the kinetic resolution of organofluorine rac-alcohols [6], the synthesis of glucoside esters [7] and the enantioselective synthesis of a β-amino acid ester via a solvent-free chemo-enzymatic reaction among others [8].

Further to this, CaLB has been shown to be an active catalyst for the synthesis of a wide range of aliphatic [9], aliphatic functional [10, 11] (e.g. polyesters containing lateral functionalities such as vinyl and hydroxy groups) and aliphatic-aromatic polyesters [12,13] and polyamides [14]. In recent years these polyesters and polyamides have been derived preferentially from renewable monomers such as 2,5-furandicarboxylic, adipic and succinic acids and 1,4-butanediol among others [5].

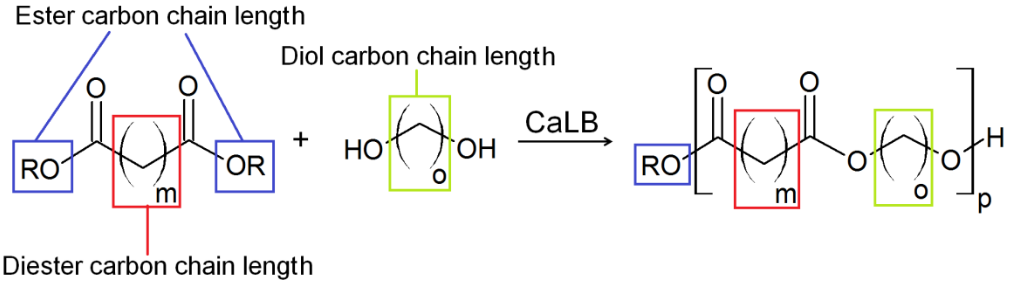
Despite several other enzymes belonging to the hydrolyses family, namely cutinases from *Humicola insolens* [15], *Fusarium solani pisi* [16] and *Thermobifida cellulosilytica* [17], being reported to be active for the synthesis of various polyesters and polyamides in their lyophilized and immobilized forms (ranging from cross-linked enzyme aggregates to covalent binding [18, 19]), the choice of the chemist is still often the readily available CaLB adsorbed on a methacrylic resin known under the tradename of Novozym® 435. This biocatalyst has been shown to be active and stable in several different conditions ranging from water-based to anhydrous organic media and up to temperatures of ~100 ºC.

Among the many synthesis studies both on polycondensation and ROPs that were performed over the years using this enzyme, we found there is a lack of comparative studies on the range of monomers shown in Scheme 1 (most of which bio-based) [20] for the synthesis of aliphatic polyesters.



Scheme 1. Dicarboxylic acid diesters (top) and linear diols (bottom) used in this study for the enzymatic synthesis of aliphatic polyesters in solvent-free systems using Candida antarctica lipase B as biocatalyst.

Despite the many studies on the topic, there remains an absence of any detailed systematic study into the implications of changing the diester and/or diol whilst applying identical methodologies for the polymerisation. In the present study we investigated the CaLB-catalyzed polycondensation of various methyl-, ethyl- and butyl- dicarboxylic acid esters with various linear diols having a carbon chain length of 4, 6 or 8, shedding light on how reactive different size diesters are when employing enzymatic catalysis in a solvent-free reaction system (Scheme 2). These results will/have help(ed) us to understand the strengths and weaknesses of using this, until now, sporadically investigated enzyme for polyester synthesis.



Scheme 2. The three parameters that were investigated in the present study, namely 1) ester carbon chain length; 2) diester carbon chain length and 3) diol carbon chain length.

Materials and methods

Chemicals and enzymes

1,4-butanediol (BDO) was purchased from Alfa Aesar. Dimethyl adipate (DMA) and 1,8-octanediol (ODO) were purchased from Acros Organics. Dimethyl succinate (DMS) was purchased from Fluka. All other chemicals and solvents were purchased from Sigma-Aldrich and used as received if not otherwise specified. *Candida antarctica* lipase B (CaLB) immobilized onto methacrylic resin was purchased from Sigma-Aldrich (product code L4777) (N435). The enzyme was dried under vacuum for 96 h at 25 ºC and stored in a desiccator prior to use.

Enzymatic polycondensation of aliphatic polyesters

0.006 mol of dicarboxylic acid ester (A) and 0.006 mol of linear diol (B) (diester:diol ratio= 1.0:1.0) were accurately weighted in a 25 mL round bottom flask. The mixture was then stirred at 85 ºC until a homogeneous melt was obtained. 10% w w^{-1} (calculated on the total amount of the monomers) of N435 was then added and the reaction was run for 6 h at 1 Atm. A vacuum of 20 mbar was subsequently applied for an additional 18 h maintaining the reaction temperature at 85 ºC (total reaction time: 24 h). The reaction product was recovered by adding THF (or DCM in the case of the 24 h succinate-BDO polymers) in order to dissolve the solid reaction products. The biocatalyst was then removed via a filtration step and the solvent evaporated under vacuum. The polymers were then characterised without additional purification steps.

Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H-NMR analyses were performed on a JEOL JNM-ECS400A spectrometer at a frequency of 400 MHz. $CDCl_3$ was used as NMR solvent for all synthesized polymers.

Gel Permeation Chromatography (GPC)

GPC was carried out using a PSS SDV High set composed of 3 analytical columns (300 × 8 mm, particle diameter 5 µm) of 1000, 1000*10^5 and 10^6 Å pore sizes, plus guard column (Polymer Standards Service GmbH, PSS) installed in a PSS SECcurity SEC system. Elution was with THF at 1 mL min^{-1} with a column temperature of 23 ºC and detection by refractive index. 20 µL of a ~2 mg mL^{-1} sample in THF, adding a drop of toluene as reference standard, was injected for each measurement and eluted for 50 min. Calibration was carried out in the molecular weight range 370-2520000 Da using the ReadyCal polystyrene standards supplied by Sigma Aldrich and referenced to the toluene peak.

Differential Scanning Calorimetry (DSC)

Traditional DSC analyses were performed on a TA Instruments Q2000 under nitrogen atmosphere. The heating rate used was 5 ºC min^{-1} over a T range of −60-200 ºC. Cooling rate was set at 5 ºC min^{-1} over the same T range. Sample mass was of 5-10 mg for all measured samples. The polymer’s melting ranges were calculated from the second heating scan using the value at peak maximum option.

Thermogravimetric analysis (TGA)

TGA was performed on a PL Thermal Sciences STA 625 thermal analyzer. ∼10 mg of sample was weighted in an aluminium pan. The sample was then placed into the furnace with a N_2 flow of 100 mL min^{-1} and heated from 21 to 625 ºC at a heating rate of 10 ºC min^{-1}. From the TGA profiles the temperatures at 5% and 50% mass loss (TD5 and TD50) were subsequently determined.

Rheology measurements

Rheological rotational measurement under controlled shear rate were recorded using a Brookfield R/S-CPS+ Rheometer fitted with a Peltier P-TS2 temperature controller and cone-plate geometry where the cone used had a 25 mm diameter, 2º angle and 0.045 mm gap height. The polymer samples were ground with a pestle and mortar prior to being loaded onto the pre-heated (80 ºC) bottom plate. In all cases, a constant shear rate of 100 s^{-1} was maintained throughout the experiments with each polymer melt being initially held at 80 ºC (under shear) for 120 s before the temperature was decreased by 0.01 ºC s^{-1}.

Results and Discussion

The CaLB-catalysed polycondensation of methyl-, ethyl- and butyl- succinic (C_4), adipic (C_6) and sebacic (C_{10}) dicarboxylic acids esters with linear diols having a carbon chain length of 4, 6 or 8 atoms were investigated. In order to be environmentally friendly, all reactions were performed in bulk (i.e. solvent-less). All monomers were melted at 85 ºC before adding the biocatalyst that allowed the initiation of the step-growth polycondensation. From the ^{1}H-NMR spectra analysis it is possible to observe that for all reactions excellent monomer conversion values (>90%) were obtained with the methyl and ethyl esters consistently giving slightly higher conversions than the equivalent butyl esters for all the dicarboxylic diesters investigated (Figure 1).

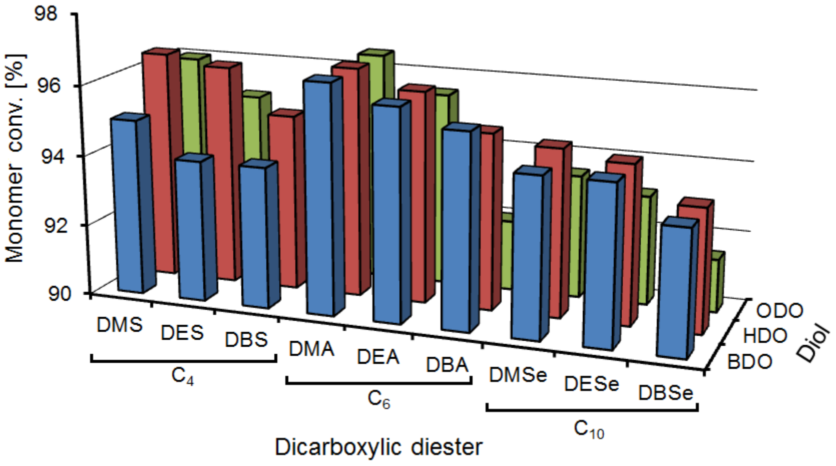
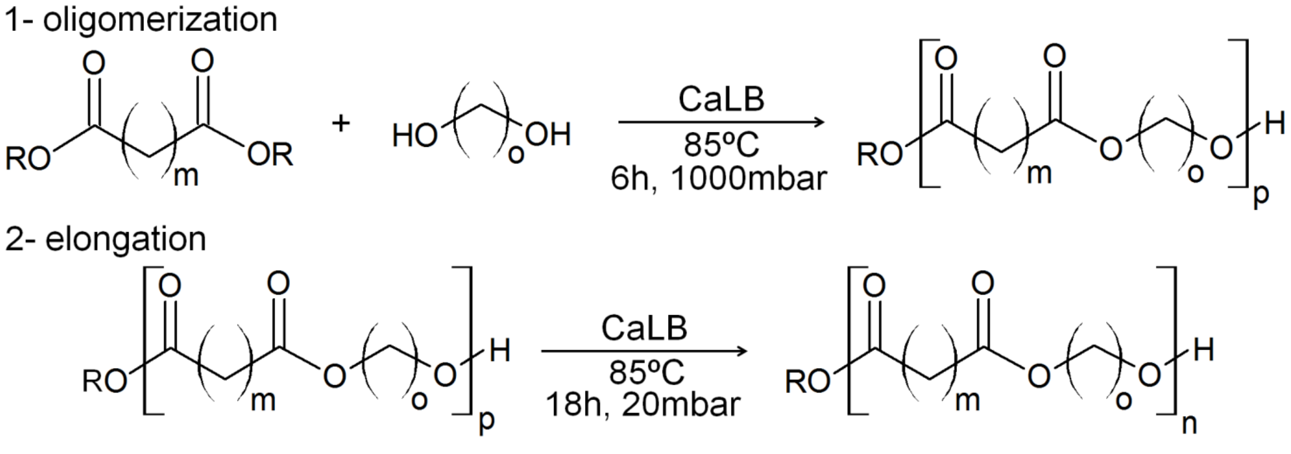


Figure 1. ^{1}H-NMR analysis of the crude polycondensation products after CaLB-catalyzed polycondensation reactions after 24 h. Reactions without catalyst led to no detectable transesterification products. All reactions were performed in duplicates. Statistical analysis reporting the mean value ± standard deviation for all reactions can be found in ESI, Figure S1 and the complete spectra assignment in Figure S5.

The 2-step polycondensation protocol (1st-oligomerization and 2nd-chain elongation) reported in Scheme 3 was found suitable for all the reactions (see Table 1). The polycondensation reactions occur via transesterification of dicarboxylic acids diesters with aliphatic diols and follow a step-growth polymerisation mechanism (the same as some other similar synthetic polymers such as polyamides and polyurethanes). In fact with low DP (~5-10) the conversion is already >80-90% and further increase in DP (two fold) influences only minor the monomer conversion. This allows the initial oligomerisation of most of the monomers without loss of any of the starting compounds. After oligomerisation is achieved, vacuum is applied to effectively remove the alcohol by-product and enables the elongation of the polymeric chain length. The biocatalyst is indeed able to catalyse synthetic reactions starting from long polymeric chains as recently reported by Vastano et al. who coupled dimethyl itaconate and poly(ethylene glycol) to poly(hydroxyalkanoates) having M_n of 50188 Da and M_w of 117720 Da produced using an engineered strain of Escherichia Coli [21].



R= -methyl, -ethyl or -butyl o= C_4, C_6 or C_8 m= C_2, C_4 or C_8

Scheme 3. The 2-step polycondensation protocol used for the synthesis of various aliphatic-aliphatic polyesters via enzymatic catalysis in bulk (n>p).

The number average molecular weight (M_n) (Figure 2) and the weight average molecular weight (M_w) (see Figure S2 in ESI) of the polymers were determined via GPC analysis. The M_n trend reported in Figure 2 agreed well with the ^{1}H-NMR spectroscopy monomer conversion analysis reported in Figure 1. The methyl and ethyl diesters lead to the synthesis of polymers having higher molecular weight relative to the butyl diesters. This effect of the size of the alkyl group of the diester (-methyl, -ethyl and -butyl) becomes less marked when increasing the diester internal chain length from C_6 to C_{10}. The lower conversions and molecular masses obtained using butyl diesters is most probably due to the relative boiling points of the alcohol by-products with methanol (BP= 64.7 ºC) and ethanol (BP= 78.4 ºC) being significantly less volatile than butanol (BP= 117.7 ºC) and therefore easier to remove from the reaction system, especially when considering a reaction temperature of 85 ºC was used. The polymers synthesized using the shortest dicarboxylic esters, dimethyl succinate (DMS), diethyl succinate (DES) and dibutyl succinate (DBS) together with 1,4-butanediol (BDO) were not soluble in the GPC mobile phase (THF) and therefore were not characterised using this technique.

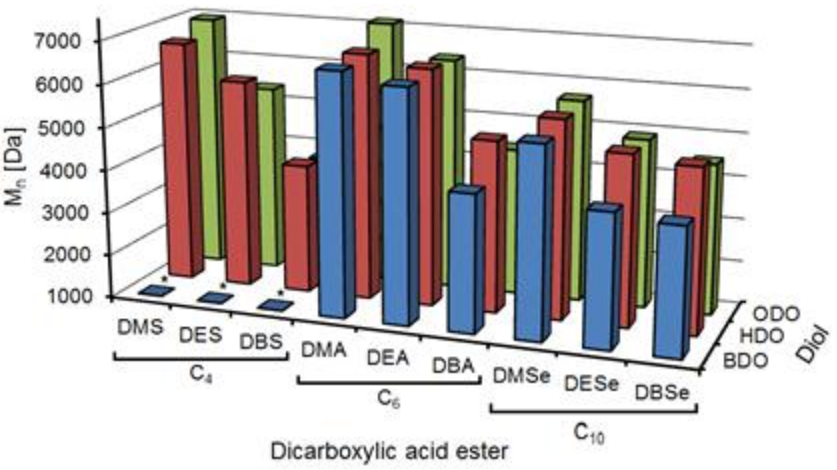


Figure 2. Number average molecular weights (M_n) calculated via GPC analysis of the crude polycondensation products using a 370-2520000 Da polystyrene calibration curve. Reactions without catalyst did not lead to detectable polyesterification products. All reactions were performed in duplicates. Statistical analysis reporting the mean value ± standard deviation for all reactions can be found in ESI, Figure S2. *=reactions non soluble in the GPC mobile phase (THF).

The generally observed trend for the synthesised polymers based on HDO and ODO having higher molecular masses than the BDO equivalents (with the exception of the adipate-based polyesters that showed similar molecular masses) reflects very well the data reported by Feder and Gross for the polycondensation of sebacic acid and various diols using N435 at 70 ºC. [15] In this previous work, a comparison between adipic and sebacic acid was made and fully agrees with our observation that the adipic acid diesters lead to higher molecular weight products than the sebacic diesters when methyl and ethyl esters were used while no significant differences were observed when butyl esters were used. It was noticed that CaLB, when operating in a solventless reaction system, led to smaller differences between the different ester chains than when using cutinases and serin-hydrolase enzymes belonging to the same family. [22] In fact, whereas CaLB leads to similar conversions with BDO, HDO and ODO, cutinases have a preferred substrate. Cutinase from *Humicola insolens* (70 °C, in bulk, diol diacid 1:1 mol/mol, 1% w/w of enzyme-to monomer, application of vacuum after 2 h) produces polyesters with higher molecular weight when ODO is used as a diol [15] while cutinase 1 from *Thermobifida cellulosilytica* (Thc_cut1) (70 °C, in bulk, diol diacid 1:1 mol/mol, 10% w w^{-1} CaLB immobilized onto methacrylic resin-to monomer, application of vacuum for 24 h) prefers BDO, leading to only short oligomers when HDO and ODO are used in combination with dimethyl adipate. [23] For Thc_cut1, it was recently reported that the structure of the diol is relevant for the chain length, but the conversion is not much affected. [17]

In order to be able to analyze the differences between all reactions, including the formation of aforementioned insoluble poly(butylene succinate), some selected reactions (reported in Table 1) were run for only 6 h at 85 ºC and atmospheric pressure in order to stop the reaction after the initial oligomerisation phase.

Table 1. Comparison of the CaLB-catalyzed polycondensation reactions after 6 h at 85 ºC and 1000 mbar and 24 h.

Diester	Diol	Time [h]	Conv. [%][#]	M_n [Da][*]	M_w [Da][*]	Đ[*]	M_0	DP_n
DES	BDO	6	89	851	1094	1.29	172.2	4.9
		24	94	ns	ns	ns		x
	HDO	6	92	2164	2398	1.26	200.2	9.8
		24	96	5853	10439	1.79		29.2
DMA	BDO	6	89	2227	2843	1.31		10.1
		24	97	6626	11549	1.71		33.1
DBA	HDO	6	83	2018	2247	1.07	228.3	9.5
		24	95	4978	8018	1.62		21.8

[#] Calculated via ^{1}H-NMR based on the conversion of the diol.

[*] Calculated via GPC using a - Da polystyrene calibration curve and toluene as internal standard.

ns= non soluble in THF

From the collected data it is possible to see that the insolubility of poly(butylene succinate) in THF, even after stirring for several days, is not due to the high molecular weight of the polymers. It is well known that the dissolution of polymers depends not only on their physical properties, but also on their polarity, molecular weight, branching, crosslinking degree and crystallinity. [24] In this particular instance, given the crystalline nature of all produced polymers it is probable that the insolubility of the succinate-based aliphatic oligoesters after 24 h reaction was indeed a result of high crystallinity rather than the oligomers as evidenced by the molecular masses data reported in Table 1 (and the DSC data reported later). The calculation of the DP of the reactions after 6 and 24 h fully confirms the trend previously reported via ^{1}H-NMR spectroscopy and GPC for all of the other reactions carried out (Table 1).

In addition to monomer conversion and molecular weight analysis, differential scanning calorimetry analysis for the determination of the polymers' melting points and thermogravimetric analysis for the determination of the mass loss were performed. Figure 3 reports the melting temperatures of all the synthesized polymers when the standard protocol (6 h at 1000 mbar, 18 h at 20 mbar, 85 ºC, 10% w w^{-1} CaLB, 24 h total reaction time) was applied. It is possible to observe a general trend where an increase in polymer melting point aligns with increasing carbon chain length of the diester, whereC_4-based polymers have lower melting points than the C_{10}-based polymers. This is due to the crystallinity of the C_4-based polymers as discussed above. A similar increase is also observed when increasing the diol carbon chain length from C_4 (BDO, blue) to C_8 (ODO, green). In this case, increasing the dicarboxylic ester length from C_4 to C_8 reduces the melting point differences among the produced polymers, analogous to number average molecular weight observations plotted in Figure 2.

Table 2. Calculated percentage of M_n reduction for any diethyl ester chain in relation to the alkyl group in relation to 1,8-octanediol.

		Diester end group		
		Me	**Et**	**Bu**
Diester carbon chain lenght	**C4**	6959 g mol^{-1}* 100%	5380 g mol^{-1}* 23%	3796g mol^{-1}* 45%
	C6	7141g mol^{-1}* 100%	6390 g mol^{-1}* 11%	4413 g mol^{-1}* 38%
	C10	5677 g mol^{-1}* 100%	4908 g mol^{-1}* 14%	4478 g mol^{-1}* 21%

* Calculated via GPC using a - Da polystyrene calibration curve and toluene as internal standard.

In Table 2 we reported the % of M_n reduction for any diethyl ester chain in relation to the alkyl group in relation to 1,8-octanediol. As we can see from the collected data, the methyl ester (Me) (taken as 100%) is always the best performing one, followed by the diethyl (Et) and the dibutyl (Bu) ones. Also to notice that the differences in the M_ns decrease for the Bu end group are lower with the increase of the carbon chain length of the diester for the considered reaction.

Further to this, the difference in polymer melting temperature between the different diol chain lengths becomes less pronounced when increasing the diester chain length (Figure 3).

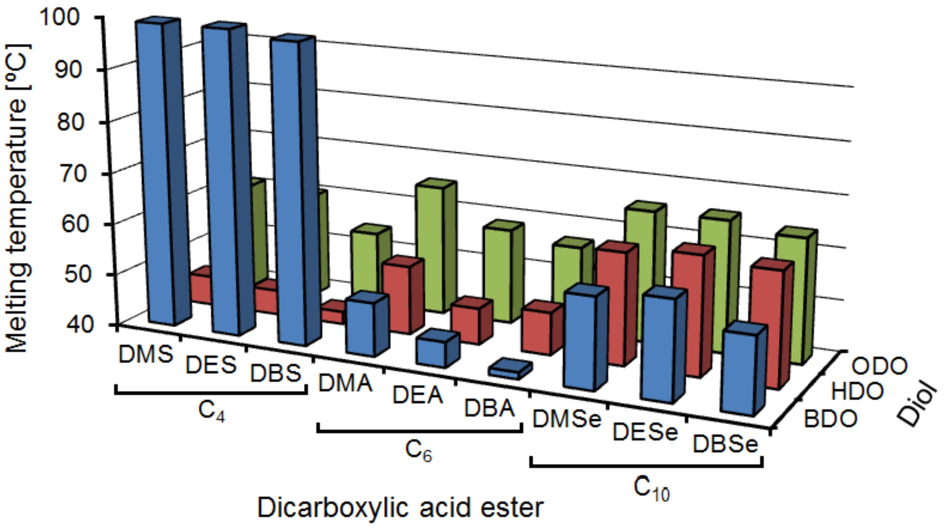


Figure 3. DSC determination of the polymer's melting points (see Table S1 for details).

The inert atmosphere (N_2) thermogravimetric profile of the polymers presented in Figure 4 is also consistent with the previously discussed molecular mass data. In fact, with the increase of the polymer's M_n and the increase of the diol's carbon chain length (see Figure 4a insert) the degradation temperature increases accordingly (Figure 4a).

A comparison of the polymerisations conducted using the same diol (1,8-octanediol, ODO, C_8) with three different diesters having methanol, ethanol or butanol alkyl groups on the monomers also show a remarkable difference in the obtained M_n (see discussion above and Figure 4b insert), with the dimethyl adipate-derived polyester proving more thermally stable than the diethyl equivalent, with this being more stable than the dibutyl. This indicates that the selection of the diester remains highly important for these transesterification reactions since the volatility of the leaving group proved to be a key point in determining the extent to the which the polyester chain grows. From the TGA profiles the temperatures at 5% and 50% mass loss (TD5 and TD50) were subsequently determined and are reported in Table S2 in ESI.

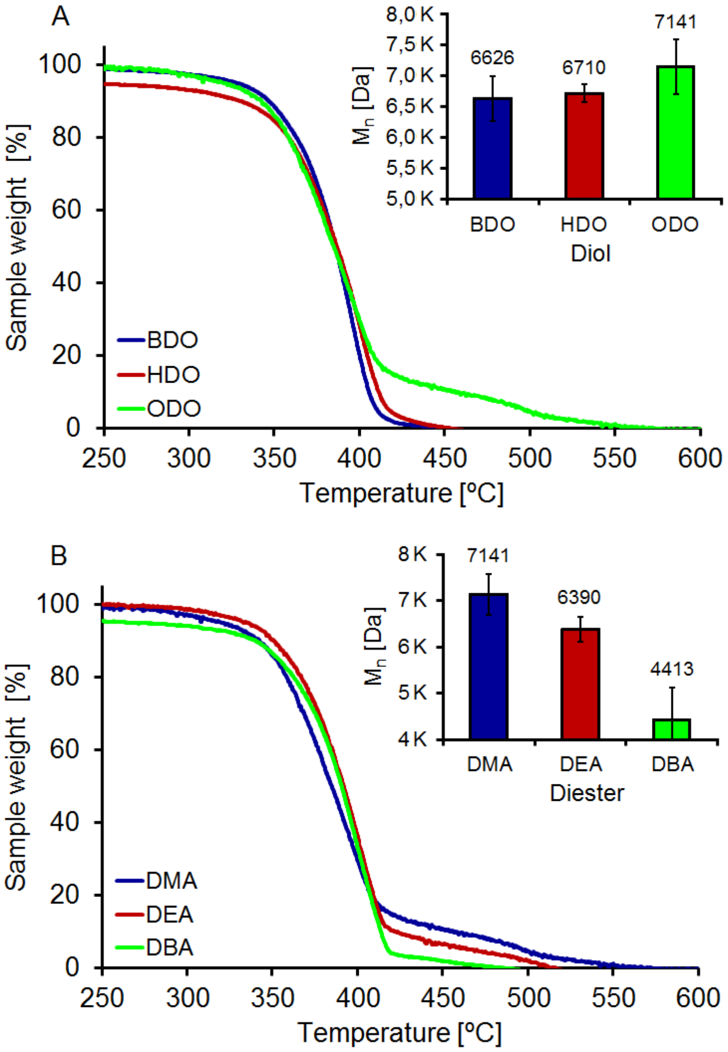


Figure 4. TGA analysis of A) polymers obtained from the polycondensation of dimethyl adipate and the three different aliphatic diols 1,4-butanediol (C4, BDO), 1,6-hexanediol (C6, HDO) and 1,8-octanediol (C8, ODO) and B) polymers obtained from the polycondensation of ODO with three different diesters dimethyl adipate (DMA), diethyl adipate (DEA) and dibutyl adipate (DBA).

Rheology was used to further characterise the polymers and identify differences in their viscosity. Figure 5 shows the viscosity change of the polymer melts based on a decrease of temperature for poly(1,6-hexylene adipate) synthesized from dimethyl adipate (blue), diethyl adipate (red) and dibutyl adipate (green). In all cases, viscosity steadily rose with decreasing temperature until the onset of freezing was reached, at which point viscosity increased rapidly. This further exemplifies how the differing molecular weights (see Fig.5 insert) lead to different viscosity profiles. The shorter chain polymer behaves remarkably different compared with the other two polymers with similar M_n values. Comparative studies of the presented reactions in organic media are needed in order to better elucidate the effect of viscosity on the reaction progression. Moreover, we noted that the viscosity of any initial mixture appeared to be below the lower detection limit (ca. <0.2 Pa.s) of the apparatus when used under the same conditions.

The produced polyesters can find applications that range from the biomedical field (as carriers in protein- and peptide-delivery systems) [25] and, if further coupled with poly(ethylene glycol), for the production of eco-friendly water-soluble polymers and coatings [26].

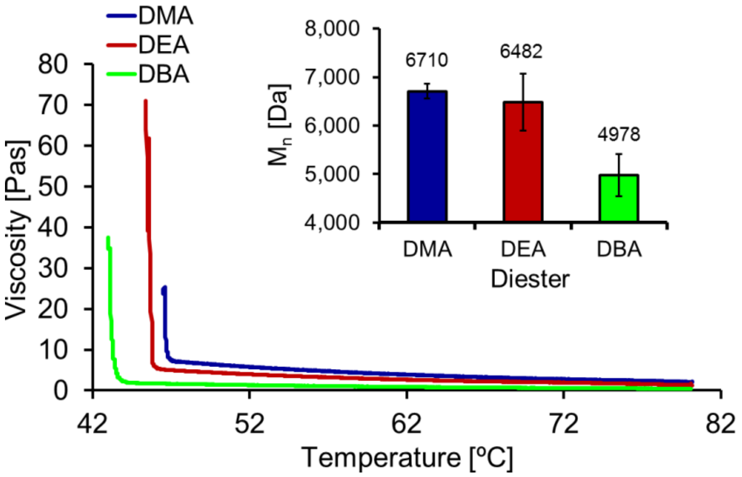


Figure 5. Change of the polymer's viscosity relative to the temperature decrease over time for different poly(hexamethylene adipate) polyesters whose chain length alters due to the chose dialkyl adipate monomer (dimethyl adipate – DMA, diethyl adipate – DEA and dibutyl adipate – DBA).

Conclusions

From the collected data we can conclude that CaLB is an effective catalyst for the synthesis of a range of aliphatic polyesters using a solventless reaction system. Polyesters based on diesters with an internal carbon chain length between 4 (succinate) and 10 (sebacate) and diols with a carbon chain length between 4 (1,4-butanediol) and 8 (1,8-octanediol) were successfully synthesized via transesterification. A strong effect of the selected alkyl group of the diester (dimethyl, diethyl and dibutyl) was observed for all polyesters. Lower molecular weights and monomer conversions were obtained using dibutyl esters since it proved more difficult to remove the butanol by-product during the reaction due to its higher boiling point (relative to methanol and ethanol). DSC, TGA and rheology measurements confirm the trend showing a large difference in degradation temperatures and viscosity of the melts due to the polymers' molecular weight. This work has sets the basis for efficient biocatalyzed syntheses of aliphatic polyesters having M_n of ~6 and M_w of ~13 KDa and with monomers conversions >94%. This also represents the first systematic study of different dialkyl aliphatic diesters with various diols under identical reaction conditions.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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Notes and references

Electronic Supplementary Information (ESI) available: Details of: ^{1}H-NMR spectroscopy conversions (Fig. S1), M_n (Fig.S3) and M_w (Fig. S2 and S4) GPC data, DSC-determined polymer's melting temperatures (Table S1) and TD5 and 50 (Table S2) are reported in ESI. All data gathered via funding from grants EP/L017393/1 and BB/N023595/1 is contained within the manuscript or the ESI.

1. S. Kobayashi, H. Uyama and S. Kimura, Enzymatic Polymerization, Chem. Rev. 101 (2001) 3793-3818.

2. S. Kobayashi, New developments of polysaccharide synthesis via enzymatic polymerization, Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 83 (2007) 215-247.

3. D. Huber, A. Pellis, A. Daxbacher, G. S. Nyanhongo and G. M Guebitz, Polymerization of Various Lignins via Immobilized Myceliophthora thermophila Laccase (MtL), Polymers 8 (2016) 280.

4. A. Pellis, E. Herrero Acero, V. Ferrario, D. Ribitsch, G. M. Guebitz and L. Gardossi., The Closure of the Cycle: Enzymatic Synthesis and Functionalization of Bio-Based Polyesters, Trends Biotechnol. 34 (2016) 316-328.

5. A. Pellis, E. Herrero Acero, L. Gardossi, V. Ferrario and G. M. Guebitz, Renewable building blocks for sustainable polyesters: new biotechnological routes for greener plastics, Polym. Int. 65 (2016) 861-871.

6. S.S. Ribeiro, C. Raminelli and A. L. M. Porto, Enzymatic resolution by CALB of organofluorine compounds under conventional condition and microwave irradiation. J. Fluor. Chem.154 (2013) 53-59.

7. O. Kirk, F. Björkling, S. E. Godtfredsen and T. O. Larsen. Fatty Acid Specificity in Lipase-Catalyzed Synthesis of Glucoside Esters, Biocatalysis 6 (1992) 127-134.

8. S. Strompen, M. Weiß, H. Gröger, L. Hilterhaus and A. Liese, Development of a Continuously Operating Process for the Enantioselective Synthesis of a β-Amino Acid Ester via a Solvent-Free Chemoenzymatic Reaction Sequence, Adv. Synth. Catal. 355 (2013) 2391-2399.

9. A. Pellis, G. M. Guebitz and T. J. Farmer, On the Effect of Microwave Energy on Lipase-Catalyzed Polycondensation Reactions, Molecules 21 (2016) 1245.

10. A. Pellis, L. Corici, L. Sinigoi, N. D'Amelio, D. Fattor, V. Ferrario, C. Ebert and L. Gardossi, Towards feasible and scalable solvent-free enzymatic polycondensations: integrating robust biocatalysts with thin film reactions, Green Chem. 17 (2015) 1756-1766.

11. H. Uyama, M. Kuwabara, T. Tsujimoto and S. Kobayashi, Enzymatic synthesis and curing of biodegradable epoxide-containing polyesters from renewable resources, Biomacromolecules 4 (2003) 211-215.

12. Y. Jiang, A. J. J. Woortman, G. O. R. Alberda van Ekenstein and K. Loos, A biocatalytic approach towards sustainable furanic–aliphatic polyesters. Polym. Chem. 6 (2015) 5198-5211.

13. Y. Jiang, A. J. J. Woortman, G. O. R. Alberda van Ekenstein, D. M. Petrović and K. Loos, Enzymatic Synthesis of Biobased Polyesters Using 2,5-Bis(hydroxymethyl)furan as the Building Block. Biomacromolecules 15 (2014) 2482-2493.

14. Y. Jiang, D. Maniar, A. J. J. Woortman and K. Loos, Enzymatic synthesis of 2,5-furandicarboxylic acid-based semi-aromatic polyamides: enzymatic polymerization kinetics, effect of diamine chain length and thermal properties. RSC Adv. 6 (2016) 67941-67953.

15. D. Feder and R. A. Gross, Exploring Chain Length Selectivity in HIC-Catalyzed Polycondensation Reactions. Biomacromolecules 11 (2010) 690-697.

16. E. Stavila, R. Z. Arsyi, D. M. Petrovic and K. Loos, Fusarium solani pisi cutinase-catalyzed synthesis of polyamides. Eur. Polym. J. 49 (2013) 834-842.

17. A. Pellis, V. Ferrario, M. Cespugli, L. Corici, A. Guarneri, B. Zartl, E. Herrero Acero, C. Ebert, G. M Guebitz and L. Gardossi, Fully renewable polyesters via polycondensation catalyzed by Thermobifida cellulosilytica cutinase 1: an integrated approach, Green Chem. 19 (2017) 490-502.

18. M. Hunsen, A. Abul, W. Xie and R. A, Gross, Humicola insolens Cutinase-Catalyzed Lactone Ring-Opening Polymerizations: Kinetic and Mechanistic Studies, Biomacromolecules 9 (2008) 518-522.

19. A. Pellis, M. Vastano, F. Quartinello, E. Herrero Acero and G. M. Guebitz, His-Tag Immobilization of Cutinase 1 From *Thermobifida cellulosilytica* for Solvent-Free Synthesis of Polyesters, Biotechnol. J. 12 (2017) 1700322

20. T. J. Farmer and M. Mascal, Platform Molecules. in Introduction to Chemicals from Biomass (eds. J. H. Clark and F. Deswarte) 89-155 (John Wiley & Sons, Ltd) 2015.

21. M. Vastano, A. Pellis, B. Immirzi, G. Dal Poggetto, M. Malinconico, G. Sannia, G. M. Guebitz and C. Pezzella, Enzymatic production of clickable and PEGylated recombinant polyhydroxyalkanoates, Green Chem. 19 (2017) 5494-5504.

22. V. Ferrario, A. Pellis, M. Cespugli, G. M. Guebitz and L. Gardossi, Nature Inspired Solutions for Polymers: Will Cutinase Enzymes Make Polyesters and Polyamides Greener?, Catalysts 6 (2016) 205.

23. A. Pellis, V. Ferrario, B. Zartl, M. Brandauer, C. Gamerith, E. Herrero Acero, C. Ebert, L. Gardossi and G. M. Guebitz, Enlarging the tools for efficient enzymatic polycondensation: structural and catalytic features of cutinase 1 from Thermobifida cellulosilytica. Catal. Sci. Technol. 6 (2016) 3430-3442.

24. C. S. Brazel and S. L. Rosen, Fundamental Principles of Polymeric Materials, 3rd Edition, Wiley, 2012.

25. S. Zhou, X. Deng, X. Li, W. Jia and L. Liu, Synthesis and Characterization of Biodegradable Low Molecular Weight Aliphatic Polyesters and Their Use in Protein-Delivery Systems, J. App. Polym. Sci. 91 (2004) 1848-1856.

26. Y. Ikada and H. Tsuji, Biodegradable polyesters for medical and ecological applications, Macromol. Rapid Commun. 21 (2000) 117-132.