Conference paper

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Lipase-catalyzed synthesis of oligoesters of 2,5-furandicarboxylic acid with aliphatic diols

Abstract: 2,5-Furandicarboxylic acid is a platform chemical for the production of biobased polymers and materials. This study reports the synthesis of furan oligoesters *via* polytransesterification of dimethyl furan-2,5-dicarboxylate and linear α, ω-aliphatic diols with chain length ranging from C2 to C12, using immobilized lipase B from *Candida antarctica* (Novozym 435) in dry organic solvents. Dimethyl furan-2,5-dicarboxylic acid (A) and 1,4-butanediol (B) were used as model substrates under different conditions producing a mixture of cyclic (CEOs) and linear (LEOs) ester oligomers up to decamers and dodecamers, respectively, with high yield. The size of the oligomers and distribution of the products is controlled by the initial concentration of substrates and temperature. While the shortest CEOs are the main cyclic compounds at 20 mM, the longest CEOs are formed at 175 mM. The chain length of the aliphatic diol co-monomers strongly influences the yield and the type of oligoesters formed. High substrate conversion of 90–95 % was obtained for C4–C12 diols, while in the case of ethylene glycol and 1,3-propanediol the conversion was moderate (i.e., 75 %). The product of the reaction between dimethyl furan-2,5-dicarboxylate and ethylene glycol (C2) and 1,3-propanediol (C3), respectively, consisted only of linear oligoesters. Longer oligoesters were obtained for alkyl chains higher than C4. The chain length and the abundance of oligoesters increases in the order: C2<C12<C10<C3<C8<C4 <C6. No substrate or product inhibition was observed in the production of furan-based oligoesters. The present biobased oligoesters are obtained via a green process and have potential application as macromonomers.

Keywords: bioplastics; enzymatic polymerization; 2,5-furandicarboxylic acid; lipase; oligoesters; POC-2014.

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Introduction

Biopolymers are extensively studied in order to diminish the petroleum dependence for plastics production [1]. In that way, biobased polyesters obtained from renewable resources are considered the most promising polymers for the future. Intensive research is in progress for the development of polyesters derived from biobased monomers, such as 1,3-propanediol, glycerol, 1,4-butanediol, succinic acid [2], citric acid [3] or 1,4:3,6-dianhydrohexitols [4]. The majority of monomers obtained from renewable resources are aliphatic molecules that

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can be used for the synthesis of a wide range of biopolymers with similar properties to common petroplastics [5]. Less options are available for biobased alternatives to aromatic polymers [6, 7]. Furan derived building-blocks that can be obtained from plant-based sugars have acquired a significant prominence in the last years, although their availability is currently limited [8–10].

In particular, 2,5-furandicarboxylic acid (FDCA) has been extensively studied as a biobased alternative to phenyl-based polymers like polyethylene terephthalate (PET) or polybutylene terephthalate (PBT). First reports on the synthesis of polyesters derived from FDCA with ethylene glycol, and tri-, tetra-, penta- and hexamethylene diols date from the early second half of the last century [8–10] but FDCA polymers did not find industrial application. Only in the past years has the interest on the development of FDCA-based polyesters been revived. There are several studies reporting the synthesis of FDCA-based polyesters. For example, Gandini et al. [8] obtained PEF by polytransesterification of 2,5-dihydroxyethyl furan-dicarboxylate catalyzed by Sb_3O_4 at 220 °C. Jiang et al. [11] catalyzed the reaction between diols and FDCA with tetrabutyl titanate at 70 PA and 235–245 °C. Ma et al. investigated the synthesis of poly [(ethylene 2,5-furandicarboxylate)-co-(butylene 2,5-furandicarboxylate)] using tetrabutyl titanate under vacuum at 200 °C in excess of ethylene glycol and 1,4-butylene glycol [12] and investigated more specifically the polymerization of poly(butylene 2,5-furandicarboxylate) and its crystalline properties. Recently it has been shown that polyethylene furanoate (PEF) has very good mechanical and thermal properties and potential applications for fibers, biobased bottles for water and packaging materials, among others [13].

The production of furan-based polymers is based on metal catalysis at high temperatures, but considering that at temperatures above 200 °C FDCA can undergo decarboxylation or side reactions leading to the formation of discolored products [14], it is necessary to extend the research for alternative catalysts for replacement of the rather toxic Sb_3O_4 and tetrabutyl titanate and furthermore perform the reactions at lower temperatures.

Indeed, an alternative to chemical catalysis is enzymatic catalysis [15]. Enzymes have been used successfully for the synthesis of many polyesters [16–18]. They offer various advantages such as lower energy requirement, enhanced selectivity and higher quality of the final product. In particular, the immobilized lipase B from *Candida antarctica* adsorbed to acrylic macroporous resin (commercially available as Novozym 435) has been used widely for polymer synthesis by ring-opening polymerization, polycondensation [16, 19] and recently the synthesis of cyclic and linear oligoesters derived from furan monomers has been reported [20].

In this work we studied the synthesis of polyesters of FDCA with aliphatic diols of different chain lengths by enzymatic catalysis. Furan based oligoesters were obtained in transesterification reactions catalyzed by Novozym 435 (N435) in organic solvent under different conditions (i.e., temperature and substrate concentration) between dimethyl furan-2,5-dicarboxylic acid (2,5-FDCA-diMe) and 1,4-butanediol (1,4-BDO). The effect of the chain length of diols (from C2 to C12) on the poly-transesterification reactions and on the product formation was also investigated.

Materials and methods

Materials

Solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Novozym 435 (N435) was a kind gift of Novozymes (The Netherlands). All solvents were purchased from Merck (Darmstadt, Germany) and equilibrated in 4 Å molecular sieves (Sigma-Aldrich, St. Luis, USA) for at least 24 h before use. *Trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-propenyl-diene] malononitrile (DCTB, >99 %) and potassium trifluoroacetate (>99 %) were obtained from Sigma-Aldrich. Dimethyl furan-2,5-dicarboxylate (2,5-FDCA-diMe, >99 %) was kindly synthesized and donated by the group of Dr. Daan van Es (Wageningen UR Food & Biobased Research, Wageningen, The Netherlands). Ethylene glycol (C2, 99.8 %), 1,3-propanediol (C3, 99.6 %), 1,4-butanediol (C4, 1,4-BDO, >99 %), 1,6-hexanediol (C6, 99 %) and 1,10-decanediol (C10, 98 %) were from Sigma-Aldrich, 1,8-octanediol (C8, >98 %) was from Merck, and 1,12-dodecanediol (C12, 99 %) was from Acros, and were used without further purification. All other chemicals (>99 %) were purchased from Merck.

Determination of the esterification activity of lipase in organic media

The esterification activity was determined using *n*-butyric acid and *n*-butanol as substrates in a toluene:*tert*-butanol solution (70:30 % wt.) at 40 °C, based on the method reported by Kiran et al. [21]. The consumption of the acid and the formation of the ester were followed by gas chromatography (GC). A Focus GC was equipped with a Restek (RXI®-5ms) capillary column (30 m × 0.25 μm i.d.) and a flame ionization detector. The carrier gas was helium at 1.5 mL min^{-1}. The oven was initially set at 50 °C and then temperature was increased to 300 °C at a rate of 20 °C min^{-1}. One unit (U) of esterase activity was the amount of enzyme capable to catalyze the formation of 1 mmol of butyl-butyrate per hour. The concentration was determined using hexadecane as internal standard.

General procedure for the enzymatic oligomerization of furan and alkyl derivatives

Routinely the reaction was carried out as follows: 2,5-FDCA-diMe, (46 mg, 0.25 mmol) and 1,4-BDO (22.5 mg, 0.25 mmol) were dissolved in toluene:*tert*-butanol mixture (5 mL, 70:30 % wt.) in 10 mL glass test tubes to obtain a final concentration of 50 mM for each substrate. The mixture was incubated at 40 °C, and the reaction was started by the addition of N435 (4 U or 2 mg). The reaction mixture was incubated for 24 h at 40 °C with continuous mixing. The reaction was stopped by separating the enzyme by filtration and the reaction medium was collected and stored at 4 °C until further analysis.

Analytical methods

HPLC

The concentration of furan derivatives was determined by HPLC, on a Waters 2690 HPLC instrument and using an ODS-2 Inertsil (250 × 3 mm i.d., Varian Inc.) column at 50 °C. Sample injection volumes of 10 μL were used. Elution of compounds was done using a gradient composed by acetonitrile (phase A) and water (phase B) as mobile phase. Both phases were supplemented with phosphoric acid (0.01 % v/v, >99 %). Gradient elution started at 35:65 (v/v) A/B and ended at 100:0 (v/v) A/B at 0.8 mL min^{-1} flow rate. FDCA was detected at 280 nm.

UPLC-MS

Samples were analyzed on a Thermo Accela UPLC system (San Jose, CA, USA) equipped with pump, autosampler and PDA detector using a Waters Acquity UPLC BEH shield RP18 column (2.1 × 150 mm, 1.7 mm particle size) with a Waters Acquity UPLC shield RP18 Vanguard pre-column (2.1 × 5 mm, 1.7 mm particle size; Waters, Milford, MA, USA). Conditions were: sample volume 3 μL; eluents: water acidified with 0.1 % (v/v) acetic acid (eluent A), and acetonitrile acidified with 0.1 % (v/v) acetic acid (eluent B); temperature: 30 °C; elution gradient was applied at 300 μL/min, and was similar with that applied for the HPLC; detection at 200–400 nm. Mass spectrometric data were obtained by analyzing samples on a Thermo Scientific LTQ-XL (San Jose, CA, USA) equipped with an ESI probe coupled to the RP-UHPLC system. The capillary temperature was set at 250 °C and the spray voltage at 4.7 kV. Settings of the MS were tuned using 2,5-dimethyl furan dicarboxylate. Data were processed using XCalibur software (Thermo Scientific).

Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis

Samples (10–20 μL) were mixed with a solution of matrix (DCTB, 10 μL, 160 mM) and trifluoroacetate (2.5 μL, 33 mM) as dopant, both dissolved in tetrahydrofuran. MALDI-TOF MS analysis was performed with Ultraflex

mass spectrometer workstation (Bruker Daltonics, Bremen, Germany), as described in [20, 22]. Polyethylene glycol standards (600, 1000, and 2000 Da) were used for calibration. Within each MALDI-TOF MS spectrum, the intensities obtained by the potassium and sodium-adducts per compound were summed up and the relative contribution of each one was calculated as the percentage of the sum of the intensities of all compounds identified in the spectrum.

Results and discussion

In this study the reaction between aliphatic α,ω-diols and 2,5-FDCA-diMe was carried out using immobilized *C. antarctica* lipase B as catalyst in an anhydrous reaction medium composed by 70 % (wt.) toluene and 30 % (wt.) *tert*-butanol. This mixture of solvents combines the stabilizing effect of toluene, a hydrophobic solvent (log P=2.5) which promotes polycondensation reactions catalyzed by lipase [23], with the increased solubility of the monomers used in the hydrophilic *tert*-butanol (log P=0.35) [24]. The synthesis of polyesters derived from 1,4-BDO and 2,5-FDCA-diMe was used as model reaction to investigate the optimum reaction conditions (i.e., temperature, substrate concentration, reaction time) and their effect on the products formed.

Effect of temperature

Transesterification reactions catalyzed by N435 were carried out at temperatures ranging from 40 to 80 °C. The time-course of 2,5-FDCA-diMe consumption is shown in Fig. 1. The reaction proceeds with high rate at all temperatures, and about 60 % conversion is attained in the first two hours. After 24 h of reaction high substrate conversion was obtained at all temperatures used, ranging from 82.5 % at 40 °C to 92.5 % at 80 °C. This result indicates the high transesterification activity and excellent thermostability of N435 in the dry organic solvent system used in these experiments.

UPLC-MS analysis of the reaction mixtures identified both cyclic (CEOs) and linear (LEOs) ester oligomers as reaction products together with the unreacted substrate, which eluted at 3.4 min (Fig. 2).

The main cyclic oligoester was the tetramer CEO_2, and various linear ester oligomers ranging from dimers to octamers were identified. The linear ester oligomers synthesized from the substrates 2,5-FDCA-diMe (A) and diols (B) showed different end-functional groups: (i) methylester and hydroxyl terminal groups (AB_n), labeled AB_1, AB_2, AB_3 and AB_4 in Fig. 2), (ii) di-methylester terminal (AA_n, labeled AA_1, AA_2, AA_3 in Fig. 2) and (iii) di-hydroxyl terminal (BB_n). The structure of the main products obtained by the enzymatic transesterification of 2,5-dimethylfuroate and 1,4-butanediol are illustrated in Scheme 1. Small amounts of oligoesters with one terminal free carboxyl group of the type AB_n (see peaks labeled AB_2-COOH, AB_3-COOH and AB_4-COOH in Fig. 2) and AA_n (i.e., AA_1-COOH, Me, AA_2-COOH, Me and AA_3-COOH, Me in Fig. 2) were found. Such

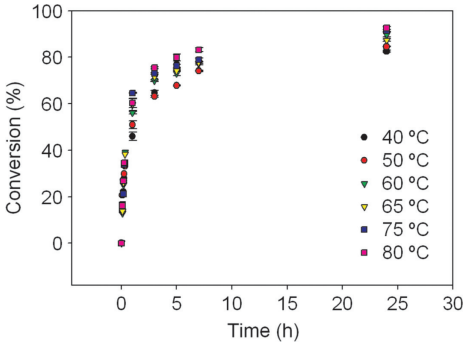


Fig. 1 Time-course of 2,5-FDCA-diMe consumption catalyzed by N435 at different temperatures. The transesterification reaction was performed using 50 mM of 2,5-FDCA-diMe and 1,4-BDO.

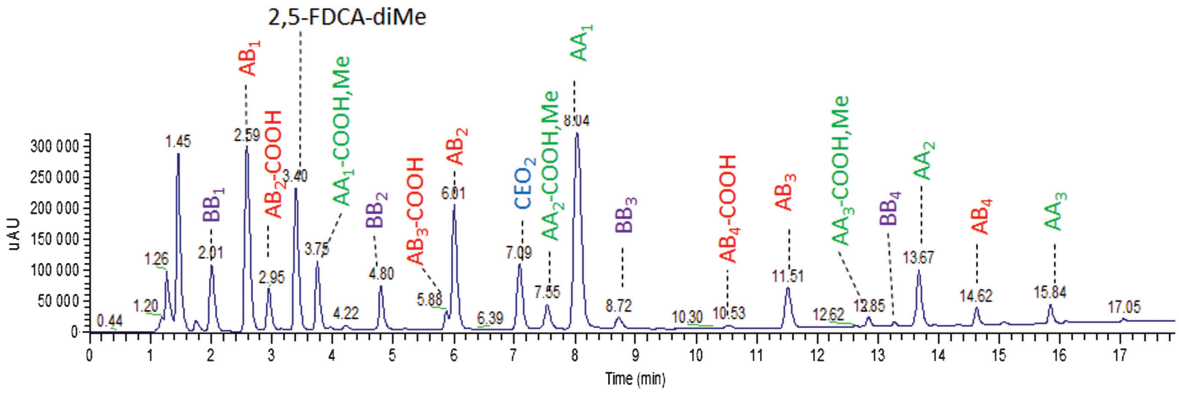
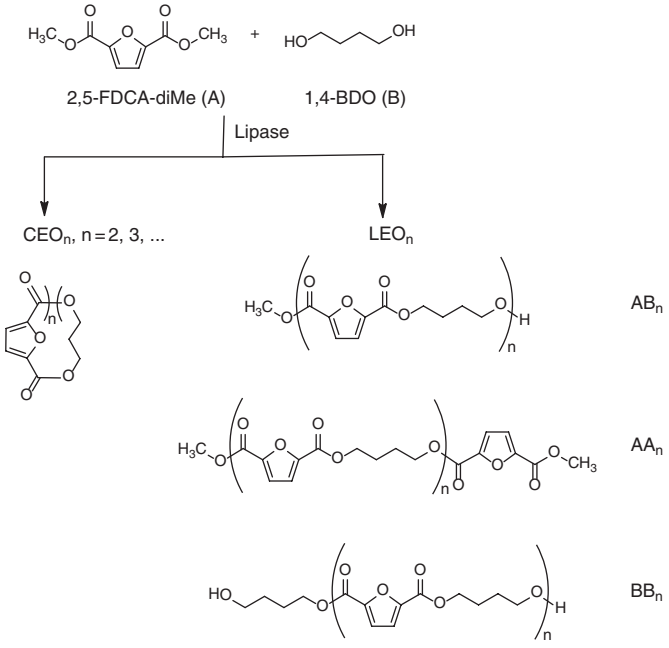


Fig. 2 UPLC-MS analysis of the products of the reaction between 1,4-BDO and 2,5-FDCA-diMe catalyzed by N435 after 24 h at 75 °C. The retention time of 2,5-FDCA-diMe was 3.40 min.

compounds are produced by the lipase-catalyzed hydrolysis of a terminal methylester group of AB_n and AA_n oligomers in the presence of traces of water. Karl-Fischer analysis of the solvent, reaction medium and reagents showed that the small amount of water that was responsible for the hydrolysis was originating from the immobilized enzyme catalyst. Maldi-TOF-MS analysis confirmed the products identified by UPLC-MS and allowed the detection of additional products, and in particular higher cyclic oligomers (Fig. S1 in the supplementary data file).

The profile of products formed at the temperatures tested (Fig. 3) is rather similar, but increasing the temperature resulted in longer oligoesters. If the cyclic tetramer CEO_2 is produced in comparable amounts at all temperatures tested, the longer cyclic esters CEO_3 and CEO_4 occur only at temperatures above 60 °C. The cyclic oligoesters CEO_2, CEO_3 and CEO_4 (i.e., tetramers, hexamers and octamers) are the minor compounds, and represent between 5 % and 15 % of the total products. No cyclic dimer (CEO_1) was detected suggesting that $[AB]_1$ is too short and/or the strain of the molecular cycle is too high and therefore the cyclic dimer is thermodynamically instable. The linear oligoesters are the major products. The linear dimer AB_1 is the major product in the first minutes of the reaction and is the principal substrate for chain elongation, so at longer



Scheme 1 Cyclic and linear ester oligomers produced by polymerization of 2,5-FDCA-diMe and 1,4-BDO catalyzed by N435.

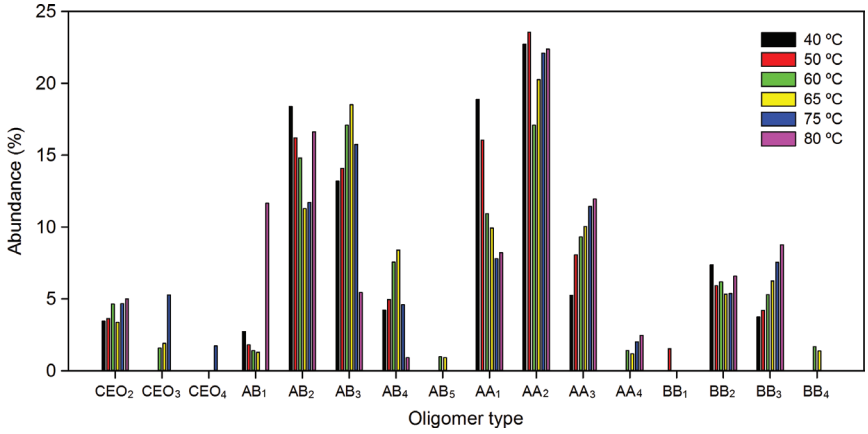


Fig. 3 Relative abundance of the produced oligomers catalyzed by N435 after 24 h using 50 mM 2,5-FDCA-diMe and 1,4-BDO at different temperatures from 40 to 80 °C as analyzed by Maldi-TOF MS.

incubation times it is only present at low concentrations or completely consumed. The main linear oligomers are AB_n and AA_n types, specifically, AB_2, AB_3, AA_2 and AA_3 oligomers represent 60 % of the total products. The dihydroxyterminal oligoesters BB_n represent between 10 % and 15 % of the total products. Not only the chain length but also the abundance of longer oligoesters increases with temperature increase. Taking into account the substrate conversion, the oligomers length and the enzyme stability, the temperature of 75 °C has been selected for further experiments.

Elongation mechanism

The time course of the lipase-catalyzed transesterification reaction between 50 mM 2,5-FDCA-diMe and 1,4-BDO at 75 °C allowed getting insight into the elongation mechanism of this reaction. The products of the short and long time incubations are shown in Fig. 4 for selected oligomers, and Table 1 shows the different formation possibilities for these oligomers.

According to the catalytic mechanism of lipase [22, 25] for the formation of the ester bond, firstly the acid constituent of substrate A binds to the serine residue of the active centre, and then the alcohol of the component B must interact with the formed tetrahedral intermediate. The requirement for an ordered and sequential mechanism for the entry of substrates in the enzyme active site and the requirements for the different oligomers production may explain in part the obtained relative abundance of the different oligoesters identified in the product mixture. In summary: $AA_n \approx AB_n$ (35–50 %) > BB_n (10–20 %) > CEO_n (5–15 %). However, these

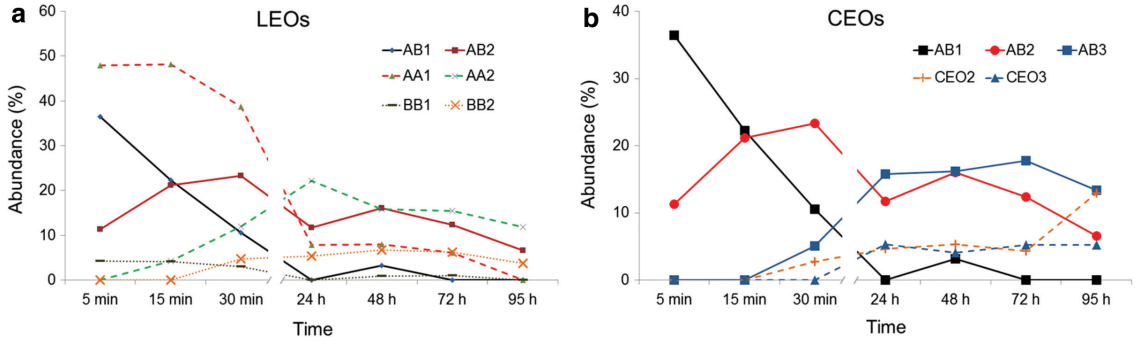


Fig. 4 Time-course of the formation of oligoesters catalyzed by N435 at 75 °C using 50 mM 2,5-FDCA-diMe and 1,4-BDO: (a) AA_n and BB_n oligomers and precursors; (b) CEO_n and precursors.

Table 1 Formation possibilities of the main oligoesters obtained during incubation of 2,5-FDCA-diMe and 1,4-BDO using N435.

Linear oligoester		Formation possibility[a]				
AB_1	AB	A+B				
AB_2	ABAB	AB_1+AB_1	AA_1+B	$A+BB_1$		
AB_3	ABABAB	AB_2+AB_1	AA_1+BB_1	AA_2+B	$A+BB_2$	
AB_4	ABABABAB	AB_2+AB_2	AA_2+BB_1	AA_3+B	$A+BB_3$	AA_1+BB_2
AA_1	ABA	$A+AB_1$				
AA_2	ABABA	AA_1+AB_1	$A+AB_2$			
AA_3	ABABABA	AA_1+AB_2	$A+AB_3$			
AA_4	ABABABABA	AA_2+AB_2	$A+AB_4$	AA_3+AB	AA_1+AB_3	
BB_1	BAB	AB_1+B				
BB_2	BABAB	AB_1+BB_1	AB_2+B			
BB_3	BABABAB	AB_2+BB_1	AB_1+BB_2			

[a]Different types of linear products may be formed but, according to the results obtained, some oligoesters are more probable than others. The probability of formation of each product is showed by different underlining styles: Most probable (-); Probably exist (-); Could exist (-); Does not exist ().

results are not explained completely with the formation probabilities summarized in Table 1. For example, in the first 5 min, when 27 % of 2,5-FDCA-diMe was consumed, the main product was AA_1 (Fig. 4a) and <5 % of BB was detected (Fig. 4a) indicating that the enzyme shows more affinity for employing the monomer AB_n as nucleophile than for B. The first cyclic oligomers appeared after 30 min of reaction, and their level remains constant at long reaction times (Fig. 4b). According to the results, N435 affinity decreases in the order: A ≈ AB > AB_n > AA_n > BB_n > CEO_n, showing preference for the shortest AB_n and AA_n products for elongating the chain. These differences in affinity could explain the relative abundances of the products obtained.

This behavior is comparable as described by Habeych et al. [22] for succinic acid and 1,4-BDO, where the enzyme affinity is higher for the α-hydroxy-ω-carboxyl oligomers (AB_n). However, in this study the affinity for α, ω-dicarboxyl oligomers (AA_n) is higher than for the α, ω-dihydroxyl oligomers (BB_n) and cyclic oligomers (CEO_n). As soon as the first reaction product is formed (AB_1), it becomes a new substrate that could follow alternative reaction pathways which include the addition to an acid (A), an alcohol (B), another AB_1 monomer and/or becomes incorporated in a cyclic product (CEO_n), as shown in Table 1. The combination orders are different, but finites and with different probabilities. The elongation of the oligomer is mainly determined by two variables: the concentrations of each intermediate at time "t" and the enzyme affinities for it [22, 26].

CEOs were not detected as the main products every time, but their abundance can shift by changing some reaction conditions, such as decreasing substrates concentration [27] or increasing the carbon chain length of diols, as it will be discussed further.

Effect of substrate concentration

In order to understand the formation of furan derived oligoesters, the reaction was assessed using different initial substrate concentrations in 1:1 molar ratio (20, 50, 100 and 175 mM). Kinetic parameters were calculated from initial rate measurements and the values of 118 mM and 5.3 mmol min^{-1} 2,5-FDCA-diMe were obtained for K_m and V_{max}, respectively. The results indicated that no substrate nor product inhibition occurs.

Preparative reactions were carried out till 72 h and different oligoesters were obtained (Fig. 5). CEOs distribution is affected by the initial concentration of substrates. While the shortest CEOs (i.e., CEO_2) are the main cyclic compounds at 20 mM, the longest CEOs are formed at 175 mM. In general, the formation of CEOs and LEOs is strongly influenced by the initial amount of 1,4-BDO and 2,5-FDCA-diMe. The formation of cyclic products is favored at concentrations below 50 mM, otherwise, the chain length of increases at higher substrate concentrations. The obtained results are in agreement with the theory described in the literature, in which the key to high cycle yields is to shift the ring-chain equilibrium at high dilution towards the

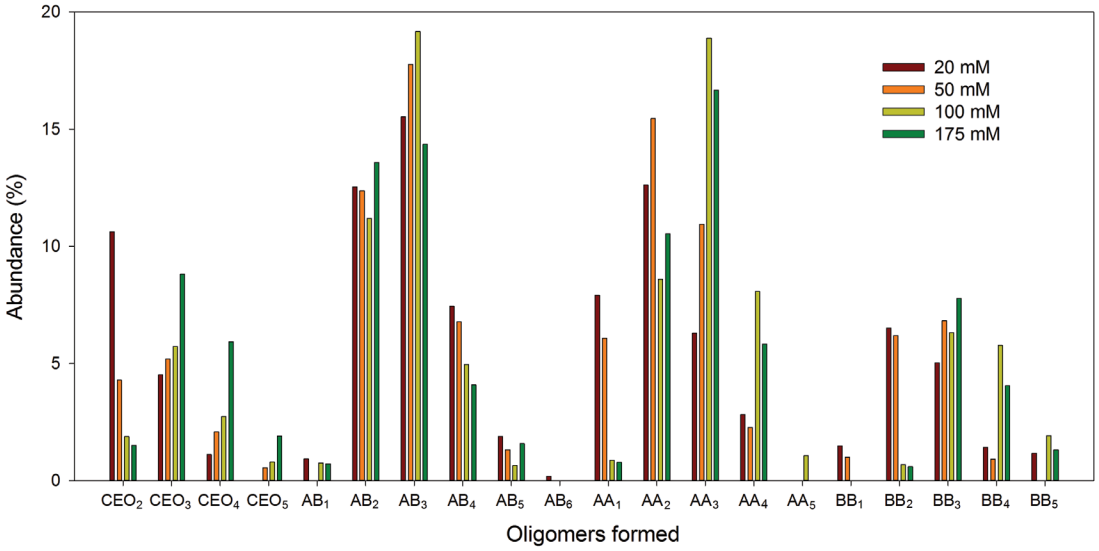


Fig. 5 Oligoesters formed after 72 h reaction between 2,5-FDCA-diMe and 1,4-BDO catalyzed by N435 at 75 °C. Different substrate concentrations were used in the reaction in ratio 1:1 (mol/mol): 20, 50, 100 and 175 mM.

formation of ring structures [28]. Hilker et al. [27] concluded from studying the synthesis of cyclic oligoesters derived from dimethyl terephthalate and diethylene glycol, that at higher concentrations than 100 mM the reaction was limited by enzyme inhibition. In our work no enzyme inhibition by the substrate 2,5-FDCA-diMe was observed. Hilker et al. [27] also described that, for lipase the cyclization of aromatic compounds with *para*-substitution is more beneficial. The 2,5-FDCA-diMe has the ester groups in *meta*-position and that may explain the small relative abundances in CEOs.

The chain length of LEOs increased at higher substrate concentrations. Two profiles in the α, ω-dicarboxy and α, ω-dihydroxy oligomers (AA_n and BB_n) were observed (Fig. 5): one for the oligoesters formed between 20 and 50 mM and another one between 100 and 175 mM. The lengths of the formed intermediates within each interval were not significantly changed; the difference appeared over 50 mM of initial substrates concentration, when longer oligomers were formed. This trend is clearly observed for the oligoesters AA_1, AA_4, BB_1, BB_2 and BB_4.

Effect of carbon chain length

Properties of polyesters depend on the type of alcohol and carbon chain length being the main factor that influences the characteristics of plastics, such as biodegradability, elasticity, melting temperature or crystallinity [7, 11, 12].

Different carbon length diols have been used for the production of phthalate-based polymers, for example: ethylene glycol is a commonly used monomer for the production of PET, and also for PEF both used for bottles. 1,3-Propanediol is a biobased monomer obtained from glycerol and glucose [28], and it is used in Sorona® [i.e., poly(trimethylene terephthalate)], commercialized by DuPont (http://www2.dupont.com/home/en-us/index.html, July 11, 2012). 1,4-BDO has been used for the production of PBT with similar properties to PET but it can be processed at lower temperatures [29, 30].

The enzymatic esterification between 2,5-FDCA-diMe and different carbon length diols from 2C to 12C was assessed with 50 mM of each substrate after 24 h at 75 °C. N435 showed 90–95 % conversion of 2,5-FDCA-diMe using diols with chain length from C4 to C12, and 75 % conversion when short-chain diols like ethylene glycol (C2) and 1,3-propane diol (C3) were used (Table 2). The results of the Maldi-TOF-MS analysis of these reactions are summarized in Fig. 6, showing large differences in the oligoester pattern as function of the chain length of the diol co-monomers.

Table 2 Effect of the diol's chain length on the conversion of 2,5-FDCA-diMe and the type of products formed during the polymerization reaction catalyzed by N435 at 75 °C after 24 h.

Entry	Diol/chain length	Conversion of 2,5-FDCA-diMe (%)	Product type/ highest DP			
			Cyclic esters CEO_n	Linear esters		
				AB_n	AA_n	BB_n
1	Ethylene glycol/C2	75.1	Not identified	3	2	2
2	1,3-propanediol/C3	75.3	Not identified	5	4	3
3	1,4-butanediol/C4	89.2	3	5	4	4
4	1,6-hexanediol/C6	91.8	3	6	5	5
5	1,8-octanediol/C8	93.1	3	5	4	5
6	1,10-decanediol/C10	91.6	2	4	3	3
7	1,12-dodecanediol/C12	92.5	3	5	4	3

The shortest diols (C2 and C3) did not produce any cyclic ester, probably because the alcohol chain is too short and rigid. The cyclic esters CEO_2 and CEO_3 were obtained for all diols with a minimum carbon chain length of 4. The amount of cyclic tetramers CEO_2 increased with the increase of the diol chain length from C4 to C12, and is approximately three-fold lower for the 1,4-butanediol than for the C6-C12 diols. A different pattern was observed for cyclic hexamers. Only C4 to C8 diols produced significant amounts of CEO_3, while the highest amount was obtained from 1,6-hexanediol. These results clearly show that both the length and the flexibility of the alcohol chain length influence the formation of cyclic esters. The ring-closure reaction occurs concurrently to the growth of linear polymer chains constituted by a flexible diol monomer and the more rigid 2,5-FDCA-diMe monomer. The oligomer chain should have some flexibility, to fold towards itself and access the acyl-serine bond through the oxoanion hole [22]. The formation of CEO_n requires intramolecular nucleophilic attack of the terminal hydroxyl group of the linear AB_n oligomer to the acyl-serine bond on the active site. Diols from C8 to C12 with the highest flexibility are expected to producing more CEOs. This is true for the shortest cyclic ester, CEO_2, but not for CEO_3, where an optimum diol chain length has been identified, as discussed above. This suggests that not only the length and flexibility of the α,ω-dihydroxyl monomer is important for cyclization, but the length and flexibility of the AB_n linear oligoester is a determining factor for ring closure.

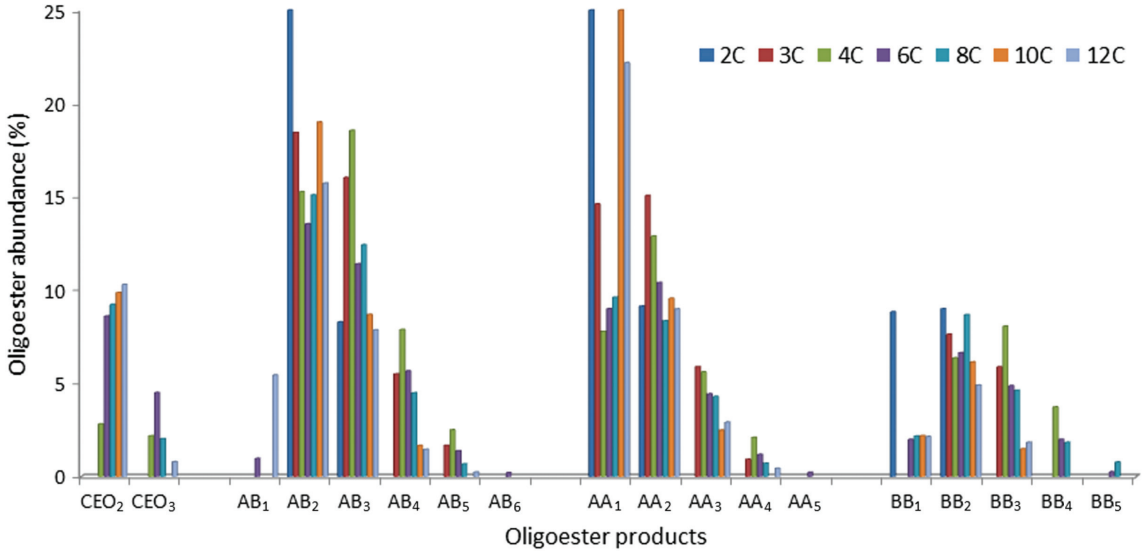


Fig. 6 Relative abundance of furan based oligoesters after 24 h of reaction at 75 °C catalyzed by N435 using 2,5-FDCA-diMe and diols of different carbon chain length ranging from C2 to C12.

Significant differences were observed in the pattern of linear oligoesters produced from 2,5-FDCA-diMe and the various aliphatic diols used. Ethylene glycol produced only short linear oligoesters like the diester-terminal and di-hydroxy-terminal pentamers of type AA_1 and BB_1 and the ester, hydroxy-terminal hexamer AB_3 (Fig. 6 and Table 2, entry 1). All other diols produced longer LEOs of all types, i.e., AA_1, BB_n and AB_n, up to undecamers and dodecamers, respectively. Small amounts of oligoesters with one free carboxyl terminal groups of the type AB_n-COOH and AA_n-COOH,Me were observed for each diol, as illustrated in Fig. S1 for the polymerization products obtained from 2,5-FDCA-diMe and 1,4-BDO (supplementary file). These minor products are not presented in Fig. 6, which describes only the major products of the reaction.

Considering the results of different products with different chain length diols, in general, the production increases in the order: C2 < C12 < C10 < C3 < C8 < C4 < C6. These results are in agreement with other reports. Thus, Mahapatro et al. [23] showed the higher reactivity for 1,8-octanediol and 1,6- hexanediol for aliphatic polyesters, and Linko et al. [31] obtained the best reaction conditions employing C6 diol and adipic acid.

Conclusions

Here we report for the first time the synthesis of furan derived oligoesters with different diols using a lipase starting from activated furan derivatives. The results are comparable with other works when phthalate-based oligoesters were used as aromatic substrate for the formation of oligomers. This work may provide the first step for the enzymatic production of novel furan-based biopolymers.

The immobilized *C. antarctica* lipase B (Novozym 435) is a suitable biocatalyst for oligomers synthesis from 2,5-FDCA-diMe and a broad range of aliphatic diols. No substrate or product inhibition was observed in the production of cyclic (CEOs) and linear (LEOs) furan-based oligoesters. The profile of formed products can be controlled by different parameters. The main variable in CEOs formation is the carbon chain length. The length and the flexibility of the linear oligomer chain is the key factor to promote ring closure and to control the size of the rings. The abundance of LEOs increased in time at high substrate concentration, especially when C4, C6 and C8 carbon length diols were used.

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