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Biocatalytic synthesis of δ-gluconolactone and ε-caprolactone copolymers*

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The biodegradability and biocompatibility properties of ε-caprolactone homopolymers place it as a valuable raw material, particularly for controlled drug delivery and tissue engineering applications. However, the usefulness of such materials is limited by their low hydrophilicity and slow biodegradation rate. In order to improve polycaprolactone properties and functionalities, copolymerization of ε-caprolactone with δ-gluconolactone was investigated. Since enzymatic reactions involving sugars are usually hindered by the low solubility of these compounds in common organic solvents, finding the best reaction medium was a major objective of this research. The optimal copolymerization conditions were set up by using different organic media (solvent and solvents mixtures), as well as solvent free systems that are able to dissolve (completely or partially) sugars, and are nontoxic for enzymes. Native and immobilized lipases by different immobilization techniques from *Candida antarctica* B and *Thermomyces lanuginosus* have been used as biocatalyst at 80°C. Although the main copolymer amount was synthesized in DMSO:*t*-BuOH (20:80) medium, the highest polymerization degrees, up to 16 for the copolymer product, were achieved in solventless conditions. The products, cyclic and linear polyesters, have been characterized by FT-IR and MALDI-TOF MS analysis. The reaction product analysis revealed the formation of cyclic products that could be the major impediment of further increase of the chain length.

Key words: ε-caprolactone, δ-gluconolactone, lipases, biopolymers

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INTRODUCTION

Synthesis of copolymers based on bio-derived monomers by *in vitro* enzymatic catalysis has attracted many research interests in the past decades (Martin *et al.*, 1992). The utilization of enzymes for synthesis of polyesters was comprehensively reviewed (Kumar *et al.*, 2001; Matsumura, 2006).

ε-Caprolactone (ECL) represents one of the most valuable cyclic monomers in ring opening polymerization reactions of lactones catalyzed by lipases. The resulting polyester, poly-(ε-caprolactone), (PCL), was intensively studied and well characterized (Gross *et al.*, 2010). Due to its biodegradability, biocompatibility and interesting mechanical properties, PCL was used in different studies as raw material for microsphere, nanoparticles, films, fibers synthesis, but mostly as drug delivery systems (Dash & Konkimalla, 2012). However, there are some limitations of PCL applicability due to its low hydrophilicity, low melting point and slow degradation rate (Sinha *et al.*, 2004). A possible solution to eliminate these drawbacks could be sugars derivatives that can introduce new functionalities and properties to the PCL hydrophobic backbone.

Enzymatic syntheses of copolymers by ring-opening polymerization of different lactones, such as ε-caprolactone and β-butyrolactone (Kobayashi, 2010) or ε-caprolactone and δ-valerolactone were previously reported (Albertsson & Srivastava, 2008).

Over the last few years, enzymatic copolymerizations of PCL with various monomers/polymers such as sugars derivatives, poly(ethylene glycol), alkyl diacids, diols have been reported (Juais *et al.*, 2010; Gross *et al.*, 2001) and, as expected, the newly introduced functionalities suggested novel potential applications. The immobilized lipase from *Candida antarctica* B, commercially available as Novozyme 435, demonstrated to be most efficient among lipases from different sources that have been screened for these reactions. Sugar-derivative based polyesters were successfully synthesized, starting from alditols. The lipases were proved to be efficient when adipic acid, 1,8-octanediol and different alditols such as: mannitol, xylitol, glucitol, galactitol, erythritol and ribitol were used as raw materials (Liu *et al.*, 2006). A different glucose derivative, isosorbide, was used as multifunctional initiator of ring-opening polymerization of ECL catalyzed by immobilized *Yarrowia lipolytica* lipase, yielding PCL-isosorbide oligomers with M_n values up to 1068 (Barrera-Rivera & Martinez-Richa, 2009). Juais and coworkers synthesized sugar-based polyesters from the same sugar derivative (isosorbide) and an aliphatic dicarboxylic acid diester (Juais *et al.*, 2010). Up to date, enzymatic synthesis of copolymers of ECL with sugar-derived lactones has not yet been reported.

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Abbreviations: α_w, water activity; ECL, ε-caprolactone; GL, D-glucono-δ-lactone; PCL, poly-(ε-caprolactone); Lipozyme-TL IM, *Thermomyces lanuginosus* lipase; CALB-Lecta, *Candida antarctica* lipase B; PPL, porcine pancreatic lipase; TMOS, tetramethoxysilane; OcTMOS, octyl-trimethoxysilane; 3-NH_2PrTMOS, 3-aminopropyl-trimethoxysilane; LC, linear copolymer; CC, cyclic copolymer; LH, linear homopolymer; CH, cyclic homopolymer; logP, partition coefficient, Mw, weight average molecular weight, Mn, number average molecular weight; PDI, polydispersity index

In this study D-glucono-δ-lactone (GL), a low priced raw material, easily available by oxidation of glucose, was selected as co-monomer for the synthesis of copolymers with ECL. Immobilized lipases from various sources and different reaction media were tested to find the suitable reaction conditions. Although sol-gel entrapped lipases were proved as very efficient catalysts for esterification and transesterification reactions (Tomin *et al.*, 2011; Ursoiu *et al.*, 2012), they were not investigated, at our best knowledge, for polyester synthesis.

EXPERIMENTAL

Materials. ε-caprolactone (ECL), D-glucono-δ-lactone (GL), *t*-butanol (~99% pure), toluene (>99%), dimethylsulfoxide (~99.7% pure), were purchased from Merck. Immobilized *Candida antarctica* lipase B on acrylic resin (Novozyme 435) and *Thermomyces lanuginosus* lipase (Lipozyme-TL IM) were from Novozyme, lyophilized *Candida antarctica* lipase B (CALB-Lecta) from C-Lecta (Leipzig, Germany), porcine pancreatic lipase (PPL) was a Sigma-Aldrich product, tetramethoxysilane TMOS 98%, octyl-trimethoxysilan OcTMOS 95%, 3-aminopropyl-trimethoxysilan 3-NH_2Pr TMOS 98% were purchased from Alfa Aesar, Brunschwig Chemie, NL.

Methods. Polymerization in organic solvents. ECL (0.445 mL, 4 mmole) and *Candida antarctica* lipase B (50 mg, Novozyme 435) were added to GL (0.356 g, 2 mmole) dissolved in 1 mL organic medium. The reactions were performed in 4 mL Micro Reactions Vessels at 80ºC, under argon atmosphere, magnetically stirred at 300 rpm. The reactions were stopped by filtration of the enzymes.

Polymerization in bulk. To 0.356 g GL and 0.445 mL ECL, 50 to 100 mg lipase, native or immobilized, were added. The reactions were performed into a 4 mL vial at 50/80°C in Ar atmosphere. At the end of reaction 1 mL of tetrahydrofurane was added and the immobilized enzyme and unsolved GL were removed by filtration. The polymer obtained by evaporating the solvent was dried overnight in vacuum, at 60°C.

Pre-equilibration of water activity. Raw materials and immobilized lipase Novozyme 435 were equilibrated with saturated salt solutions at 25°C in separate containers. The salts chosen for this study were $MgCl_2$ (α_w 0.225), K_2CO_3 (α_w 0.432), Na_2SO_4 (α_w 0.95) and K_2SO_4 (α_w 0.973). The equilibration was performed overnight previous to the starting of the reaction.

Lipase sol-gel immobilization. Lipase from *Candida antarctica* B was immobilized by sol-gel entrapment method, as described before (Corici *et al.*, 2011). In a 4 ml glass vial, 780 μL lipase solution, 200 μL PEG 20000 (4%), 100 μL NaF (1M) and 200 μL isopropyl alcohol were mixed (magnetic stirring, 600 rot/min), and 6 mmol silane precursors were added. The resulting mixture was mixed at ambient temperature until the gel formation was observed, and the gel was kept for 24 h at 4°C for a complete polymerization. The bulk gel was washed and dried at room temperature as described elsewhere (Zarcula *et al.*, 2010), and kept at 4°C until further use.

Products characterization. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). Fourier Transform Infrared (FT-IR) spectra of the samples were obtained in attenuated total reflectance (ATR) mode on a Bruker Vertex 70 (Bruker Daltonik GmbH, Germany) spectrometer equipped with a Platinium ATR, Bruker Diamond Type A225/Q. Spectra were collected in the range 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and with 64 co-added scans.

MALDI-TOF MS analysis. MALDI-TOF MS analysis of products was carried out using Bruker BIFLEX III matrix assisted laser desorption ionization time-of-light mass spectrometry (Bruker Daltonik GmbH, Germany) at an acceleration voltage of 20 kV using 2,5-dihydroxybenzoic acid as matrix. Sample preparation, weight average (M_w) and number average (M_n) molecular numbers, and the product relative content were determined as described elsewhere (Kakasi-Zsurka *et al.*, 2011). Within every MALDI-TOF spectrum, we estimated the relative composition of the reaction product assuming that the ionization efficiency was not significantly different for the oligomeric species present in the mixture.

RESULTS AND DISCUSSION

Lipase-catalyzed copolymerization of ECL and δ-gluconolactone and identification of the products

Synthesis of different bio-polymers by ECL grafting/insertion was intensively studied in the last years, using the chemical route and $Sn(Oct)_2$ as a catalyst. Li and coworkers (2005) described the starch-*g*-poly(ε-caprolactone) chemical synthesis by three different polymerization methods, in bulk, in toluene suspension or in suspension/bulk polymerization, at temperatures higher than 120°C. The highest PCL grafting efficiency obtained was 40% (wt), and the homopolymer synthesis could not be avoided (Li *et al.*, 2005).

The use of lipases as biocatalysts for copolymers synthesis can lead to highly ordered repeat-unit chain sequence (Matsumura, 2006), and lipases can facilitate copolymerizations of monomers that are difficult to be achieved by traditional methods (Kumar *et al.*, 2000).

Recently, our group firstly demonstrated the ability of lipase to use GL, together with 3-hydroxybutyric acid, as substrate for copolymer formation (Kakasi-Zsurka *et al.*, 2011). In the present study, ECL was used as co-monomer, anticipating higher cycle reactivity due to the higher polymerization degree of PCL synthesized by lipases, compared to polyhydroxybutyrate (Mee van der *et al.*, 2006).

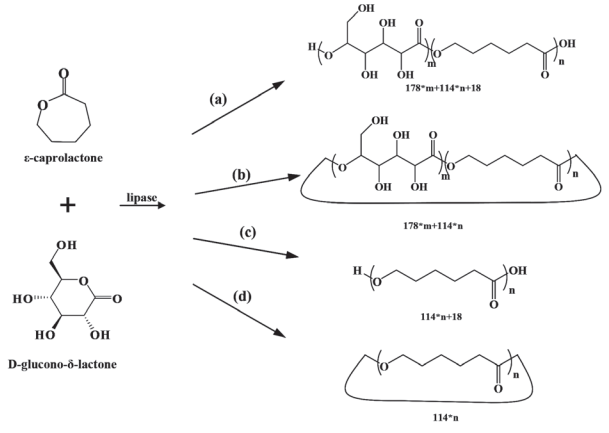

Figure 1. Formation of linear (a) and cyclic (b) copolymers and linear (c) and cyclic (d) homopolymers, as possible reaction products.
The structures indicate the masses of the corresponding oligomers, m and n meaning the numbers of GL and ECL repeat units, respectively. 178 and 114 are the masses of the GL and ECL units, respectively, and 18 is the mass of the H_2O end-group.

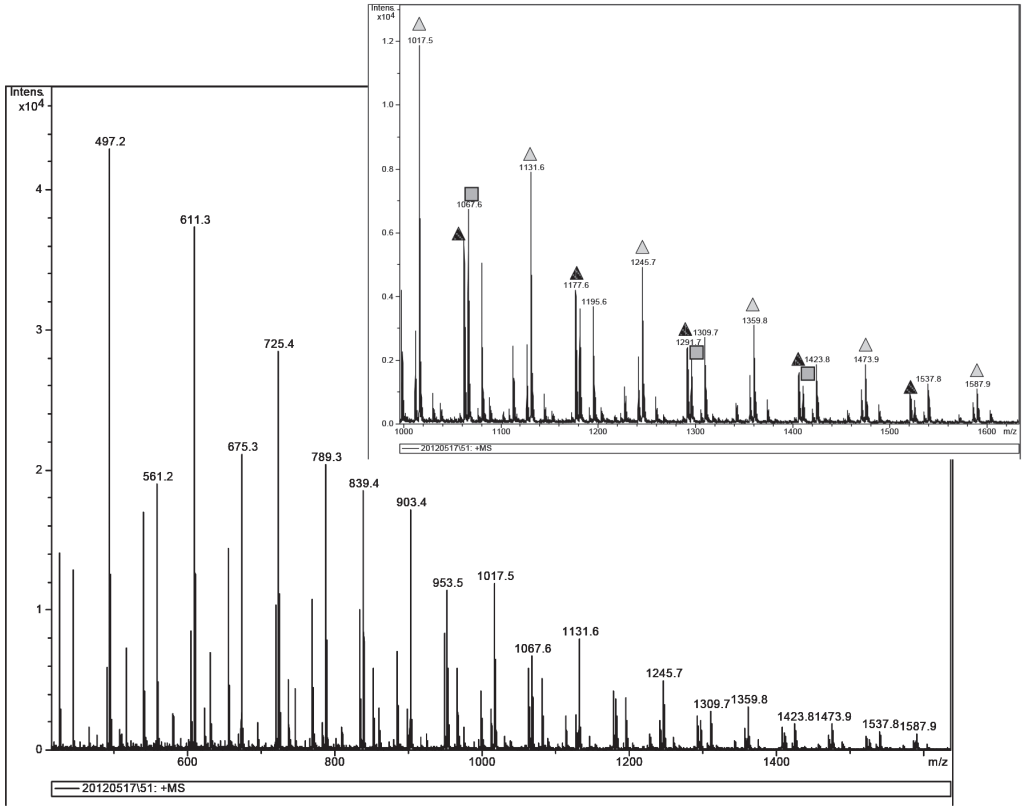

Figure 2. MALDI-TOF spectrum of the ECL and GL based copolymer catalyzed by sol-gel entrapped *CalB* lipase at 50°C, at 24 h reaction time.
Inset: the 1000–1600 *m/z* region (△) linear copolymer; (▲) cyclic copolymer; (□) linear homopolymer).

The possible reaction products can be linear (a) or cyclic copolymers (b), together with possible linear (c) or cyclic homopolymers of ECL (d) (Fig. 1). The homopolymer formation as co-product is explained by (i) higher solubility of ECL in non-polar organic solvents compared to the less soluble sugar derivatives, resulting in easier accessibility into the lipase catalytic site; (ii) higher reactivity of the 7-membered cycle (Mee van der *et al*., 2006).

The product formation was monitored by infrared spectroscopy. A shift of the bands corresponding to the carbonyl group stretching vibrations was observed during the polymerization reaction, from 1721 cm^{-1} in ECL and 1722 cm^{-1} in GL raw materials, to 1766 cm^{-1} in the ester product.

The products were identified based on MALDI-TOF MS spectra. A typical MALDI-TOF MS spectrum is depicted in Fig. 2, for a product obtained in solvent-less system using sol-gel immobilized lipase. The peak series which can be assigned to the sodium adduct ions of different oligomer series ($[M+Na]^+$) indicate the formation of linear and cyclic copolymers containing GL unit into the backbone (as depicted in Fig. 1a, b), together with ECL homopolymers (Fig. 1c, d). For example (inset of Fig. 2), the peak at m/z 1131.6 corresponds to the sodium adduct ion of a linear oligomer with n=8 and m=1 and the peak at m/z 1177.6 corresponds to the sodium adduct ion of a cyclic oligomer with n=7 and m=2 (i.e. two inserted GL units). PCL homopolymer series were also detected, e.g. the peak at m/z 725.4 demonstrates the presence of the oligomer with n=9.

The identification of the reaction products by MALDI-TOF MS demonstrates, for the first time, the lipase-catalyzed insertion of gluconolactone, as 2,3,4,5,6-pentahydroxy-caproic unit, into a PCL backbone. The formation of linear and cyclic copolymers was also confirmed by NMR analysis (data given in the Supplementary material).

Influence of the reaction medium and the water content

The reaction medium in the copolymers synthesis catalyzed by lipases represents an essential parameter, particularly when the solubility of the raw materials in the non-polar solvent is low.

Organic solvents with different partition coefficient value, logP, as well as solvent-less systems were investigated as reaction media. Since the suitable solvents for sugar dissolution such as dimethylsulfoxide, pyridine, dimethylformamide can significantly decrease the lipase activity due to the strip off the necessary water from the enzyme microenvironment (Kennedy *et al.,* 2006), in this study mixtures of DMSO and *t*-BuOH were tested. Previous studies concerning syntheses of sugar esters indicated that a concentration up to 20% DMSO in the reaction media can be tolerated by lipases, with partial loss of activity (Croitoru *et al*., 2012). Our experiments were conducted 24 h, using Novozyme 435 as catalyst. At increasing log P values only a slight decrease of the number average molecular weight (M_n) and weight average molecular weight (M_w) was observed (Table 1), excepting at high DMSO concentration (DMSO:*t*-BuOH =1:4) when the above-mentioned sensitivity of lipases toward DMSO resulted in lower molecular weights. However, important differences concerning the product composition were observed. The relative content of linear (LC)

Table 1. Influence of reaction medium on the composition and molecular weight of the product, in the copolymerization reaction of ECL with GL catalyzed by Novozyme 435 lipase

Reaction medium	log P[a]	Mn[b]	Mw[c]	PDI[d]	Relative content in the product, [%]			
					LC	CC	LH	CH
A. Organic solvent/solvent mixture								
Dioxane	–0.31	827.05	933.80	1.13	21.6	22.0	14.8	46.5
DMSO:t-BuOH 1:4	0.33	539.40	559.31	1.03	45.4	17.9	34.4	2.3
DMSO:t-BuOH 1:5	0.60	760.72	851.99	1.12	19.0	35.5	42.0	3.4
Toluene	2.73	714.71	791.92	1.11	n.d	n.d	93.3	6.7
B. Solventless/controlled water activity (salt)	α_w[e]							
Solventless		841.43	954.06	1.13	20.5	11.3	66.9	1.4
K_2SO_4	0.973	877.13	974.35	1.11	16.0	10.1	73.9	n.d
Na_2SO_4	0.95	1108.78	1210.81	1.09	22.9	16.9	60.14	n.d
K_2CO_3	0.432	832.56	936.58	1.12	6.0	24.6	60.7	8.8
$MgCl_2$	0.225	808.71	858.72	1.06	16.7	12.8	66.6	3.9

[a]logP-partition coefficient value (Du, 2000; Sangster, 1989,) [b]number average molecular weight; [c]weight average molecular weight; [d]polydispersity index, LC, linear copolymer; CC, cyclic copolymer; LH, linear homopolymer; CH, cyclic homopolymer; α_w, water activity values (Adlercreutz, 2008)

and cyclic (CC) copolymers was higher than previously found in the reaction products obtained from 3HBA and GL (Kakasi-Zsurka *et al.,* 2011). The formation of linear (LH) and cyclic (CH) homopolymers could not be avoided, possibly due to the ECL excess employed, particularly in the solvent-less systems, where ECL had the role of reaction medium as well. The relatively high amount of cyclic copolymers explains the lower polymerization degree of ECL compared to the experiments with non-sugar raw materials (Matsumura, 2006).

Based on the higher relative linear copolymer/cyclic copolymer ratio (LC/CC=1.8) achieved in solvent-less system compared to organic reaction media (dioxane LC/CC=0.98, DMSO:*t*-BuOH 1:5, LC/CC=0.53), the forthcoming experiments were carried out in solventless systems.

The presence of water is essential in lipase-catalyzed synthesis reactions, as small amounts of water are necessary to preserve the activity of the enzyme in organic solvents. Water was also proved to be an important nucleophilic reagent in the chain initiation step of ring-opening polymerization reactions (Ma *et al.,* 2009). In the studied process, water could have two opposite effects, facilitating the GL solubility but shifting the reversible process toward hydrolysis. Therefore, determination of the optimum water level was imperious. Thus, the substrates and enzyme were pre-equilibrated separately with saturated salt solutions with different water activity prior to the reaction. All experiments were carried out 24 h at 80°C under argon atmosphere. The distribution of product species and the PDI were estimated by MALDI-TOF MS analysis. As shown in Table 1B, the highest M_n value was obtained at 0.95 water activity. At low water activity values only traces of cyclic homopolymers were detected, but the formation of cyclic copolymer could not be avoided, regardless of the water activity value in the reaction system. Mei *et al.* have found that the increase of water content of the enzyme (Novozyme 435) led to increase of the molecular weight of the synthesized ECL homopolymers (Mei *et al.*, 2002). In our case, such a dependence could not be evidenced, because of the more complex polymerization pathway.

Influence of the lipase immobilization method

The investigation of the influence of reaction medium and water activity on the copolymerization reaction was carried out using Novozyme 435. In fact, this extremely versatile immobilized lipase has been used in the majority of studies involving lipase-catalyzed polymerization (Miletic *et al.,* 2012). However, since the copolymerization reaction also yielded considerable amounts of homopolymers, a screening study was considered useful in order to increase the copolymer content, as well as the polymerization degree.

This screening was carried out by using two native lipases, lyophilized *Candida antarctica* lipase B (C-Lecta) and porcine pancreatic lipase (PPL), as well as four immobilized lipases, Novozyme 435 (CALB physically immobilized within a macroporous resin of poly(methylmethacrylate), Lipozyme TL IM (silica granulated *Thermomyces lanuginosus* lipase), Sol-gel CALB 1 (sol-gel entrapped CALB using OcTMOS:TMOS=1:1 as silane precursors), and Sol-gel CALB 2 (sol-gel entrapped CALB using 3-NH_2PrTMOS:TMOS=1:1 as silane precursors). The last two immobilized lipases were prepared in our laboratory. All experiments were conducted at 50°C due to the native lipases stability, under magnetic stirring (300 rpm) and argon atmosphere, at about 25 U enzyme/mmol substrate, after a pre-equilibration of the substrates and enzyme over night at α_w=0.95.

The results depicted in Fig. 3, estimations of the relative composition of the products based on m/z intensities from the MALDI-TOF MS spectra (Kakasi-Zsurka *et al.*, 2011), indicate that Lipozyme TL IM and the Sol-gel CALB 2 lipase were more effective for the synthesis of the ECL-GL copolymer than Novozyme 435, the lipase most frequently used for polymer synthesis. Porcine pancreas lipase was not appropriate for this reaction, as copolymer could not be detected and the homopolymer polymerization degree was not higher than 5 (data not shown). Other authors reported ECL copolymer

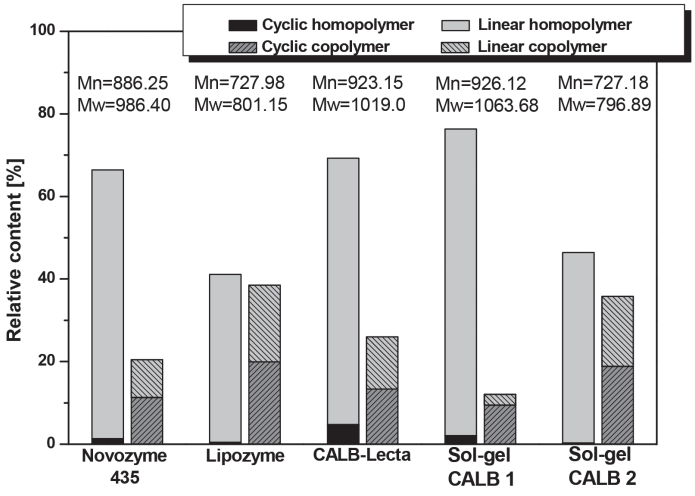

Figure 3. Estimated relative composition of ε-caprolactone copolymers with δ-gluconolactone (2:1 molar ratio), catalyzed by lipases from different sources, at 50°C and 24 h reaction times.

formation at higher molecular weights with more reactive derivatized sugars (ethyl glucoside) in the presence of different lipases, but using the sugar derivative only as initiator for the ring-opening polymerization of ECL (Bisht, 1998). The other native lipase, CALB yielded a relative content of about 30% copolymer, in 1:1 cyclic to linear ratio.

The enzyme immobilization method together with the characteristics of the support material has important influence on the immobilized enzyme activity and substrate specificity. Important differences were observed for our reaction system, probably as a result of different immobilization methods and supports, leading to particular support porosities and other characteristics that influenced the immobilized enzyme activity. Using Novozyme 435 as biocatalyst, linear homopolymer synthesis was facilitated, while the copolymer content was not higher than 20% and the highest polymerization degree obtained for the copolymer was 15.

In the best experiments with sol-gel entrapped *Candida antarctica* lipase (Sol gel CALB 2), a polymerization degree of 13 for the linear copolymer (m/z value 1587, Fig. 2), as well as about 50% relative content of linear copolymer was obtained. Replacing 3-NH_2PrTMOS with OcTMOS as silane precursor during the immobilization process resulted in higher M_n values, but the copolymer content was lower, probably because of the increased hydrophobicity of the matrix that can influence the diffusion of the substrate to and through the lipase catalytic site. A more accurate correlation between the biocatalyst activity and structure of the support is difficult to achieve, since several characteristics that influence the enzyme conformation and mass transfer during the catalyzed process should be taken in account. A morphological characterization of the entrapped lipases, providing information about the microstructure, porosity and texture of the matrix, will be realized in our forthcoming study and hopefully will help to better elucidate the mechanism of copolymer synthesis by sol-gel immobilized lipases.

Another important outcome of the studied process is the linear/cyclic ratio in the copolymer product. At the studied conditions, it was not possible to lower the cyclic polymer content below 50%. Cyclic polymer formation was reported by other authors, as well. Wahlberg *et al.,* using ECL and D,L-lactide as raw materials, detected cyclic copolymers with one or two D,L-lactide units during the initial stage of the reaction, that gradually disappeared at long reaction time (up to 696 h) (Wahlberg *et al.*, 2003). As our experiments with immobilized enzymes were carried out at a quite low temperature (50°C), the presence of cyclic species in all formed products cannot be considered as the effect of the temperature.

CONCLUSIONS

The insertion of GL into the hydrophobic backbone of poly(ε-caprolactone) was demonstrated for the first time, leading to oligomers containing 2,3,4,5,6-pentahydroxy-caproate units. Such new copolymers can exhibit new and more useful properties compared to PCL. Among the tested lipases, sol-gel entrapped CALB has proved higher activity for the synthesis of copolymers, compared to Novozyme 435.

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REFERENCES

Adlercreutz P (2008) Fundamentals of biocatalysis in neat organic solvents. In *Organic synthesis with enzymes in non-aqueous media*. Carrea G, Riva S, eds, Wiley-VCH, Weinheim.

Albertsson AC, Srivastava RK (2008) Recent developments in enzyme-catalyzed ring-opening polymerization. *Adv Drug Deliv Rev* **60**: 1077–1093.

Barrera-Rivera KA, Martinez-Richa A (2009) One-pot biocatalytic synthesis of sugar based poly (ε-caprolactone). *Macromol Symp* **283–284**: 144–151.

Bisht KS, Deng F, Gross RA, Kaplan DL, Swift G (1998) Ethyl glucoside as a multifunctional initiator for enzyme-catalyzed regioselective lactone ring-opening polymerization. *J Am Chem Soc* **120**: 1363–1367.

Croitoru R, Fitigau F, van den Broek LAM, Frissen AE, Davidescu CM, Boeriu CG, Peter F (2012) Biocatalytic acylation of sugar alcohols by 3-(4-hydroxyphenyl)propionic acid. *Process Biochem* **47**: 1894–1902.

Dash TK, Konkimalla VB (2012) Poly-ε-caprolactone based formulations for drug delivery and tissue engineering: A review. *J Control Release* **158**: 115–33.

Du CM, Valko K, Bevan C, Reynolds D, Abraham MH (2000) Characterizing the selectivity of stationary phases and organic modifiers in reversed-phase high-performance liquid chromatographic systems by a general solvation equation using gradient elution. *J Chromatogr Sci* **38**: 503–511.

Gross RA, Ganesh M, Lu W (2010) Enzyme-catalysis breathes new life into polyester condensation polymerizations. *Trends Biotechnol* **28**: 435–443.

Gross RA, Kumar A, Kalra B (2001) Polymer synthesis by in vitro enzyme catalysis, *Chem Rev* **101**: 2097–2124.

Hu J, Gao W, Kulshrestha A, Gross RA (2006) "Sweet polyesters": lipase-catalyzed condensation-polymerizations of alditols. *Macromolecules* **39**: 6789–6792.

Juais D, Naves AF, Li C, Gross RA, Catalani LH, (2010) Isosorbide polyesters from enzymatic catalysis. *Macromolecules* **43**: 10315–10319.

Kakasi-Zsurka S, Todea A, But A, Paul C, Boeriu CG, Davidescu C, Nagy L, Kuki A, Kéki S, Péter F (2011) Biocatalytic synthesis of new copolymers from 3 hydroxybutyric acid and a carboydrate lactone. *J Mol Cat B:Enzym* **71**: 22–28.

Kennedy JF, Kumar H, Panesar PS, Marwaha SS, Goyal R, Parmar A, Kaur S (2006) Enzyme catalyzed regioselective synthesis of sugar esters and related compounds. *J Chem Technol Biotechnol* **81**: 866–876.

Kobayashi S (2010) Lipase-catalyzed polyester synthesis-a green polymer chemistry. *Proc Jpn Acad Ser B* **86**: 338–365.

Kumar A, Kalra B, Dekhterman A, Gross RA (2000) Efficient ring-opening polymerization and copolymerization of ε-caprolactone and ω-pentadecalactone catalyzed by *Candida antartica* lipase B. *Macromolecules*, **33**: 6303–6309.

Li C, Yushan N, Xinchao B, Xueyu Q, Xiuli Z, Xuesi C, Xiabin J (2005) A novel approach to grafting polymerization of ε-caprolactone onto starch granules. *Carbohyd Polym* **60**: 103–109.

Liu C, Qian Z, Gu YC, Fan LY, Li J, Chao GT, Jia WJ, Tu MJ (2006) Synthesis, characterization, and thermal properties of biodegradable aliphatic copolyester based on ε-caprolactone, adipic acid, and 1,6-hexanediol. *Mat Lett* **60**: 31–38.

Ma J, Li Q, Song B, Liu D, Zheng B, Zhang Z, Feng Y (2009) Ring-opening polymerization of ε-caprolactone catalyzed by a novel thermophilic esterase from the archaeon *Archaeoglobus fulgidus*. *J Mol Cat B:Enzym* **56**: 151–157.

Martin BD, Ampofo SA, Linhardt RJ, Dordick JS (1992) Biocatalytic synthesis of sugar-containing polyacrylates. *Macromolecules* **25**: 7081–7085.

Matsumura S (2006) Enzymatic synthesis of polyesters via ring-opening polymerization. *Adv Polym Sci* **194**: 95–132.

Mee van der L, Helmich F, Bruijn R, Vekemans JAJM, Palmans ARA, Meijer EW (2006) investigation of lipase-catalyzed ring-opening polymerizations of lactones with various ring sizes: kinetic evaluation. *Macromolecules* **39**: 5021–5027.

Mei Y, Kumar A, Gross RA (2002) Probing water-temperature relationships for lipase-catalyzed lactone ring-opening polymerizations. *Macromolecules* **35**: 5444–5448.

Miletic N, Nastasovic A, Loos K (2012) Immobilization of biocatalysts for enzymatic polymerizations: possibilities, advantages, applications. *Bioresource Technol* **115**: 26–135.

Poojari Y & Clarson SJ (2009) Lipase catalyzed synthesis of poly(ε-caprolactone)-poly(dimethylsiloxane)-poly(ε-caprolactone) triblock copolymers. *Silicon* **1**: 165–172.

Sinha VR, Bansal K, Kaushik R, Kumria R, Trehan A (2004) Poly-ε-caprolactone microspheres and nanospheres: an overview. *Int J Pharm* **278**: 1–23.

Tomin A, Weiser D, Hellner G, Bata Zs, Corici L, Peter F, Koczka B, Poppe L (2011) Fine tuning the second generation sol-gel lipase immobilization with ternary alkoxysilane precursor systems. *Process Biochem* **46**: 52–58.

Ursoiu A, Paul C, Kurtán T, Peter F (2012) Sol-gel entrapped *Candida antarctica* lipase B — a biocatalyst with excellent stability for kinetic resolution of secondary alcohols. *Molecules* **17**: 13045–13061.

Wahlberg J, Persson PV, Olsson T, Hedenstrom E, Iversen T (2003) Structural characterization of a lipase-catalyzed copolymerization of ε-caprolactone and D,L-lactide. *Biomacromolecules* **4**: 1068–1071.

Zarcula C, Corici L, Croitoru R, Ursoiu A, Peter F (2010) Preparation and properties of xerogels obtained by ionic liquid incorporation during the immobilization of lipase by the sol–gel method. *J Mol Cat B: Enzym* **65**: 79–86.