Supporting Information

Enzymatic Ring Opening Polymerization of ω-Pentadecalactone by Reactive Extrusion

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Contents:

1. General Experimental:

a) Materials and Methods:

The monomer ω-pentadecalactone (PDL, 98%) was purchased from Aldrich and used without further purification. Novozym 435 (N435, specific activity 10,000 PLU/g) was a gift from Novozymes (Bagsvaerd, Denmark) and consists of *Candida antarctica* Lipase B (CALB) physically adsorbed within the macroporous resin Lewatit VPOC 1600 (poly[methyl methacrylate-co-butyl methacrylate], supplied by Bayer).

b) Characterization

Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra were recorded in $CDCl_3$ using a Bruker AMX-300 apparatus at a frequency of 300 MHz. Size-exclusion chromatography (SEC) was performed using chloroform (sample concentration: 1 wt.%) at 35°C using a polymer laboratories (PL) liquid chromatography equipped with a PL-DG802 de-gas system, an isocratic HPLC pump (LC1120, flow rate: 1 mL/min), a Basic-Marathon Autosampler, a PL-RI refractive index detector and three columns: a guard column PLgel 10 lm (50 x7.5 mm) and two columns PLgel mixed-B 10 lm (300 x 7.5 mm). Molar mass and molar mass distribution was calculated by reference to a calibration curve constructed from polystyrene standards.

X-ray photoelectron spectroscopy (XPS) was performed on recovered enzyme beads. XPS analysis was performed using an Axis Ultra spectrometer (Kratos Analytical). Samples were irradiated with monochromated X-rays (Al Ka, 1486.6 eV) with photoelectrons analyzed from a selected area 700 lm by 300 lm, with a take-off-angle of 90°. CasaXPS (Casa Software Ltd.) data processing software was used to calculate the area under peaks representative of elements detected, which were then normalized to take into account relative sensitivity to provide relative concentrations. The measured binding energy of these peaks (BEmeas) was used with a relevant reference binding energy (BERef) by the Casa-XPS (Casa Software Ltd.) data processing software to apply a correction energy, D_{corr}, to all the spectra acquired from the same analysis position.

The particle size distributions (PSD) of N435 beads before and after e-REX were measured by using a Malvern Mastersizer 3000 laser diffraction particle size analyzer (Malvern Instruments, UK). Laser diffraction measurements were performed in wet-mode using water as the suspension medium. The samples were measured ten times, without ultrasonication, using a stirrer speed of 2000 rpm. The measurements provide the size distribution on a volume (or mass) basis and the statistical diameters (for non-spherical particles), D10, D50, and D90. $D_{10,50,90}$ refer to the particle size for which 10, 50 or 90% of the particles by weight are finer. The average mean particle size are reported.

c) Reactive extrusion polymerization of ω-pentadecalactone

Both ω-pentadecalactone (PDL) and N435 were dried in a vacuum overnight at 60 °C. Following this, N435 (2.5 - 10% by weight) and PDL were physically dry-mixed and then introduced into a DSM twin-screw mini-compounder (capacity: 15 cc) under nitrogen flow in three minutes. Reactions were conducted at 90 – 130 °C and then terminated at a given point by quenching the reaction on fast cooling and removing the polymer that adhered to the screws. Beads of N435 were primarily located in the recirculation pathway. For reactions conducted at temperatures exceeding 100°C, the polymer could be extruded out of the die, while most of the enzyme was left in the recirculation pathway.

d) Residual enzyme activity

The activity of fresh and recovered N435 was compared by an assay that follows the catalysis of ε-CL ring-opening polymerization. For this assay, ε-CL (1 mL) was dissolved in toluene (2 mL) in the presence of 100 mg immobilized N435 bio-catalyst. The reaction mixture was magnetically stirred and the temperature was maintained at 70 ^{o}C. Sample aliquots were withdrawn at 15, 30 and 60 minutes to monitor the conversion of ε-CL to PCL by ^{1}H-NMR.

2. Figures/Tables

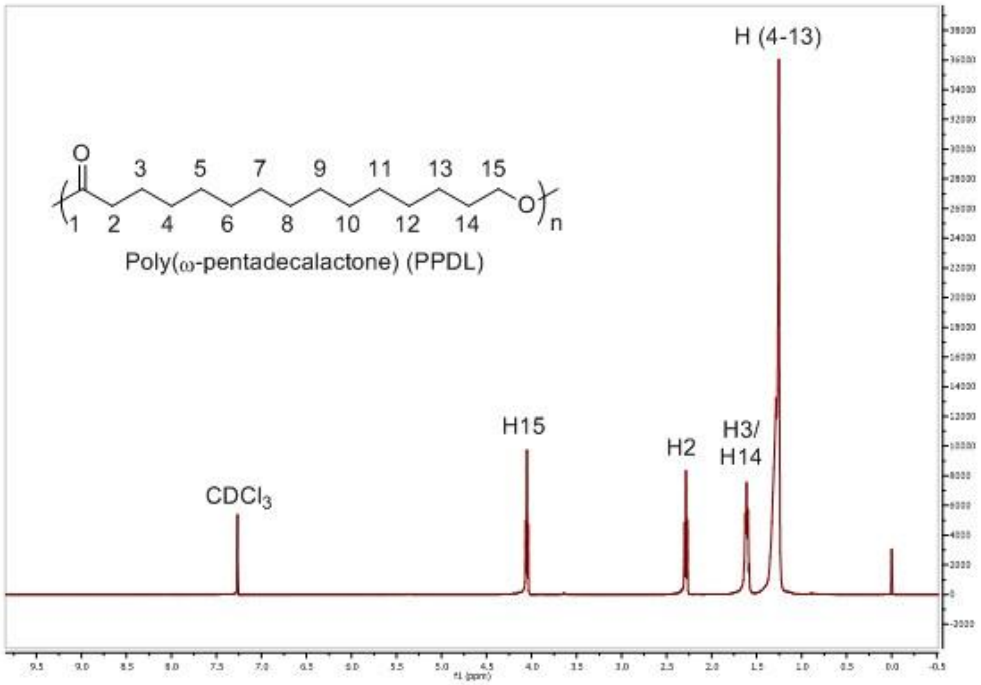

Figure SI-1: ^{1}H NMR (500 MHz, $CDCl_3$) of PPDL prepared by e-REX polymerization of PDL (20 min, 90 ^{o}C, 10% N435)

Table SI-1. Monomer conversion and molecular weight *versus* time for the reactive extrusion polymerization of PDL catalyzed by 2.5% N435 performed at 110 ^{o}C at a mixing rate of 60 RPM.

time (min)	M_n [a] (g/mol)	M_w [a] (g/mol)	M_w/M_n [a]	conv.[b] (%)
10	1,600	1,700	1.4	10
20	20,000	45,000	2.2	36
30	40,000	81,000	2.0	50
40	74,000	155,000	2.1	72
50	121,000	242,000	2.0	95
60	142,000	296,000	2.0	>99

a) Molecular weight averages and dispersity ($Đ=M_w/M_n$) values were determined by size exclusion chromatography with chloroform as the eluent using narrow dispersity polystyrene standards

b) Determined by ^{1}H NMR

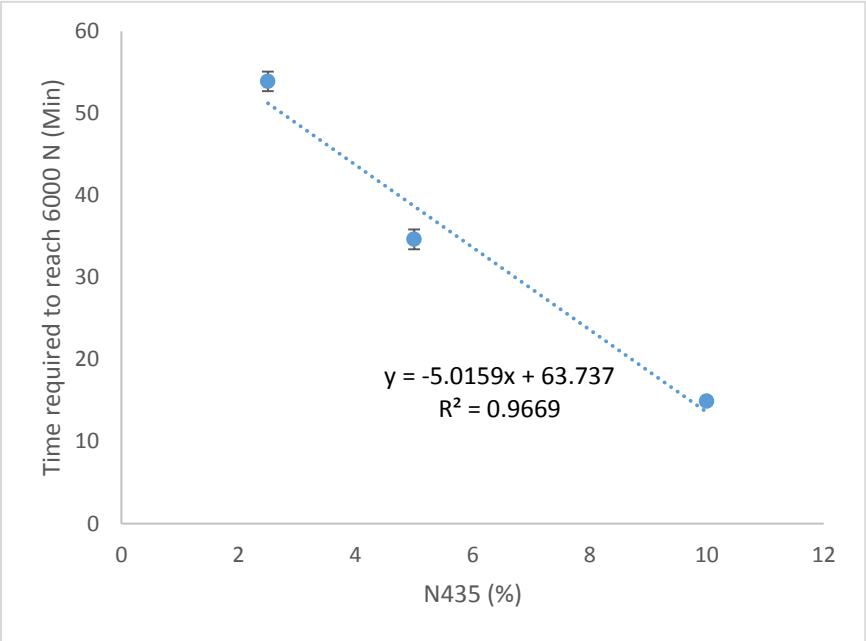

Figure SI-2: Linear regression of N435 concentration vs. time necessary to reach 6000 N

Table SI-2. N435 bead dimensions determined from granulometry (unused beads and beads recovered from reactive extrusion polymerization of PDL (10%-N435, 90 °C, 15 min, mixing rate 60 RPM)

Enzyme Particle	D x 10 (μm)	D x 50 (μm)	D x 90 (μm)
N435 unused	372	546	793
N435 recovered	138	428	825

Table SI-3: Residual enzyme activity of unused N435 and N435 beads recovered from e-REX (10%-N435, 90 °C, 15 min, mixing rate of 60 RPM).[a]

Sample	Time point (min)	Monomer Conversion (%)[b]
N435 - Unused	15	41.4
N435 – Unused	30	73.6
N435 – Unused	60	97.7
Recovered N435	15	31.0 ± 3.5
Recovered N435	30	54.8 ± 1.3
Recovered N435	60	94.2 ± 2.0

a) Unused and recovered N435 beads were assayed to determine monomer conversion for an ε-caprolactone polymerization conducted in toluene-d^8 at 80°C.

b) Monomer conversion was determined by ^{1}H-NMR

Table SI-4. Amount of enzyme remaining within of unused N435 and N435 beads recovered from e-REX (10%-N435, 90 °C, 15 min, mixing rate of 60 RPM).[a]

Sample	protein content[a] (%-by-wt)
N435	8.4 ± 1 %
Recovered N435	4.5 ± 0.5 %

a) determined by XPS