ACTPAC

Funded by the European Union

ACTPAC	
Project number:	101135289
Project name:	A Complete Transformation Path for C-C backboned plastic wastes to high-value Chemicals and materials
Topic:	HORIZON-CL6-2023-ZEROPOLLUTION-01-5
Type of action:	HORIZON-IA
Starting date of action:	01.01.2024
Project duration:	48 months
Project end date:	31.12.2027
Deliverable number:	D8.1
Deliverable title:	Methods established for the polycondensation of the developed long chain monomers
Document version:	Ver2
WP number:	WP8
Lead beneficiary:	08-UG
Main author(s):	Katja Loos (08-UG), Georgios Karchilakis (08-UG)
Internal reviewers:	Henri Cramail (04-CNRS), Frédéric Peruch (04-CNRS)
Nature of deliverable:	R
Dissemination level:	PU
Delivery date from Annex 1:	30/07/2024
Actual delivery date:	30/07/2024

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or REA. Neither the European Union nor the granting authority can be held responsible for them.

Document history

Version	Date	Beneficiary	Description
0.1	23.05.2025	08-UG 04-CNRS	For Internar Review
0.2	27.06.2025	08-UG	Eddited after Internal review
1.0	30.06.2025	08-UG	Submitted

Executive Summary

Petroleum-derived plastics have gained massive popularity thanks to their resilience and cost-effectiveness, making them essential in every day applications. Among these, PE stand out as one of the most extensively produced and consumed plastics, owning its excellent durability, flexibility and chemical resistance. However, its widespread use also presents serious risks to environmental sustainability and human health. Due to inefficient recycling systems, millions of tons of carbon-carbon backbone-based polymer waste accumulate annually in both terrestrial and marine ecosystems. Hence, developing sustainable pathways to integrate PE into new value-added products has become increasingly important. ACTPAC consortium is focused on establishing a comprehensive pathway for the recycling and upcycling of PE-based waste, by intergrading innovative chemical and biological processes to convert PE into high-value chemicals. This approach involves the initial breakdown of PE into defined-length alkanes, followed by the selective functionalization into versatile building blocks suitable for repolymerization into biodegradable polymers with tailored properties.

Focusing on enzymatic catalysts as a sustainable alternative to traditional metal-based catalyst finds lipases as the ideal biocatalysts, owning to its environmental compatibility, reusability and great catalytic selectivity. In this context, N435 stands out as one of the most widely used enzymes for polyester production. Notably, this biocatalyst combines broad range substrate tolerance with high regio- and enantio-selectivity, especially favoring hydrophobic aliphatic monomers. Therefore, N435-catalyzed polycondensation for the production of polyesters is pursued. Novel biobased short-chain diols, such as 1,4-butanediol, along with long-chain length biobased diacids are used to produce aliphatic polyester under melt conditions. The key variables such as the enzyme concentration, reaction time and temperature are optimized to maximize yields and control molecular weights of the final products.

Contents

Figures

Tables

Acronyms & Abbreviations

Term	Description
DX.X	Deliverable X.X
WP	Work Package
PE	Polyethylene
CALB	Candida antarctica lipase B
N435	Novozym 435
HiC	Immobilized cutinase from *Humicola insolens*
Mn	Number-average molecular-weight
Mw	Weight-average molecular-weight
NMR	Nuclear magnetic resonance
SEC	Size exclusion chromatography
ATR-FTIR	Attenuated total reflection-Fourier transform infrared
TGA	Thermogravimetric analysis
DSC	Differential scanning calorimetry
WAXD	Wide-angle X-ray diffraction

1 Introduction

1.1 Overview

The main objective of WP8 is to define efficient enzymatic polycondensation routes to produce aliphatic polyesters. In specific, the development of novel biobased polyester with polyolefin-like properties via bulk-enzymatic polymerization is being pursued, aiming to enhance properties, provide biodegradability and recyclability. Novel biocatalysts are utilized, as they have emerged as green alternatives to traditional metal-based catalyzed polymerizations, aligning well with green chemistry principles through milder conditions and high efficiency. In particular, immobilized CALB has demonstrated excellent catalytic performance, making it especially promising for the synthesis of high-molecular weight polyester. Therefore, enzyme-catalyzed melt polycondensation of aliphatic polyesters, utilizing upcycled long-chain length diacids is being researched to produce new materials with tailored properties.

The scope of the first deliverable is to identify and obtain information on possible methods to synthesize biobased aliphatic polyesters via melt-enzymatic polycondensation. This includes a thorough literature review to evaluate the most effective enzymatic systems, particularly focusing on immobilized biocatalysts and their performance under various reaction conditions. Factor such as chain length, reaction temperature, enzymes stability in melt conditions were analyzed to determine their influence on polyester production. The insights gained served as a foundation for developing a robust, scalable synthetic strategy, guiding the experimental phase for the production of high performance, aliphatic polyesters.

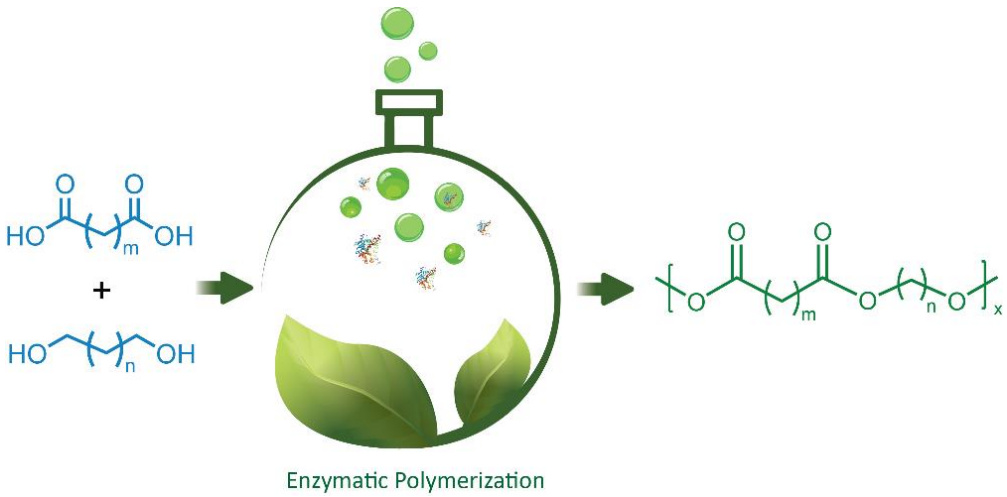

Figure 1: Schematic overview of the enzyme-catalyzed synthesis of biobased polyesters.

1.2 Relation to other tasks and deliverables

As the initial deliverable, this task does not rely on input from preceding tasks or deliverables. However, it provides essential output to the subsequent tasks and deliverables listed in Table 2.

Provides outputs to:

Table 1: D8.1 Output for other tasks and deliverables.

Deliverable	Due Date	Output from D8.1
D8.2	M30	Production of 3 polyesters through an enzymatic route at g scale
D11.3	M46	Scale-up of 3 target polyester production by biocatalytic polymerization and demonstration of production at TRL6 level (confidential)
D11.4	M46	Scale-up of 3 target polyester production by biocatalytic polymerization and demonstration of production at TRL6 level (confidential)

1.3 Structure of the deliverable

In this deliverable, methods established for the enzymatic-polycondensation of the developed long-chain polyesters will be discussed and divided into 3 sub-sections:

1. Biotic synthesis of polyesters via step-growth melt polymerization process.
2. Exploration of biocatalysts - reaction parameters on polyester synthesis.
3. Establishment of synthetic procedure of long-chain aliphatic polyesters.

2 Methods established for the polycondensation of the developed long chain monomers.

2.1 Biotic synthesis of polyesters via a step-growth polymerization process

2.1.1 Two-step melt polycondensation

As mentioned before, the main objective of this deliverable is to explore, evaluate and establish methods for enzymatically catalyzing the polycondensation reaction of aliphatic monomers obtained from the depolymerization of polyethylene. Pending the availability of these monomers, commercially available monomers were used as model compounds.

In general, the synthesis of polyesters from functional monomers via step-growth polymerization involves the stoichiometric, stepwise reaction between bifunctional reactants, which is accompanied by the formation of a low molar condensate. Aliphatic polyesters are typically synthesized through two primary routes in melt (Figure 1): (i) direct esterification of a dicarboxylic acid with a diol (or a hydroxyacid) followed by a polycondensation step to achieve a high molar mass polymer, or (ii) transesterification of a diester dicarboxylate with a diol (or a hydroxyester). Both direct esterification and transesterification reactions proceed slow at low temperature, necessitating the use of catalysts or high temperature and high end-group (i.e., reactive group) concentration, ideally for solvent-free conditions. Vacuum is generally applied during the last steps of reaction to facilitate the removal of reactions condensate (i.e., water in direct polyesterification or a volatile alcohol in transesterification polymerization). This constant removal is critical for driving the equilibrium toward the formation of high-molar-mass polyesters.

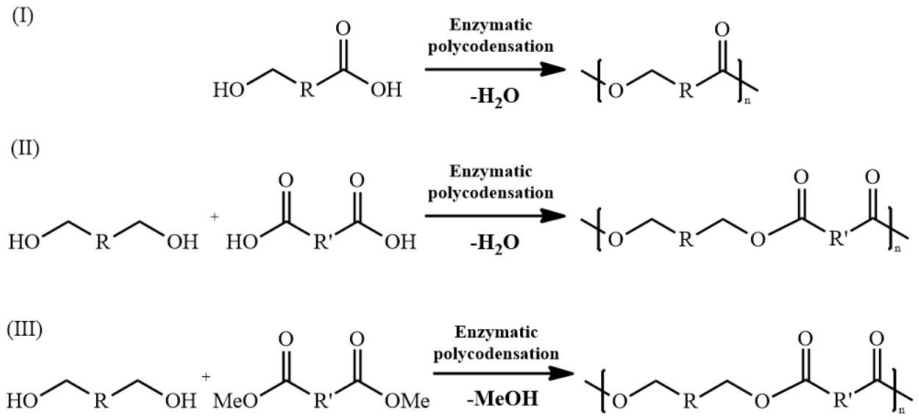

Figure 2: Enzyme-catalyzed polycondensation of (i) hydroxy acids, (ii) diols with diacids, and (iii) diols with diacid derivatives (such as methyl esters).

According to the literature, a variety of strategies have been considered by researchers in order to overcome this drawback and prepare high-molecular-weight polyesters. The most effective method for achieving a high-degree of esterification involves the use of several vacuum steps in a stepwise manner.[1] However, in the case of volatile monomers, such as short-chain diols or diacids, it is important to avoid using strong vacuum during the initial stages of polymerization. This precaution prevents the removal of unreacted monomers and low-molecular-weight oligomers, which could alter the molar ratio of the two substrates. Jiang et al. underlined that by starting a high vacuum procedure after oligomers have been formed, a significant increase in polymer molecular weight can be achieved.[2,3] In addition to vacuum, drying agents such as preactivated molecular sieves can be utilized to absorb condensation products.[4,5] However, molecular sieves are more frequently used is solvent-based systems, where lower viscosity facilitates easier stirring and enhances the removal of by-products.

2.2 Exploration of biocatalysts – reaction parameters on polyester synthesis.

Within the class of hydrolases, lipases belong to the subclass known as efficient biocatalysts for enzymatic polymerization. Among them, CALB serves as the most widely used biocatalyst due to its exceptional enantioselectivity, chemo selectivity and regioselectivity.[6–8] It efficiently catalyze various-reactions, including esterification, transesterification and amination making it highly suitable for linear polyester synthesis. Despite its many advantages, CALB notably suffers from limited long-term operational stability and presents challenges in recovery and reuse. Immobilization has emerged as an effective strategy to address these issues, significantly enhancing the enzyme's activity, thermal stability and solvent tolerance.[6] As a result, immobilized CALB, commercially available as N435, has been widely employed in polymerization process, allowing to function effectively at temperatures up to 120°C.[9] These advances underscore N435's growing relevance is sustainable polymer chemistry.

2.2.1 Influence of the substrate length.

Research on N435-catalyzed polymerization has highlighted that substrate specificity is a crucial factor in determing its effectiveness. Given that lipases primary function is to hydrolyze lipids into fatty acids, lipases have a natural tendency for hydrophobic substrates with long, linear alkyl chains. This preference extents to polyesters synthesis, where lipases generally exhibit increased catalytic activity as the chain length of the dicarboxylic acid or the diol increases. Longer alkyl chains also enhance substrate flexibility, facilitating easier access to the enzyme's active site. Mahapatro et al. showed that the average-molar-mass of polyesters synthesized via N435-catalyzed polycondensation of adipic acid both in bulk and in solvent systems, increase with the diol chain length ranging from 1,4-butanediol to 1,8-octanediol.[10] Furthermore, a similar trend was observed for the polycondensation of various long alkylene chain diacids with 1,8-octanediol, in diphenyl ether and in bulk. More recently, Campisano et al. further supported the substrate preference, showing that longer-chain diacids such as azelaic and sebacic acids ,along with diols up to 1,8-octanediol generally favored over their shorter-chain counterparts such as oxalic, malonic, succinic, glutaric and adipic acid and diols such as ethylene diol and 1,3-propanediol.[11] Feder et al. compared the substrate specificity of two enzymes, N435 and HiC for aliphatic polyester synthesis.[12] HiC exhibited high activity for ω-hydroxyalkanoic acid substrates

ranging from C10 to C13, with limited activity for shorter chain acids. In contrast, N435 demonstrated consistent catalytic activity for a broader range of substrates, highlighting its greater substrate preference.

2.2.2 Influence of the reaction temperature

Generally, lipase-catalyzed synthesis of aliphatic polyesters is performed at temperature up to 90°C to prevent enzyme denaturation and maintain catalytic activity. Early studies often used free-enzymes, which limited the reaction temperature to around 40-60°C.[13,14] However, the immobilization of lipase, such N435, significantly improves their thermal stability, allowing reactions to proceed at higher temperatures for extended durations, thereby enhancing reaction rates and polymer molecular weights. Uyama et al. observed that by increasing the reaction temperature from 50°C to 60°C during the CALB-catalyzed polymerization of sebacic acid and 1,4-butanediol, a notable increase in the M_n was achieved.[15] Mahapatro et al. conducted bulk polymerization of 1,8-octanediol and adipic acid using N435, with reaction temperatures ranging from 65 to 90°C.[10] While temperature had minimal impact on the M_n during the first 24 hours, a notable effect was observe at longer reaction times. The highest M_n was achieved at 90°C, indicating that elevated temperatures can enhance polymer growth over extended reaction durations. Temperature control is an important aspect of enzymatic polymerization, especially when working with monomers that have limited thermal stability or high melting-point. Operating within an optimal temperature range ensures sufficient molecular mobility and reaction rates while preserving the structural integrity and long-term activity of the biocatalyst.

Therefore, after evaluation of possible methods to achieve aliphatic polyesters, the most appropriate reaction parameters have been identified. These optimized conditions aim to ensure efficient enzymatic polycondensation, polymer molecular weight and reaction feasibility. The selection of bulk polycondensation over solvent- based methods is supported by its environmental and operational benefits. While higher viscosity can limit mass transfer, this is outweighed by the method's ability to achieve polymer without the introduction of unnecessary reagents, making it favorable for the enzymatic processes.

3 Establishment of synthetic procedure of Long-chain aliphatic polyesters

3.1.1 Materials

Novozym® 435 immobilized lipase from Candida antarctica was kindly donated by Novozymes. Ethylene glycol (99%, Sigma-Aldrich), 1,3-propane diol (98%, TCI Europe N.V.), 1,4-butanediol (polymer-grade, TCI Europe N.V.), 1,5-pentane diol (98%, Fisher Emergo B.V.), 1,6-hexane diol (98, Merck Life Science N.V.), suberic acid, (99%, Fisher Emergo B.V.), sebacic acid (98%, Fisher Emergo B.V.), dodecanedioic acid (99%, Merck Life Science N.V.), tetradecanedioic acid (99%, TCI Europe N.V.), hexadecanedioic acid (98%, Merck Life Science N.V.), chloroform (amylene stabilized, HPLC grade, >99.8%, Merck Life Science N.V.), and methanol (HPLC grade, >99.8%, Merck Life Science N.V) were all used as received.

3.1.2 Synthesis of Long-chain aliphatic polyesters

The long-chain polyesters were prepared following the two-step melt polycondensation method. In the first step of the esterification reaction, the reaction of diacids and diols resulted in formation of oligomers. In the second step, polycondensation of the oligomers gave polymer of high-molecular weight. The setup was consisted of a 25 ml two-necked flask equipped with a stirring device and a fitted to a distillation line. The system was connected to a Schlenk line, to switch between vacuum and argon flow. As an example, the enzymatic synthesis of poly (butylene suberate) is given: 1.648 gr of suberic acid (1.0 eq.), 0.852 gr of 1,4-butanediol (1.0 eq.) and 10% w/w N435 were added to the flask. The reaction mixture was maintained under a mild argon flow, magnetically stirred in an oil bath and heated to 120 °C for 24 h. Subsequently, the argon flow was closed, and the system was gently switched to vacuum (2 × 10−2 mbar), while the temperature was kept at 120 °C for another 55 h. The obtained polyesters were dissolved in chloroform, separated from the immobilized enzyme particles using a thin needle and syringe, and subsequently precipitated in cold methanol. The polymers were collected via centrifugation (4500 rpm, 10 min, 1 °C) and dried in a fume hood.

3.1.3 Characterization of Long-chain aliphatic polyesters

Proton NMR (^{1}H NMR; 600 MHz) spectra are recorded on a Bruker Ascend NMR600 spectrometer using $CDCl_3$ as the solvent.

The molecular weights (number-average, M_n, and weight-average, M_w) of the aliphatic polyesters are determined via a SEC instrument equipped with a triple detector, consisting of a Malvern Dual detector and Schambeck RI2012, a refractive index detector. Separation is carried out by utilizing two PLgel 5 µm MIXED-C 300 mm columns from Agilent Technologies at 35 °C. HPLC grade chloroform is used as the eluent with a flow rate of 0.5 mL min^{-1}. The samples are filtered through a 0.2 µm PTFE filter prior to injection. Molecular weights are determined based on a conventional calibration curve generated from narrow dispersity polystyrene standards (Agilent and Polymer Laboratories, M_w = 645–3,001,000 g mol^{-1}).

ATR-FTIR spectra is recorded on a Bruker VERTEX 70 spectrometer equipped with an ATR diamond single reflection accessory. The measurement resolution is at 4 cm^{-1}, and the spectra is collected in the range of 4000–400 cm^{-1}, with 16 scans for each sample. Atmospheric compensation and baseline correction is applied to the collected spectra using OPUS spectroscopy software (v7.0) (Bruker Optics).

The thermal stability and degradation temperature is analyzed via TGA on a TA-Instruments Discovery TGA 5500 instrument using a heating rate of 10 °C min^{-1} in a nitrogen environment.

DSC analysis is carried out to determine the glass transition temperatures (T_g) and melting points (T_m). The measurements are performed on a TA-Instruments Q1000 DSC using a heating and cooling rate of 10 °C min^{-1}, under a mild nitrogen flow, for a temperature range of −70 to 120 °C with a 5 mg sample size.

The relative crystallinities of the enzymatically produced aliphatic polyesters are obtained via WAXD analysis of compression molded discs (10 mm diameter, 1.0 mm thickness). The discs are pressed at a Specac Atlas 15T manual hydraulic press for approximately 5 min at 25 kN and stored at room temperature prior to measurement. The WAXD measurements are performed on a Bruker D8 Advance diffractometer ($Cu_{K\alpha}$ radiation, λ = 0.1541 nm, 40 kV, 40 mA) in the angular range (2ϑ) of 4 to 51 at ambient conditions. The obtained WAXD patterns are analyzed with the software Origin 2018, wherein the crystalline and amorphous peaks are separated via peak deconvolution. The degree of crystallinity (χ_c) is calculated through the following formula: $\chi c\ (\%) = (Area_C / (Area_C + Area_a))*100$, where $Area_c$ and $Area_a$ are the sum of the integrated crystalline and amorphous peaks, respectively.

The same compression molded discs are used for water contact angle measurements. These experiments are performed on a Dataphysics OCA 15EC device, and the software SCA20 is used to determine the left, right and mean water contact angles. A water droplet of 5.00 µL is dispensed on the surface at a rate of 5.00 µL s^{-1}. The droplet is directly photographed, and each polymer sample is measured in triplicate.

4 Conclusions

As the starting point of WP8 within the ACTPAC project, we first analyzed the available enzymatic polymerization methods, identifying bulk polymerization as a more favorable than solvent-based systems, due to its alignment with green chemistry principles. Furthermore, N435 has emerged as the optimal biocatalysts due to its broad substrate range and superior performance with long-chain, hydrophobic aliphatic monomers. Therefore, biobased short-chain diols and long-chain diacids are catalyzed by N435 to produce aliphatic polyesters. Prior to obtaining the target compounds generated within the ACTPAC project, reference compounds are employed to establish and optimize the experiments methodology. The key variables such as the enzyme stability reaction time and temperature are optimized to maximize yields and control molecular weights of the final products. The chemical structures are verified by ^{1}H, ^{13}C and 2D NMR and FTIR analyses and SEC is used to determine the molecular weight. WAXD method is employed to investigate the crystal structures of the samples. Final, the thermal behavior of the polyesters is analyzed via TGA and DSC. The findings will lay a strong foundation for scalable, sustainable production of novel biobased polyesters via alternative synthetic routes for polycondensation, creating materials with improved properties.

References

1. Hevilla V, Sonseca A, Echeverría C, Muñoz-Bonilla A, Fernández-García M. Enzymatic Synthesis of Polyesters and Their Bioapplications: Recent Advances and Perspectives. *Macromol Biosci*. 2021;21(10):2100156. doi:10.1002/mabi.202100156

2. Jiang Y, Woortman A, Van Ekenstein G, Loos K. Enzyme-Catalyzed Synthesis of Unsaturated Aliphatic Polyesters Based on Green Monomers from Renewable Resources. *Biomolecules*. 2013;3(3):461-480. doi:10.3390/biom3030461

3. Jiang Y, Loos K. Enzymatic Synthesis of Biobased Polyesters and Polyamides. *Polymers*. 2016;8(7):243. doi:10.3390/polym8070243

4. Dahu Yao, Guangji Li, Tapas Kuila, Peng Li, Nam Hoon Kim, Seong-Il Kim, Joong Hee Lee. Lipase-catalyzed synthesis and characterization of biodegradable polyester. Journal of Applied Polymer Sci.2010:120(2):1114-1120. https://doi.org/10.1002/app.33257.

5. Stamatina N. Vouyiouka, Evangelos Topakas, Adamantia Katsini, Constantine D. Papaspyrides, Paul Christakopoulos. A Green Route for the Preparation of Aliphatic Polyesters via Lipase-catalyzed.Macro Materials Eng.2012:298(6)679-689. https://doi.org/10.1002/mame.201200188.

6. Ortiz C, Ferreira ML, Barbosa O, et al. Novozym 435: the “perfect” lipase immobilized biocatalyst? *Catal Sci Technol*. 2019;9(10):2380-2420. doi:10.1039/C9CY00415G

7. Wang H, Li H, Lee CK, Mat Nanyan NS, Tay GS. Recent Advances in the Enzymatic Synthesis of Polyester. *Polymers*. 2022;14(23):5059. doi:10.3390/polym14235059

8. Liu Y, Song L, Feng N, Jiang W, Jin Y, Li X. Recent advances in the synthesis of biodegradable polyesters by sustainable polymerization: lipase-catalyzed polymerization. *RSC Adv*. 2020;10(59):36230-36240. doi:10.1039/D0RA07138B

9. Post C, Maniar D, Jongstra JA, et al. Enzymatic bulk synthesis, characterization, rheology, and biodegradability of biobased 2,5-bis(hydroxymethyl)furan polyesters. *Green Chem*. 2024;26(15):8744-8757. doi:10.1039/d4gc01512f

10. Mahapatro A, Kalra B, Kumar A, Gross RA. Lipase-Catalyzed Polycondensations: Effect of Substrates and Solvent on Chain Formation, Dispersity, and End-Group Structure. *Biomacromolecules*. 2003;4(3):544-551. doi:10.1021/bm0257208

11. Campisano ISP, De Queiros Eugenio E, De Oliveira Veloso C, Dias ML, De Castro AM, Langone MAP. Solvent-free lipase-catalyzed synthesis of linear and thermally stable polyesters obtained from diacids and diols. *Braz J Chem Eng*. 2021;38(3):549-562. doi:10.1007/s43153-021-00137-y

12. Feder D, Gross RA. Exploring Chain Length Selectivity in HIC-Catalyzed Polycondensation Reactions. *Biomacromolecules*. 2010;11(3):690-697. doi:10.1021/bm901272r

13. Uyama H, Yaguchi S, Kobayashi S. Lipase-catalyzed polycondensation of dicarboxylic acid-divinyl esters and glycols to aliphatic polyesters. *J Polym Sci Part Polym Chem*. 1999;37(15):2737-2745. doi:10.1002/(sici)1099-0518(19990801)37:15<2737::aid-pola7>3.0.co;2-m

14. Uyama H, Yaguchi S, Kobayashi S. Enzymatic Synthesis of Aromatic Polyesters by Lipase-Catalyzed Polymerization of Dicarboxylic Acid Divinyl Esters and Glycols. *Polym J*. 1999;31(4):380-383. doi:10.1295/polymj.31.380

15. Uyama H, Inada K, Kobayashi S. Lipase-Catalyzed Synthesis of Aliphatic Polyesters by Polycondensation of Dicarboxylic Acids and Glycols in Solvent-Free System. *Polym J*. 2000;32(5):440-443. doi:10.1295/polymj.32.440