Enzymatic synthesis of polyesters and their bioapplications: Recent advances and perspectives

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Abstract

This article reviews the most important advances in the enzymatic synthesis of polyesters. In first place, the different processes of polyester enzymatic synthesis, i.e. polycondensation, ring opening and chemoenzymatic polymerizations, and the key parameters affecting these reactions, such as enzyme, concentration, solvent or temperature, are analyzed. Then, the latest articles on the preparation of polyesters either by direct synthesis or *via* modification are commented. Finally, the main bioapplications of enzymatically obtained polyesters, i.e. antimicrobial, drug delivery or tissue engineering, are described. It is intended to point out the great advantages that enzymatic polymerization present to obtain polymers and the disadvantages found to develop applied materials.

Keywords: Enzymatic polymerization, CALB, polyesters, bioapplications, drug delivery, tissue engineering, antimicrobial.

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1. Enzymes in Polymer Chemistry

1.1. Introduction

Enzymes in nature catalyze the breakdown of natural polymers such as lignocellulose and cutin. However, scientific advances have also demonstrated their potential as biocatalysts for polymer synthesis and functionalization.[1–3] Therefore, enzyme-based polymerization is arising as greener alternative strategy to more conventional polymer synthesis methods that use chemical catalysts. Enzymatic polymer synthesis is motivated by a latent search of alternative strategies to classical synthesis routes, which imply metallic catalysts, and/or harsh processing conditions. Metal catalysts can cause negative environmental effects as well as toxicity in biomedical materials,[4] and harsh processing conditions can cause undesired side reactions[5–7] and largely limit the incorporation of many interesting but thermal sensitive functional groups (vinyl, epoxy, etc.) and biological materials (drugs, proteins, cells, etc.).[2,3,8]

Therefore, enzymatic polymerization has emerged in the last decades as an important synthetic technique for the development of biodegradable polymers, especially aliphatic polyesters, with exceptional architectures able to fulfill the demands of as advanced application fields as biomedical one, where the toxicity of solvents and catalyst residues needs to be considered specially when biodegradation processes are involved

Main advantages of enzymatic polymer synthesis are related with the mild reaction conditions needed for finely tune and control the polymerization and functionalization processes, thanks to the high enantio-, regio- and chemoselectivity of these catalysts and the few by-products resulting from the synthesis. Additionally, when combined with chemical methods, known as chemoenzymatic polymerization, the variety and complexity of resulting macromolecules can be overwhelming. However, suitable activity and specificity of enzymes only partially ensure their efficient application to polyester synthesis. The competitiveness and sustainability of enzymatic production and processing of polyesters at an industrial scale can only be guaranteed

by proper catalyst immobilization, what will allow its recovery and reuse. In this way, immobilization protocols have to prevent the protein detachment from the support by preserving its integrity under stirring conditions[9,10] at the same time that protect the enzyme against pH, solvent or temperature. Although it is not the intention of the current review to center the attention over the enzymes immobilization protocol, it is worth to at least mention that to overcome these challenges, new research efforts have arisen devoted to develop new covalent immobilization methods as well as reactor configurations.[2,3] In line with this, new studies are exploring the use of greener reaction media as supercritical carbon dioxide[11] and ionic liquids[12] as an alternative to conventional toxic organic solvents.

Nevertheless, enzymatic polymer synthesis and functionalization provides without any doubt a growing platform for greener and more creative polymers with tailor made structures for fulfilling specific applications [] In this sense, polyester synthesis has gained attention in the field of enzymatic catalysis thanks to their unique properties such as biodegradability and biocompatibility. The combination of their properties together with the enzymatic synthesis methods, have raised polyesters as ideal candidates in biomedical and packaging fields where tailor made structures and properties are more and more demanded. The aim of this review is therefore, to present the most recent polyester developments using biocatalysis. The work will mainly focus in advances in lipase-catalyzed polyesters with insights in synthesis methods, parameters of importance and monomers for polyester synthesis and functionalization, while exposing the trends in their promising bioapplications and future perspectives in this field of enzymatic polymerization.

1.2. Enzymatic Polyester Synthesis

Hydrolases, transferases and oxidoreductases are the main enzymes employed as catalysts for enzymatic polymerizations. Interestingly hydrolases, also known as esterases, in aqueous media catalyze the hydrolysis of fatty acid esters, while in organic solvents they are able to catalyze esterifications and transesterifications. Therefore, they have been studied for polyester

synthesis.[13,14] Okumura *et al.* reported in 1984 the first study in which several dicarboxylic acids and polyols were polycondensated obtaining short oligomers by means of a lipase from *Aspergillus niger*.[15] Since then, lipases have been intensively investigated as catalyst for polyester synthesis,[14] and nowadays, hydrolases typically employed for polyester production are relatively stable, commercially available and easily produced.

Among lipases, lipase B from *Candida antarctica* (CALB), is without any doubt the most important and widely employed biocatalyst for esterification and transesterification in polyester synthesis both, in its free and immobilized forms. From 90´s, there have been conducted many studies devoted to compare different lipases for polyester synthesis, and from all this work, CALB arose and still remains as the most promising biocatalyst widely used for polyester synthesis and functionalization.[16,17] Due to that, although the present review is not limited to works employing CALB as catalyst for polyester synthesis and functionalization, they represent the vast majority.

As mentioned before, immobilization of enzymes is a key factor to ensure their biological function in organic media and, therefore, to successfully polymerize and functionalize polyesters in good yields and molecular weights. For many years, the most employed CALB has been the commercially available Novozym® 435 that immobilizes the lipase physically adsorbed on a macroporous acrylic resin.[2] Although physical adsorption onto hydrophobic supports is the most used immobilization method, this approach has some drawbacks such as leaching of the enzyme, fragility of the support under stirring, and its dissolution in polar solvents as ethanol and methanol. In this direction, few more strategies have been followed over recent years in order to improve immobilization of CALB either by physically and covalently methods.

On one side physically immobilization methods to entrap CALB into poly(glycidol) based microgels, have been reported to improve the performance of the enzyme in aqueous media favoring the esterification activity over the hydrolytic one.[18] On the other side, covalent

anchoring of the protein into an epoxy-functionalized methacrylic resin has been demonstrated to improve the robustness of the immobilization overcoming most of the problems related with enzyme leaching from the substrate.[2] The studies of covalent anchoring of CALB in epoxy functionalized resins have led into new commercial variations of this catalyst, such as Fermase CALB 10000™.[19,20] Moreover, covalently immobilized enzymes formulations favor stability, recyclability and simplified separation of the catalyst from the polymer.

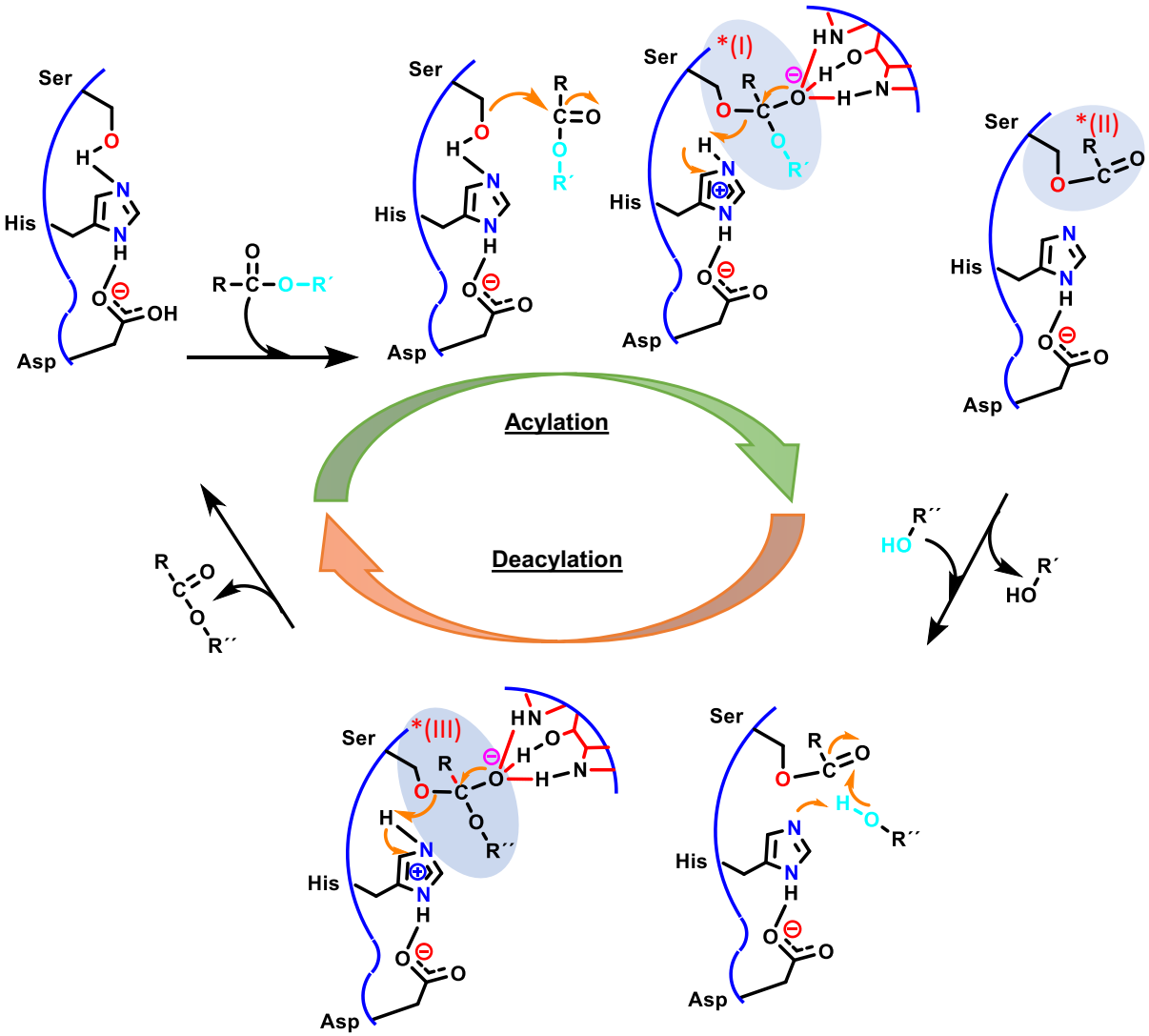
Although greener media alternatives for enzymatic catalyzed polyester synthesis are being explored (supercritical carbon dioxide and ionic liquids), typically those have been mainly conducted in solvent-less conditions or in organic solvents and biphasic organic media (solvent-aqueous mixtures). In this sense, CALB has successfully been applied over the last two decades as a catalyst for obtaining polyesters *via* two major polymerization strategies, polycondensation and ring-opening polymerization (ROP) of lactones.[21–23] CALB as a polyester catalyst can produce polymerization under mild reaction conditions, i.e. at low temperatures, resulting in good quality polyesters with controlled structures thanks to its high enantio-, regio- and chemoselectivity. This fact, together with steric hindrance at active sites, restricts side reactions and allows the synthesis of nearly linear polyesters when the starting monomers possess functionality ≥ 3.

Many reviews have been reported describing the enzyme-catalyzed polyester synthesis from the beginning of the field in the early 90´s; therefore, the following sections although will include the most important early works in the field, will highlight the most pioneer works done over the past years, which have supposed an advance in the enzymatic polyester polycondensation and ROP.

1.2.1. Lipase-Catalyzed Polycondensation (PC)

The catalytic cycle of CALB-catalyzed polycondensation is visualized in **Scheme 1**. Active centre of the enzyme is formed by a catalytic triad consisting of three amino acids residues electronically stabilized: serine (Ser105), histidine (His224) and aspartic acid (Asp187). A large

hydrophobic pocket (oxyanion hole) and a medium-sized one are located above and below the catalytic triad, respectively. These residues and the pockets work together in order to promote esterification/transesterification reactions under the proper conditions as commented above.



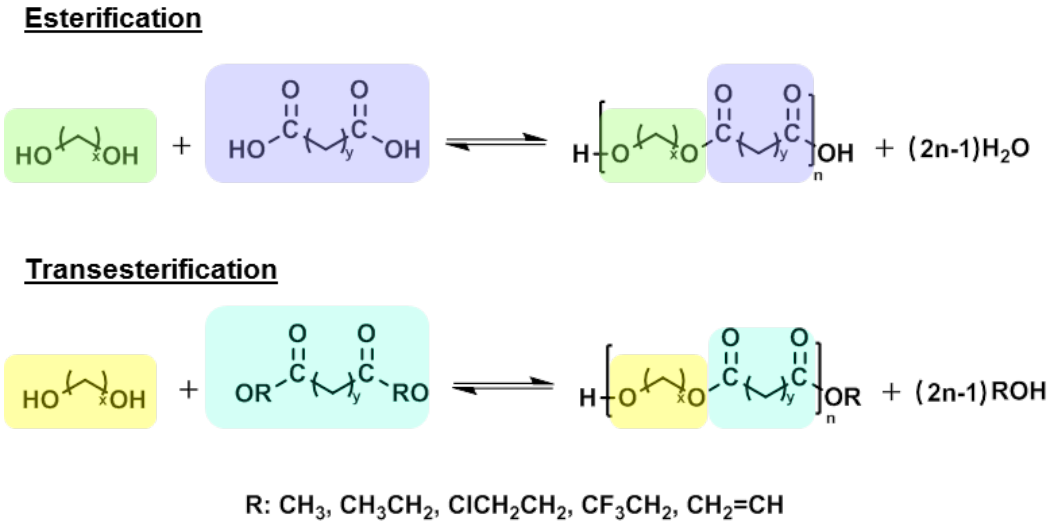
Scheme 1. Enzymatic polycondensation in the presence of CALB. (*I) Tetrahedral intermediate, (*II) acyl-enzyme complex (AEC), (*III) tetrahedral intermediate.

First, the primary alcohol from the nucleophilic serine (-CH_2OH) in the active site, interacts with the carbonyl group of a substrate molecule (carboxylic acid or its esters) RC(=O)-OR′, forming a tetrahedral intermediate (*I) stabilized through three hydrogen bonds from glutamine

(Gln 106, 1 hydrogen bond) and threonine (Thr40, 2 hydrogen bonds) located in the oxyanion hole. During the reaction, the imidazole group of His224 residue acts as a base pulling the proton from Ser105 and therefore, increasing the nucleophilicity of the oxygen to attack the carbonyl carbon of the substrate. At the same time, the carboxylate group of Asp187, helps the His224 residue to pull the proton and the acyl-enzyme complex (AEC, *II), also known as enzyme-activated monomer specie (EAM), is formed liberating R´O-H (acylating step), at the same time that the by-product will be removed from His224. In the deacylating step, also the imidazole and carboxylate groups as well as the oxyanion hole facilitate the production of RC(=O)-R´´. Subsequently, a nucleophile i.e. a primary alcohol from a diol (R´´O-H), reacts with the AEC carbonyl carbon forming a new intermediate specie. Thus, a second tetrahedral intermediate (*III) is formed and stabilized too by the oxyanion hole. In the last step, the enzyme is deacylated releasing the product (ester, polyester) and regenerated being ready for the next catalytic cycle.

Enzyme immobilization allows advances not only in polycondensation reactions for research purposes but also industrially. Thanks to that, enzymatic formation of polyesters, represents the majority of published research documents in the field.[24,25,34–36,26–33] Esterification (dehydration) and transesterification reactions occur during polycondensation to form polyesters in the presence of CALB, as shown in **Scheme 2**. Aliphatic polyesters based on dicarboxylic acids and diols/polyols have been extensively produced from these reactions (esterification and transesterification), however, these syntheses often result in the formation of low molecular weight products, as they are all reversible. Dehydration between a series of dicarboxylic acids (C6~C14) and diols to produce polyesters after 16 h of reaction at 30 °C by using enzymatic catalyst was reported for the first time by Okumura *et al*.[15] In this process, only oligomers of low molecular weight (three to seven units) were obtained due probably to the water by-product affecting the formation of polyesters in a good yield and molecular weight. Nowadays, it is well known that in polycondensation type reactions, especially when enzymatic

catalyst is used, the most important parameter affecting synthesis equilibrium is the by-product removal.[26,37]



Scheme 2. Lipase-catalyzed polycondensation reactions to form polyesters from diols and diacids or its derivatives.

Hence, some strategies have been considered by researchers in order to overcome this drawback mainly involving the usage of activated esters. The most commonly used activated esters are alkyl, haloalkyl and vinyl esters that are ordinarily obtained by esterification of the acid group in a carboxylic. In this sense, the equilibrium in the reaction can be shifted towards polymerization by properly removing by-product (water) produced during the reaction, and more efficiently by using alkyl diesters (*e.g.* diethyl and dimethyl succinate and adipate, dimethyl 3,3′-thiodipropionate, dimethyl 2-mercaptosuccinate) instead of dicarboxylic acids, as it results in much volatile by-product (alcohol), in combination with vacuum conditions.[38,39] When dealing with volatile short chain monomers, the best results in terms of number-average molecular weight (M_n) have been obtained when several vacuum steps are applied stepwise in order to reach high vacuum, often together with high temperature. In that way, removal of unreacted monomers and small oligomers is avoided retaining the proper molar ratio during the

reaction. In general, high boiling point solvents, *e.g.* diphenyl ether, and two or three polycondensation/transesterification steps are preferred in which high vacuum and temperature are achieved after at least 20 h of reaction.[26,40] Sonseca *et al.*[41] proposed a three stage synthesis for the enzymatic polymerization of diethyl succinate (DES), and aliphatic diols (dilinoleic diol (DLD) and 1,4-butanediol (BDO)) based copolyesters, similarly to what Azim *et al.*[42] did for obtaining enzymatic poly(butylene succinate) homopolymer (PBS). The first stage was carried out at atmospheric pressure and 95 °C under N_2 flow and after 3 h, the vacuum was set to 600 Torr for 21 h. After this oligomerization time, in a second stage, the vacuum was increased to 2 Torr until the experiment was finished after 4 days. The M_n increased slightly during the first 21 h to values around 3000-7000 g mol^{-1} (depending on the monomers ratio). On the other hand, the application of high vacuum in the last stage significantly affected the M_n for all the copolymers and increased by at least 2 fold after 24-48 h. Juais *et al.*[43] with the purpose of enzymatically catalyze (CALB) copolyesters from DES and BDO containing unsaturated itaconate moieties, tested different vacuum conditions after carrying a first stage for oligomerization at 80 °C under N_2 during 2 h. Vacuum variation during the second synthesis stage was confirmed to had a marked influence on final product M_n (~ 2900-3900 g mol^{-1}) with the highest vacuum (2 mm Hg) yielding the highest M_n for all of the studied compositions. Consistently, other numerous studies employed the two-stage polymerization strategy with increased vacuum/temperature in a second polycondensation/transesterification step in order to obtain polyesters with higher molecular weights compared to single stage process without vacuum. In that way, dimethyl 2,5-furandicarboxylate (DMFDCA) was polymerized with several aliphatic diols with increasing M_n with diol chain length (diol length from 4 to 8 M_n ~1200-3300 g mol^{-1} and M_n up to 23700 g mol^{-1} for diol length up to 10).[44] Although azeotropic distillation was demonstrated to be more successful for this purpose,[43] the use of diesters (dimethyl and diethyl) together with vacuum application in a two stage

polycondensation step synthesis, is still the most common way of by-product removal during enzymatic synthesis of polyesters.

Another strategy studied in order to shift the equilibrium towards polyester synthesis, is to use halo esters such as bis(2,2,2-trichloroethyl) alkanedioates with primary alcohols to obtain halogenated alcohols (2,2,2-trichloroethanol) as a by-product. In this way, authors stated that the increase on the electrophilicity of the acyl carbonyl will avoid significant alcoholysis of the obtained products' reactive end-groups as the nucleophilicity of the leaving alkoxy group decreased (compared to the formation of methanol or ethanol as by-product).[45] However, halogenated alcohols obtained as by-products were demonstrated to accelerate the enzyme bounded water release driving the hydrolysis of activated ester end-groups, meaning that this activation strategy did not result as effective as expected to obtain high molecular weight polymers.[46]

All the transesterifications mentioned above using lipase catalysts are often very slow due to the reversible nature of the reactions. In addition, the assistance of high vacuum is usually needed to ensure the proper removal of by-products which favours polymer molecular weight growth. Among activated diester monomers, alkylene esters of dicarboxylic acids (vinyl esters), have been gaining attention in the last few years, for the lipase-catalyzed acylations, as they have been demonstrated to be the most effective monomers to achieve higher molecular weight products avoiding vacuum and high reaction temperatures.[39,47] The vinyl ester represents a polycondensation leaving group that liberates unstable enols (vinyl alcohols) as by-product that rapidly tautomerizes to an aldehyde and therefore, shifts the equilibrium towards the product as the reaction becomes irreversible.[48] It is well known that increasing concentration of acetaldehyde, which is formed during the reactions with vinyl esters, implies a decrease in enzyme activity affecting the biocatalyst reuse, even though it is one of the most effective ways of increasing the polymer molecular weight and reaction yields. In contrast with the general effect of inactivation that acetaldehyde from vinyl esters has over most of the enzymes, this

occurs less intensively over CALB that has been demonstrated highly stable when exposed to it.[49] In this sense, divinyl esters were found in the middle 90s to be the most reactive ones forming polyesters in the presence of different diols at mild conditions (45 °C in diisopropyl ether for 48 h), with M_n around 7000-30000 g mol^{-1} depending on the synthesis conditions and the monomers employed.[39,47,50,51] By contrast, under the same reaction conditions, the dicarboxylic acids and diethyl esters counterparts of the divinyl esters did not produce polyesters.

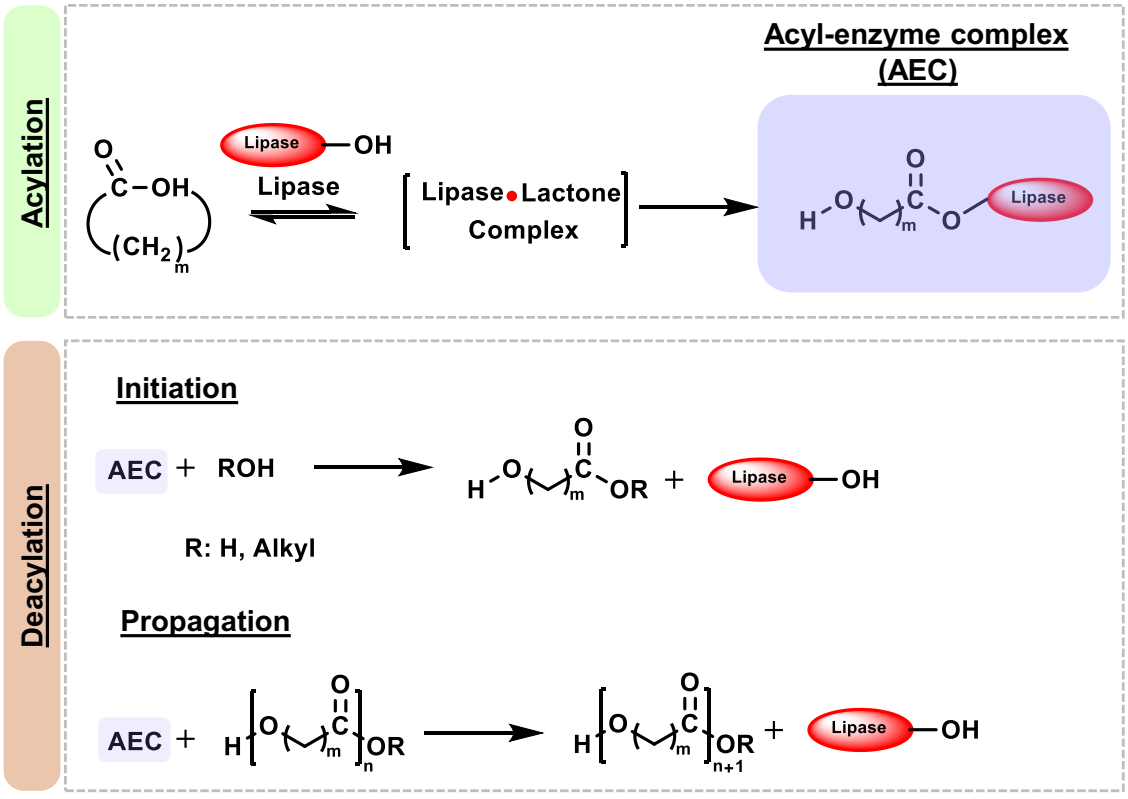
The main advantage of using vinyl esters in enzymatic polycondensations is that the esterification proceeds much faster than with alkyl or haloalkyl esters to form the desired products with higher molecular weights and yields. Additionally, the use of vacuum or high temperatures for by-product removal is not needed. This fact allows to better preserve the regioselectivity of the lipases[52,53] and synthesis procedures can be easily extended to the usage of polyols as potential monomers for obtaining linear polyesters bearing pendant hydroxyl functional groups.[54] Additionally, the mild reaction conditions open the possibility of better control the reaction of hydrazide- containing monomers,[55] the construction of epoxide-containing polyesters,[56] the efficient production of aromatic polyesters,[57] and the preparation of reactive polyesters preserving the unsaturated moieties from renewable plant oils for further crosslinking reactions.[58]

1.2.2. Lipase-catalyzed ring-opening polymerization (ROP)

Lipases, are the main class of enzymes used for ring-opening polymerization of polyesters.[59] **Scheme 1** shows the mechanism of enzymatic transesterification from which the mechanism of enzymatic ring-opening polymerization (ROP) is derived. **Scheme 3** summarizes the mechanism of enzymatic ROP.

Briefly, enzyme opens cyclic monomers used as substrate (cyclic esters as lactones, or other cyclic monomers). The lactone, forms an enzyme-lactone complex through the Ser105 residue, resulting in an active acyl-enzyme complex (AEC or EAM from enzyme activated monomer),

which corresponds to the acylating step of the enzyme. The initiator of the polymerization is a nucleophile, that can be a water molecule (HO-H) or any other nucleophile such a primary alcohol from a diol (R´´O-H), necessary to regenerate the enzyme and create the ring-opened product attacking nucleophilically the acyl carbon of the intermediate producing the ω-hydroxycarboxylic acid. After one catalytic cycle, the ring-opened product formed contains a hydroxyl-moiety at one end and the initiator-functionality at the other end. In a subsequent catalytic cycle, a new substrate is activated by the enzyme and propagation occurs by a nucleophilic attack of the hydroxyl-moiety of the previous cycle ring-opened product. Both, initiation and propagation steps correspond to a deacylating step of the enzyme. Kinetic analysis performed over lipase catalyzed ROP, determined that the lipase-lactone complex formation step (acylation step) and the AEC intermediate formation and reactivity are crucial to ensure a high polymerization ratio.[59]



Scheme 3. Lipase-catalyzed ring-opening polymerization to form polyesters from cyclic esters.

Contrary to polycondensation, ROP does not produce any leaving group; however, it is well known that the initiation of the polymerization is facilitated by enzyme bounded catalytic amounts of water and similarly to PC, removal of excess water from the reaction bath is crucial to get good conversions and hence, high molecular weight products.[60]

Lactones, lactides, cyclic carbonates and cyclic depsipeptides are the most common monomers employed for enzymatic ROP of polyesters.[61] First enzymatic ring opening polymerization (ROP) was performed in 1993 with lactones to achieve the corresponding polyesters (ε-caprolactone 7 membered ring, δ-valerolactone 6 membered ring).[62–64] The obtained polyesters possessed a carboxylic acid group and a hydroxyl group at one and other end, respectively, what was an indicator of the water role for initiate and terminate the reaction. Thereafter, several lactones differing in ring-size, substituted and unsubstituted, as well as other cyclic monomers have been polymerized, copolymerized and catalyzed from different lipases.[39,65–67]

Unsubstituted lactones with a 4-17 carbon atoms ring size have been polymerized using several lipases in bulk and solution conditions.[68] Among all, the most representative enzymatic ROP, is the synthesis of poly(ε-caprolactone) (PCL) starting from ε-caprolactone in toluene as a solvent able to solubilize substrates as well as products while the activity of biocatalyst is retained.[69] Regarding the catalyst, as occurs for PC, *Candida antarctica* (CALB) immobilized on an acrylic resin has been found to be the most effective for the ROP of ε-caprolactone and other lactones differing in ring sizes.[70,71]

In general, ROP of lactones is preferred to the enzymatic PC of their linear hydroxyl esters counterparts as higher molecular weights and conversions can be achieved without side products.[64,72] Additionally, construction of end-functionalized polyesters, macromonomers and telechelics of practical importance in polymer chemistry are easily achieved in a single-step synthesis by ROP. The common approaches are the initiator and terminator methods. The

first uses a functional alcohol to initiate the ROP of lactones,[73] while the second occurs in the presence of vinyl esters that react with hydroxyl group "terminating" the polymerization.[74]

1.2.3. Combination of Lipase-Catalyzed ROP and PC

As mentioned above, enzymatic polycondensation and ring-opening polymerization occur through similar mechanisms and reaction intermediates (acyl-enzyme intermediates). Therefore, their combination can result in novel and complex polymeric structures that cannot be obtained by any of these processes isolated. Thus, copolymerizations of monomers such as lactones, divinyl esters and glycols in the presence of lipase were demonstrated to occur in a single process resulting in polyester macromonomers and telechelics. During the synthesis, ROP of the lactones occurred and condensation of the vinyl esters with the terminal alcohol from the ROP polymerized lactones took place.[75] Afterwards, many different monomers have been used for this kind of terpolymerization such as, ω-pentadecalactone (PDL), DES and (BDO), by first carrying out oligomerization under low vacuum and subsequently, increasing the vacuum in order to enhance the molecular weight of the product.[76] Other approaches also involving two vacuum stage synthesis, copolymerized L-lactide (LLA), with different diesters (diethyl adipate (DEA) and diethyl dodecanedioate (DED) and diols (BDO and 1,6-hexanediol (HDO))[76] or obtained copolyesters of PBS containing *p*-dioxanone (PDO). Noteworthy, new functional polyesters bearing disulphide functional groups were enzymatically synthesized by copolymerizing PDL with BDO and dimethyl 3,3′-dithiodipropionate (DMD), a disulphide containing diester.[77]

1.2.4. Chemoenzymatic Polymerization

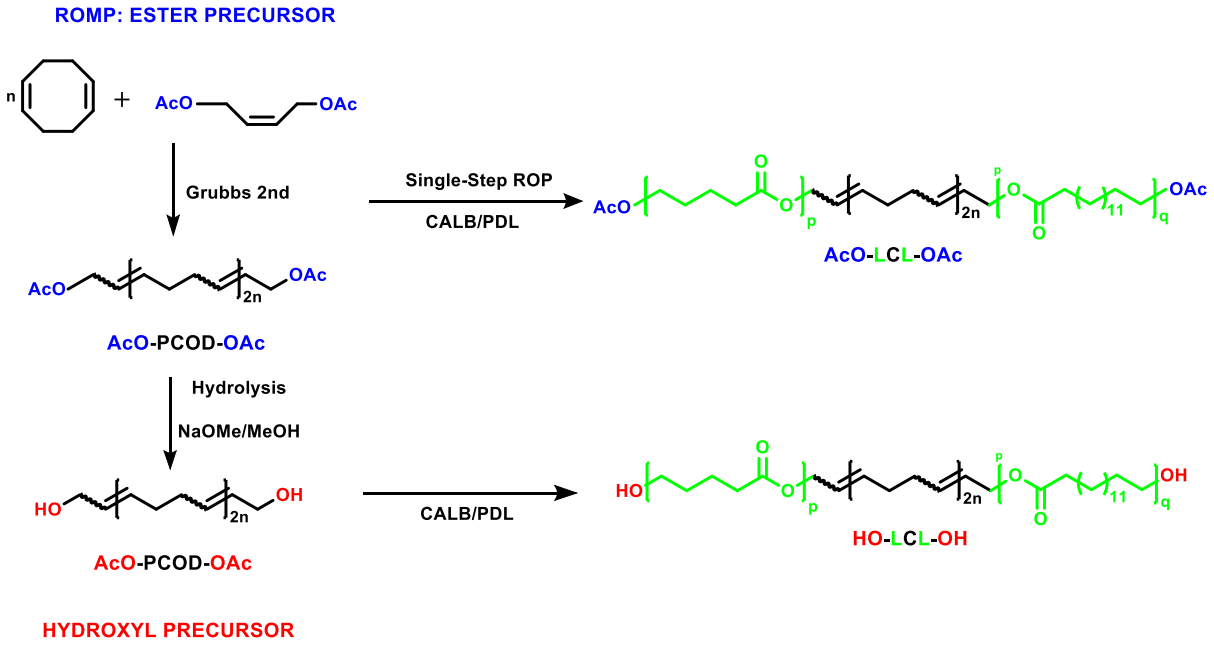
In the finding of novel materials with unique properties, "green" natural enzymatic catalysts have been used in combination with "chemical" synthetic organometallic catalysts, giving rise to chemoenzymatic methodologies based on multiple sequential or simultaneous reactions. These approaches represent sophisticate strategies for production of a wide range of polymeric

products with unique properties that cannot be obtained *via* either, enzymatic or chemical protocols alone.

On one hand, enzyme catalysis is of interest for imparting high reaction selectivity and high purity to the production of polymers and more specifically to polyesters. On the other hand, the use of synthetic catalysts provides advantages for commercial-scale production of polyesters. In order to combine these advantages, chemoenzymatic methods on which selective step(s) by enzymatic catalyst precede non-selective chemical step(s) have been developed for the synthesis on new polymeric materials difficult to prepare in other way. In this sense, enzymatic catalyst is mainly employed for modifying the monomers properties while chemical one polymerizes the functional groups enzymatically added to the monomers.

The first attempt in chemoenzymatic method combined lipase-catalyzed ring-opening polymerization of lactones (ROP) with atom transfer radical polymerization (ATRP) for the synthesis of block copolymers. A functional dual-headed initiator containing a primary alcohol and an activated bromide to initiate ROP of ε-CL, and ATRP of styrene, respectively, was prepared. Firstly, poly(ε-caprolactone) was obtained enzymatically bearing proper end groups to initiate ATRP of styrene in a consecutive step, obtaining poly(ε-caprolactone-*b*-styrene) copolymers.[78] Additionally, both polymerizations were proved to have limitations when integrated in one-pot type reaction due to the simultaneous initiation of both reaction types, and occurring an inhibiting effect of the ATRP catalyst over the enzymatic activity. The limitations observed in one-pot enzymatic ROP and ATRP were solved by employing nitroxide-mediated polymerization (NMP) instead of ATRP. Living free radical polymerization (LFRP) occurs in a specific temperature window (95-120 °C) allowing for enzymatic ROP to occur first at 60 °C, followed by LFRP without intermediate steps.[79] More recently, simultaneous multicomponent polymerizations (MCP) were carried out by several research groups in order to efficiently prepare functional polyesters with the desired functional-groups/configurations. Wu *et al*, performed the chemoenzymatic synthesis of unsaturated polyesters with stereoregular (R)- or

(S)-configuration. To that end, enzymatic dynamic kinetic resolution (DKR) using wild-type *Candida antarctica* lipase B (WT-CALB) was combined with hydrogenation of homoallylic alcohols and olefin metathesis to obtain enantiopure ω-substituted unsaturated hydroxyvalerate (R)-configurational. (S)-configuration was obtained by using engineered CALB mutant. Subsequently, chemical polycondensation gave rise to chiral polyester with either aromatic or aliphatic side chains.[80] More recently, Xiang *et al.*[81] combined enzymatic ROP with ring-opening metathesis polymerization (ROMP), a non-radical polymerization using Grubbs catalyst (ruthenium alkylidene). As represented in **Scheme 4**, either acetoxy or hydroxyl terminated poly(butadiene)s were prepared by ROMP and employed to initiate enzymatic ROP of PDL to construct polyester block copolymers.



Scheme 4. Synthesis of poly(butadiene) block copolymers by ROMP of 1,5-cyclooctadiene and subsequent enzymatic ROP of ω-pentadecalactone (PDL).

Summing up, the most common chemoenzymatic approach to synthesize polyesters is the combination of ROP of lactones with radical polymerization, i.e. ATRP,[78,82,83] NMP[84] and reversible addition-fragmentation chain transfer (RAFT).[85] Besides, the chemoenzymatic

synthesis, in which an enzymatic modification of a monomer is followed by conventional chemical polymerization, is the most prominent example found on the literature, and although some attempts have been made for the chemoenzymatic synthesis of polyesters, integrating all the steps in a one-pot fashion, this is only beginning to be exploited. Nevertheless, the combination of enzymatic polymerization processes with chemical polymerization ones would help, for sure, to enhance the availability of complex functional polyester polymers of commercial interest.

1.3. Polyesters Enzymatic Polymerization Parameters

The enzymatic polymerization is affected by several parameters such as the type of enzyme, its concentration, the monomer structure, the reaction temperature and time and, the absence or presence of solvent during the reaction. Main effects of these parameters over enzymatic synthesis of polyesters will be discussed in the following sections.

1.3.1. Enzyme Type and Concentration

For several decades, many authors have shown that the enzyme concentration in the reaction media is directly related to the polymerization rate, and therefore, it has a great impact on the molecular weight of the synthesized polymers. In this regards, different enzymes have been tested for polyester synthesis. In particular, Linko *et al.*[26] investigated the polymerization of a diacid derivative; bis (2,2,2-trifluoroethyl) sebacate was reacted with BDO in the presence of four different lipases, *Pseudomonas fluorescens* (PF), *Mucor miehei* (MM), *Candida rugose* (CR) and porcine pancreas lipase (PPL) at 37 °C under vacuum in the presence of solvent. Although the four enzymes produced polyesters with reasonable molecular weights (M_n > 12000 g mol^{-1}), the highest molecular weight polyester was obtained in the presence of MM. In this line, Uyama *et al.*[24] corroborated that even within the lipase family, enzymes demonstrated different catalytic activity. In particular, they investigated the polymerization of BDO and sebacic acid at 60 °C for 8 h in solvent-less condition using five different non-immobilized commercial lipases from different origin, CALB, PF, MM, *Pseudomonas cepacia* (Pc), and

PPL. Among the five enzymes studied, only CALB resulted in polyester formation under the conditions tested. Authors stated that polyester formation was observed even from the combination of solid monomers by the addition of a small amount of adjuvant (inert organic liquid). Thus, as mentioned before, many research results similar to these ones support the fact of CALB being the most employed and convenient enzymatic catalyst in polyester synthesis.

Apart from the enzyme choice, its concentration is crucial and in most of the cases decisive for high molecular weight polyesters synthesis. Generally, molecular weight tends to increase with increasing the concentration of enzyme, as many authors proved. For instance, Mahapatro *et al.* [86] observed a clear increase in M_n of polyesters obtained from 1,8-octanediol and adipic acid in bulk conditions with increasing immobilized CALB concentration. M_n raised from 5480 g mol^{-1} to 22600 g mol^{-1} with enzyme increasing amount from 0.1 to 10 wt%. However, higher concentration of enzyme not always results in higher molecular weight as demonstrated by Kanelli *et al.*[87] They studied the effect of 10 and 20 wt% of immobilized CALB over the polymerization reaction of 1,8-octanediol and 1,12-dodecanedioic acid. Although an improvement in the reaction yield was observed with higher amount of enzyme as catalyst, both reactions produced polymers with M_n differing only by about 500 g mol^{-1}; M_n reached a plateau for the reaction conditions studied despite of the enzyme concentration. Authors believe that this fact was due to a variation in catalytic activity of the enzyme as a function of substrate chain length. Thus, the reaction rate slows down as molecular weight of the formed polyester increases with the time, due to higher steric hindrance of the long chain macromolecules as well as the lower diffusion in the reaction media.

It is well known that lipase-regioselectivity allows the conversion of polymers based on multifunctional monomers (functionality ≥ 3), such as polyols, into linear or nearly linear polyesters. However, the regioselectivity starts to be compromised when high amounts of enzyme are present in the reaction media. For example, Perin *et al.*[88] obtained polymers with slightly higher amount of dendritic units during the polycondensation of equimolar amounts of

glycerol and sebacic acid, to reach poly(glycerol sebacate) (PGSe), in acetone at 40 ºC for 24 h by increasing CALB amount from ~7 to ~14 wt%. This effect was more marked when polyesters were obtained from sebacic acid/glycerol ratios differing from equimolarity (higher polyol content).

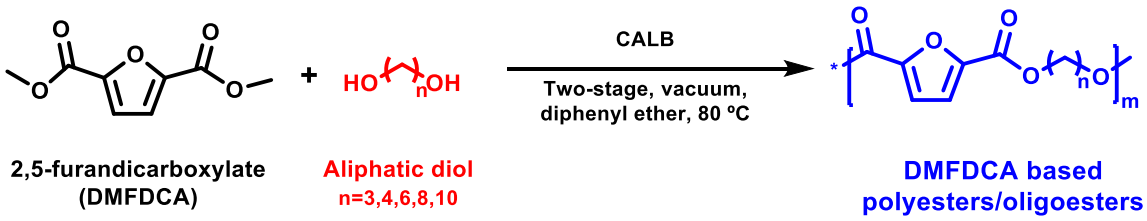
1.3.2. Monomers Chain Length and Ratio

Monomer Chain Length

An important parameter that affects the enzymatic synthesis of polyesters is the chain length of the monomers used. Since the mid-1990s, several researchers have studied how this parameter affects enzymatic polycondensation reactions to obtain polyesters. Linko *et al.*[26] polymerized *bis*(2,2,2-trifluoroethyl) sebacate with five different diols, whose aliphatic skeleton length varied from two to six carbon atoms (C2-C6), in the presence of MM lipase. Results showed that an increase in length of the diol carbon chain resulted in an increase in the M_w of the polyester. Thus, the highest molecular weight and degree of polymerization (DP) were obtained when 1,5-pentanediol was the diol (M_w ~40000 g mol^{-1}, DP 155) followed by HDO, with values not differing much from the ones obtained with its five carbon atoms counterpart. Other authors made similar observations and stated that, beyond the six carbon length of the diol, the effect over the M_w of the polyester was negligible in the polymerization of *bis*(2,2,2-trifluoroethyl) sebacate in 1,2-dimethoxybenzene with pancreatic lipase.[89] Years later, similar studies with diacids (C4-C12) and diols (C2-C6) in the presence of other enzymes, *Rhizomucor miehei* (RM lipase)[90] and immobilized CALB[91] obtained similar results. These studies confirmed that systems with long chain diacids such as sebacic and adipic acids and diols, such as 1,8-octanediol and 1,6-hexanediol, possessed greater reactivity than systems with shorter diacids and BDO. To this end, a series of polymerizations of the different diacids with the different diols (C4, C6 and C8) were carried out under vacuum in diphenyl ether and in bulk at 70 ºC in the presence of immobilized CALB and as a general trend data showed that M_w increased with the diol length. Regarding the effect of the diacid chain length, at short reaction times (4-8 h)

polymerization proceeded faster with the longest diacid (C10) relative to what happened with shorter ones. However, after 24 h the DP obtained in polyesters containing either sebacic or adipic acid (C10 and C6, respectively) was statistically equivalent. The same phenomenon was observed for polymerizations of 1,8-octanediol either with glutaric and succinic acid. In addition, Kanelli *et al.*[87] observed an increasing trend in the M_n after the enzymatic polymerization for 4 h of 1,8-octanediol with dicarboxylic acids of C4 and C10 lengths in diphenyl ether. In this particular case, a further increase of the diacid length from C10 to C14 decreased the M_n from 8020 to 5360 g mol^{-1}.

Recent publications have studied the enzymatic polymerization of aliphatic diols of various lengths with acid derivatives in order to obtain furanic-aliphatic polyesters as more sustainable alternative to aromatic-aliphatic ones. For this purpose, Jiang *et al.*[44] reacted DMFDCA with diols varying from 3 to 10 carbons length (C3, C4, C6, C8 and C10) in diphenyl ether using immobilized CALB (see **Scheme 5**).



Scheme 5. CALB catalyzed synthesis of furanic-aliphatic polyesters/oligoesters from 2,5-furandicarboxylate (DMFDCA) and aliphatic diols with different lengths.

The obtained M_n steady increased with the diol length from C4 to C8 from 1200 to 1700 g mol^{-1}. Further diol chain length (C10) significantly raised the obtained M_n to a value of 23700 g mol^{-1}. This change in the M_n trend from diol length up to C10 was due to the lower solubility of shorter diols in the reaction media that produced an early precipitation of the products. In contrast, Cruz-Izquierdo *et al.*[92] reported different results using the same acid derivative and

C2-C12 chain length diols, at 40 °C in a toluene:*tert*-butanol mixture (70-30 wt%). The longest oligoesters were obtained when HDO (C6) was the diol, while diols with C3, C4 and C8 length gave better results than C12 one. Thus, chain length and abundance of oligoesters increased as follows: C2<C12<C10<C3<C8<C4<C6. These significant variations in the results obtained in both studies may be due to differences in reaction temperature and solvent-system employed.

Debuissy *et al.*[93] immobilized-CALB enzymatically polymerized two diols, 1,3-propanediol (PDO) and BDO, with two other acid derivatives, DES and DEA to obtain homopolymers and their respective copolymers. In the case of homopolymers, they found that enzyme showed a preference for those containing BDO, a diol with more than 3 carbons, that leads to higher degrees of polymerization in comparison with PDO containing polyesters and in agreement with previously reported observations by Jiang *et al*.[44] No preference was observed between DES and DEA during the homopolyesters synthesis.[94] For copolymers including both, PDO and BDO, a decrease of M_n was observed with increasing the amount of PDO, corroborating the higher affinity of enzyme with C4 diol, whereas no clear preference was observed towards one or another acid derivative.

In a further study, Jiang *et al.*[95] apart from DES (C4) and DEA (C6), used other acids, diacid ethyl esters such as diethyl glutarate (DEG, C5), diethyl suberate (DESu, C8), diethyl sebacate (DESe, C10) and diethyl dodecanedioate (DED, C12) to CALB enzymatically synthesize poly(butylene dicarboxylates) in diphenyl ether. In contrast, their results showed a significant influence on the molecular weights of the obtained aliphatic polyesters. The lowest M_w was obtained in polymers containing succinate while, an increase in diacid ethyl ester chain length from C4 to C6, resulted in the highest M_w. However, further increase from C6 to C12 of the diacid derivative length lowered the obtained M_w. These observations are consistent with previously studies reported by Juais *et al.*[43] about the enzymatic synthesis of isosorbide based polyesters. Similarly, an increase in the diacid ethyl ester chain length from C2 to C4

significantly increased the M_w of the polyesters from 3000 to 21000 g mol^{-1}, while diacid derivatives lengths of C6 and C8 dramatically reduced the M_w to ~10000 g mol^{-1}.

Although enzymatic polymerization is affected by many other factors apart from enzyme specificity for the different monomers, with the presented background it is evident that CALB reacts at different rates as a function of the substrates chain lengths. As a general trend, diacids and its derivatives as well as diols of medium chain length are preferred for high activity.

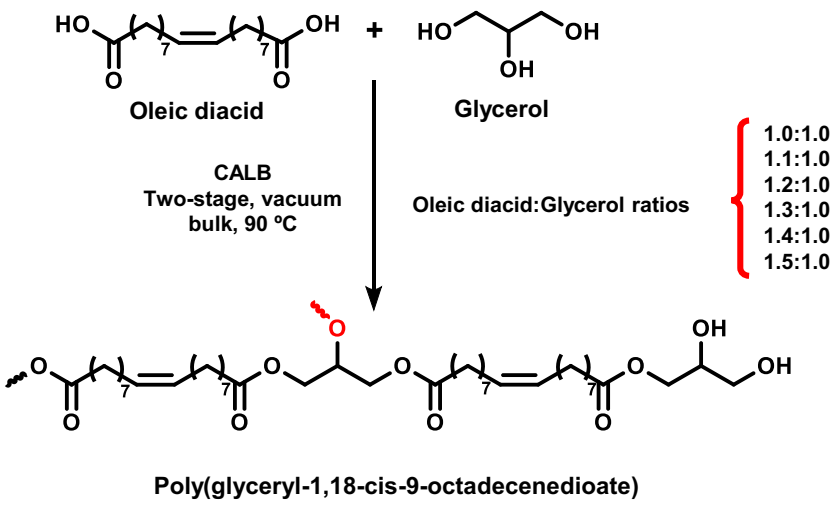
Ratio of Monomers

The ratio of monomers is another critical parameter in the enzymatic reactions for polyester formation. In enzymatic transesterification reactions involving the use of acid derivatives such as methyl and vinyl esters with alcohols, an increase in the ester concentration has been shown to increase the rate and conversion of transesterification.[96,97] Nevertheless, an increase in the alcohol concentration can cause reduced rates and conversions due to competitive inhibition by alcohol that can bind reversibly to the active enzyme site and avoid binding of the substrate ester,[98] especially when using CALB as a catalyst. For example, Yadav and Trivedi[96] studied the transesterification of *n*-octanol with vinyl acetate at 30 ºC using CALB, and varied the concentration of *n*-octanol while keeping the amount of vinyl acetate constant. An increase in the reaction rate when the *n*-octanol concentration augmented from 0.0075 to 0.03 mol was observed; however, beyond this *n*-octanol amount, the rate decreased probably due to a complex formation between *n*-octanol and the lipase.

As mentioned before, one of the main advantages of using enzymes as catalysts for polyester synthesis is their inherent regioselectivity that allows to extend monomers with functionalities higher than 2 (functionality ≥ 3) as potential ones for obtaining nearly linear structures. Thus, the initial ratio of monomers becomes critical when the enzymatic catalysis of polyesters involves a polyol instead of a diol, especially when linear structures bearing pendant functional groups are preferred. In this regard, Perin *et al.*[88] studied the synthesis of PGSe using different sebacic acid/glycerol ratios: 1.5:1.0, 1.2:1.0, 1.0:1.0, 1.0:1.2 and 1.2:1.5. Authors analyzed the

effect of monomers molar ratio over the structure (from linear to dendrimer-like), molecular weight, degree of branching, and end-group functionality of the obtained polyesters. Results showed that the higher the polyol feed was, the fewer the dendritic units in the resultant polyesters were and, therefore, more linear were the polymers. Contrary, as the diacid ratio increased the proportion of dendritic units increased and, as expected, the fraction of glyceridic end-groups decreased. Regarding the molecular weight, the highest was obtained for equimolar concentrations of diacid and polyol, while any deviation from this ratio leads to a decrease in M_w. Yang *et al.*[29] studied the enzymatic polymerization of glycerol and oleic acid using CALB with molar ratios varying as follows: 1.0:1.0, 1.1:1.0, 1.2:1.0, 1.3:1.0, 1.4:1.0 and 1.5:1.0 (see **Scheme 6**). They observed that, up to 6 h synthesis, products' M_n and PDI were similar for all the oleic acid:glycerol feed ratios studied (M_n~5000 g mol^{-1}, PDI ~3). For further reaction time (24 h), polyester obtained from equimolar monomer ratio reached M_n of 9100 g mol^{-1}, while the M_n increased much more slow for the rest of the polyesters (non-equimolar ratio) reaching values of about 6500 g mol^{-1} after 24 h, in agreement with Perin *et al.* observations.[88] Similarly, the higher the ratio of oleic diacid in the feed resulted in higher dendritic units and lower terminal glycerol units.

Lang *et al.*[99] immobilized-CALB enzymatically synthesized a series of poly(glycerol-1,8-octanediol-sebacate) (PGOS) copolymers in bulk, varying the molar ratios between glycerol and 1,8-octanediol as follows: 0.50:0.50; 0.33:0.66; 0.25:0.75 and 0.2:0.8. The highest M_n (9500 and 7300 g mol^{-1}) and the lowest PDI (8.3 and 7.8) were achieved when glycerol:1,8-octanediol molar ratios were 0.25:0.75 and 0.2:0.8, respectively.



Scheme 6. Enzymatic synthesis of poly(glyceryl-1,18-*cis*-9-octadecenedioate) with various oleic diacid:glycerol ratios

In the line of monomer feed effect over enzymatic copolymerization, Jiang *et al.*[40] studied the possibility of obtaining copolymers bearing unsaturated moieties from itaconate. To this intention, DES, dimethyl itaconate and BDO were copolymerized in the presence of immobilized CALB with dimethyl itaconate molar ratio varying from 0% to 30%. No polymers were obtained for higher concentrations. Interestingly, although a bit lower, the theoretical feed of itaconate was near to the experimental calculated one and the double bonds were well preserved during enzymatic synthesis. Additionally, increasing itaconate content up to 15 mol% decreased the M_n from 13300 to 8400 g mol^{-1}, probably due to a low enzyme activity towards dimethyl itaconate compared to DES, limiting the itaconate incorporation into the polymeric growing chain. In the same study, the reactivity of itaconic acid and derivatives (diethyl itaconate and dibutyl itaconate) was tested, also at different molar ratios. In all these cases, the calculated percentage of itaconate introduced into the obtained polyesters was lower than the theoretical one.

Interestingly, 2,3-butanediol (2,3-BDO), a monomer bearing only secondary hydroxyl functionalities, was copolymerized using CALB with DES and BDO at different molar

ratios.[94] Similarly to the results obtained with previous mentioned itaconate polymerizations, the experimentally calculated 2,3-BDO contents were only slightly lower than the theoretical feed ones. Also, increasing the content of this monomer in the feed, lowered the molecular weight of the copolymers: M_n values ranged from 13500 g mol^{-1} when only BDO was in the feed, to 4300 g mol^{-1} when 2,3-BDO constituted the 100 mol% of the diol feed. Additionally, the polydispersity was found to decrease as a function of the increasing molar percentage of 2,3-BDO. Thus, results showed the lower reactivity of 2,3-BDO with respect to BDO and highlighted the preference of CALB towards diols with longer chain lengths as well as primary hydroxyl functions.

Variation on the monomers feed ratio in polyester enzymatic synthesis is a clear strategy towards the control of molecular weight, end group functionality as well as degree of branching when monomers with functionality higher than two are considered. As deduced from the analyzed literature above, enzymatic polyesters obtained by the reaction of two monomers are commonly carried out in equimolar molar ratios in order to obtain the highest possible polymer molecular weight. On the other hand, studies related to the synthesis of polyesters involving the reaction of three monomers, deepen much more into the understanding of the varying ratio of monomers effect over the polymer properties.

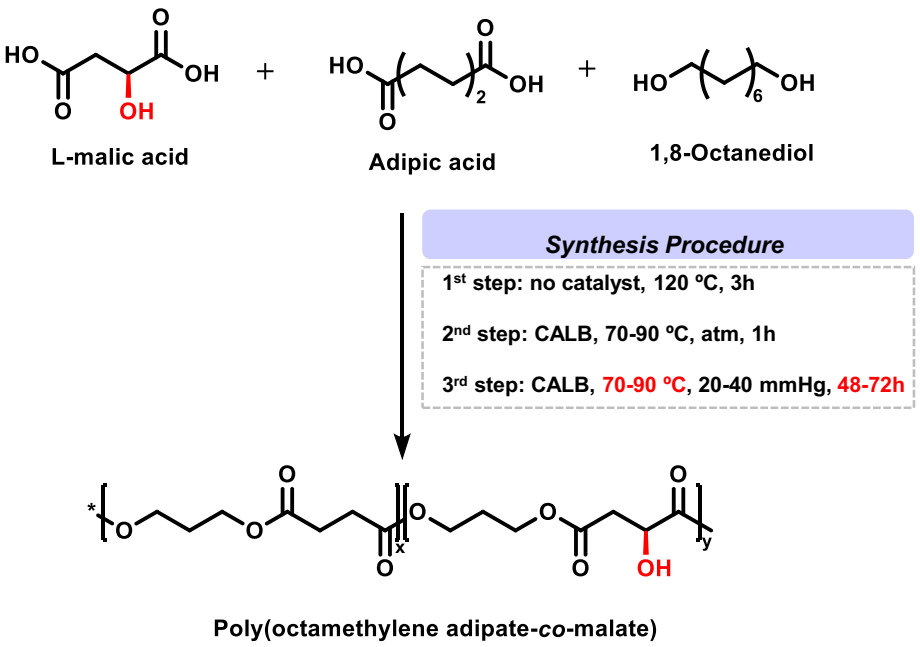
1.3.3. Temperature and Time

Temperature and reaction time are both synthetic parameters that greatly affect enzyme reactivity (catalytic activity, dimensional structure). In view of the literature, by increasing the reaction temperature a greater growth of polymer chains occurs and, therefore, higher molecular weights are achieved. However, especial care has to be taken particularly at high temperatures as the enzyme activity and thermal stability can be compromised.[24,27,42,100]

Azim *et al.*[42] studied different temperatures (60, 70, 80 and 90 ºC) during the synthesis of PBS with CALB in diphenyl ether in a two stage-process. Authors observed that for this specific system, at 80 ºC precipitation of PBS occurred at 5-10 h of reaction, limiting the growth of

polymer chains. In order to keep the polymer soluble in the reaction mixture, temperature was raised to 95 ºC in a second stage after 21 h of reaction time. An increase in molecular weight up to M_n= 27400 g mol^{-1} was observed from the M_n ~8000-10000 g mol^{-1} obtained after 21 h at 80 ºC. Regarding the reaction time, extending the reaction from 24 to 72 h, did not produce a significant increase in M_n; nevertheless, PBS with exceptionally low PDI was produced by diffusion and reaction between low-molecular weight oligomers seemed to be favored.

During the solvent free enzymatic synthesis of functional polyesters bearing pendant hydroxyl groups from L-malic acid, adipic acid and 1,8-octanediol (see **Scheme 7**), Li *et al.*[101] observed that rising the reaction temperature from 70 to 80 ºC, M_w increased from 4700 to 7400 g mol^{-1}.



Scheme 7. Enzymatic synthesis of functional polyesters containing pendant hydroxyl groups varying temperature and reaction time.

However, further increase from 80 to 90 ºC, produced a drop of M_w to 6500 g mol^{-1}. Extending the reaction time from 48 to 78 h while holding the reaction at 75 ºC, increased the M_w from 5200 to 6600 g mol^{-1}, while longer times than 48 h for higher temperatures tested, 80-85 ºC, did not produced significant growth in the obtained M_w.

Similar behavior was also found by Moreno *et al.*[102] during the synthesis of polyketoesters derived from hydroxyesters, in which the highest molecular weight values were obtained at 80 ºC, and by increasing temperature to 90 ºC, a decrease was observed. Authors explained this fact due to a protein denaturation causing the loss of enzymatic activity at 90 ºC. On the other hand, Wu et *al.*[103] during the immobilized-CALB enzymatic polycondensation of methyl 3-((11-hydroxyundecyl)thio) propanoate (MHUTP) derived from castor oil in diphenyl ether at different temperatures (50, 70 and 90 ºC) and times (2-72 h) observed that the highest M_n values were obtained for the highest temperature and time (90 ºC and 72 h). Thus, the polymer growth at 50 ºC was observed to significantly slow down, in comparison with the growth at 70 and 90 ºC, probably due to difficulties in elimination of the methanol by-product together with lower enzyme activity at this temperature.

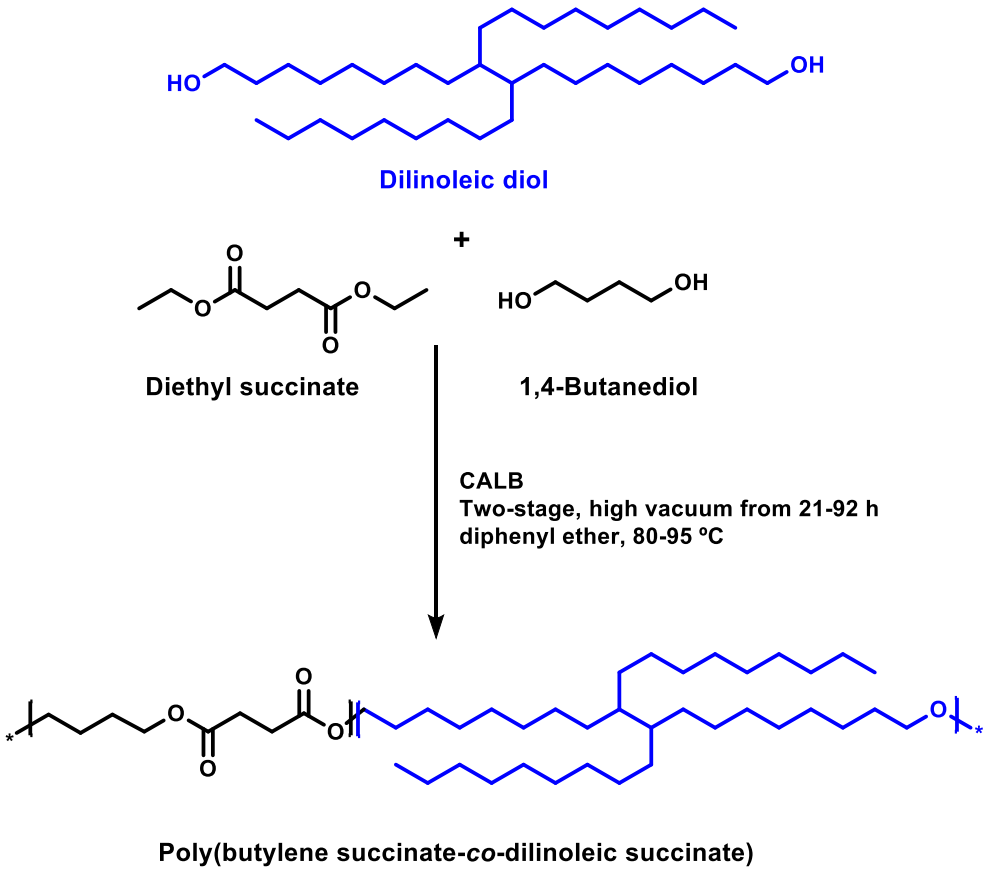
Mahapatro *et al.*[86] enzymatically polymerized in bulk 1,8-octanediol and adipic acid in the presence of immobilized CALB, varying the reaction temperature from 65 to 90 ºC. For times up to 24 h, no significant effect of temperature over M_n was observed. However, after 48 h the achieved M_n at 90 ºC, the highest temperature tested, was 24700 g mol^{-1}, significantly higher than the M_n obtained at 60 ºC (18800 g mol^{-1}). Intermediate temperatures from 70-90 ºC produced small or not changes at all in the M_n of the obtained polymers, in agreement with previous observations made in the same laboratory during enzymatically CALB mediated ring opening polymerization of ε-caprolactone, in which little variation over monomer conversion was observed along an even broader temperature range (from 20 to 180 ºC).[104] In the analysis of the enzymatic catalyst activity at 4, 24 and 48 h, the authors observed that after 48 h of reaction at 70 ºC the catalyst retained almost 80% of the catalytic activity, indicating the possibility of reusing it for one more catalytic cycle. About the temperature behaviour, likewise, Uyama *et al.*[24] observed invariability of M_n between 60 and 70 ºC during the polymerization of sebacic acid and BDO with CALB, while an increase in M_n occurred when the temperature

was raised from 50 to 60 ºC. Thus, at some temperature intervals it seems that enzymatic polymerization proceeds almost independently to the reaction temperature.

More recently, Beyazkilic *et al.*[105] was studied the homopolymerization of vinylsulfide-containing hydroxyacid (VHSA) from 10-undecenoic fatty acid, and ketone-containing hydroxyester (KHE) from methyl oleate in diphenyl ether with immobilized CALB at 80 and 90 ºC. Additionally, copolymers containing 1:1 molar ratio of these comonomers were enzymatically obtained in bulk and in diphenyl ether at 80 and 90 ºC. For all the polymers, molecular weight increased as a function of time (until 48 h), and in the case of KHE and VHSA:KHE copolyester, the increase in temperature did not result in a significant difference in the obtained M_w at any of the reaction times tested. However, increasing the temperature in the case of VHSA resulted in a greater difference in the M_w for longer reaction times, achieving the highest M_w at 90 ºC after 48 h of reaction.

Jiang *et al.*[106] studied the immobilized-CALB catalyzed polymerization of 2,5-bis(hydroxymethyl)furan (BHMF) with various diacid ethyl esters in a three-stage method *via* stepwise application of high vacuum. In the third stage, high vacuum (2 mmHg) was applied after 6 h of reaction time at 80 ºC. Increasing reaction time from 6 to 31 h, enhanced significantly the M_n of the products from 300 g/mol to 1200-1400 g mol^{-1}, reaching a plateau value at around 1300-1500 g mol^{-1} after that. Sonseca *et al.*[41] synthesized a series of poly(butylene succinate-*co*-dilinoleic succinate) copolyesters via one-pot enzymatic synthesis procedure with stepwise decrease of vacuum until 2 Torr (see **Scheme 8**). During the high vacuum step (from 21 to 92 h of reaction time), the highest increase of M_n was occurred for all the polymers between 21 and 48 h. After that time, the M_n of the copolyester containing the lowest amount of long chain dilinoleic diol reached a plateau at around M_n~10000 g mol^{-1}, while for the rest of the formulations with increasing dilinoleic diol content, M_n continued increasing until 72-92 h reaching values of 18400 and 32000 g mol^{-1}, respectively. Thus, in the formulations with the highest content of long aliphatic chain diol, longer reaction times

favoured its incorporation into the main formed polymer chains contributing to the continuous increase in M_n.



Scheme 8. Enzymatic synthesis of poly(butylene succinate) containing fully bio-based dilinoleic diol at different molar amounts.

In general, unless denaturation of the protein or lower affinity with the substrates, higher reaction times and temperatures help to increase the molecular weight of the products during enzymatic synthesis. Although clear differences exist between studies found in the literature, they are mainly due to other different reaction conditions such as amount of catalyst or solvent-system used or the absence of it, as well as the structural differences of the monomers employed. All of them are variables that have to be taken into account together with the reaction temperature and time as they are also known to greatly affect the polymer chain growth evolution.

Apart from the influence of reaction temperature and time over molecular weight of the obtained polyesters, other authors have also studied the relationship of these reaction parameters with the end-groups of the polymer chain and the dendritic units when monomers with functionality ≥ 3 are involved. Yang *et al.*[29] studied the immobilized CALB polymerization of oleic diacid with glycerol in an equimolar ratio at 90 ºC in bulk. Aliquots were withdrawn at different reaction times (2, 4, 6, 8, 10 and 24 h) showing that after 24 h of reaction time, glycerol terminal groups decreased from 46 to 27%, while not dendritic units were observed during the first 4 hours of reaction. Debuissy *et al.*[94] studied the enzymatic synthesis of poly(1,4-butylene succinate-*ran*-2,3-butylene succinate) copolyesters with a 50/50 BDO/2,3-butanediol (2,3-BDO) molar ratio at different temperatures (50-90 ºC). The overall trend observed for the molar mass evolution was: the higher the temperature, the higher the molecular weight; however this effect was less visible between 60 and 80 ºC. Additionally, no significant effect over end-groups proportion was observed with increasing temperature although, mostly ester end-groups (75-87%) were present as terminal groups in the majority of the polymers. Interestingly, hydroxyl end-groups proportion were mainly due to 2,3-BDO confirming the lower reactivity of CALB towards secondary OH groups.

Taresco *et al.*[107] studied the synthesis of poly(glycerol adipate) from glycerol and divinyl adipate at different times and temperatures using immobilized CALB. The average molecular weight of the polyester was observed to decrease from 11400 to 5200 g mol^{-1} when the polymerization temperature increased from 40 to 70 ºC respectively, while the polydispersity values increased from 2.7 to 4.2. Authors consider this effect as a result of several phenomena combination such as the loss of catalyst selectivity for the primary hydroxyl groups with the temperature, what led to polymers with increasing branching degree (from 5 up to ~30%) and, therefore, a lower apparent molecular weight than their linear counterparts.

1.3.4. Solvent

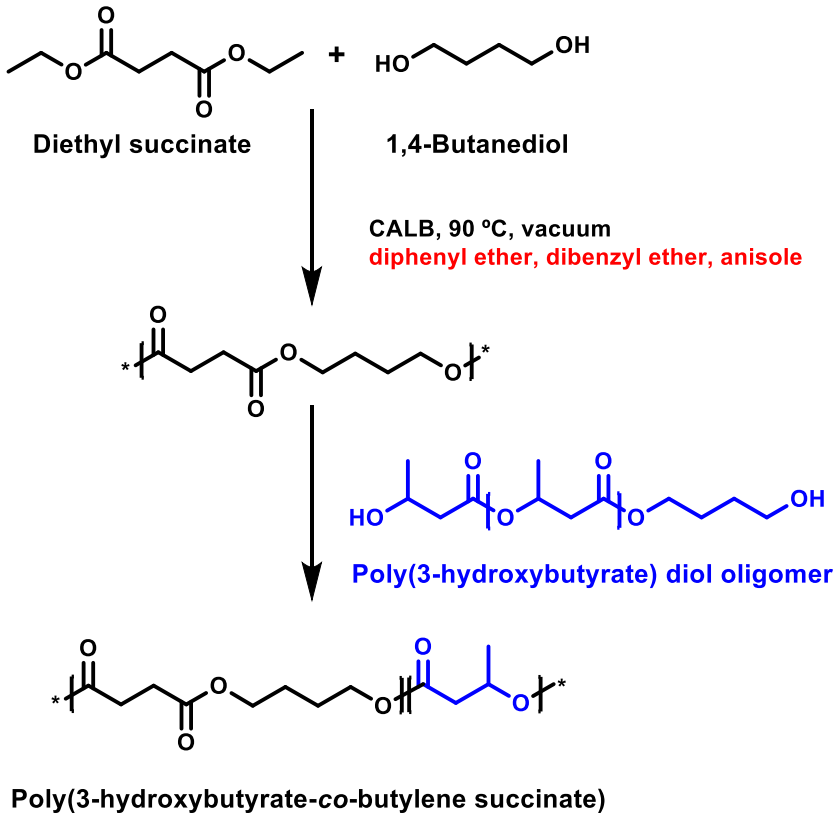
The enzymatic polymerization of polyesters can be typically carried out in solvent-based or solvent-free systems. In solvent-free systems, the enzyme is dispersed within the reagents themselves in the absence of solvent, leading to efficient polymerization,[108] but making mass transfer difficult. For this reason, in most of the studies, higher molecular weights are achieved in the presence of solvent. In solvent-based systems, the physical properties of the solvent such as polarity, water solubility, dielectric constant and log *P* (*P* = octanol/water partition coefficient) among others, must be taken into account, since they may or may not favor the polymerization reaction. The polarity of the solvent can either cause the deactivation of the enzyme producing conformational changes or facilitate the release of water bound to the enzyme, which, in turn, can be used in hydrolysis reactions, impairing the polymerization reaction. Therefore, in enzymatic syntheses, solvents with a low polarity are usually preferred (high log *P* value, between 1.9 and 4.5).[69,101,109,110] Additionally, high enzyme-solvent affinity is required to have the optimal 3D shape of the binding site in order to be accessible to reactive groups.[94] It is also important to take into account other solvent properties such as its reactivity under the selected reaction conditions and its boiling point. Thus, the solvent must be chemically inert to avoid secondary reactions that interfere with the polymerization, and it must have a boiling point that allows it to remain within the reaction medium.[69]

One of the first investigations concerning the effect of solvent-system in enzymatic synthesis was carried out by Linko *et al.*[111] Their work studied the polymerization of sebacic acid or its derivatives (*bis*(2,2,2-trifluoroethyl)sebacate and DESe) with BDO to synthesize poly(1,4-butylene sebacate) using MM lipase in various solvents with relatively high boiling points, such as diphenyl ether and 1,2-dimethoxybenzene (veratrole). Diphenyl ether resulted a better solvent when underivatized sebacic acid and *bis*(2,2,2-trifluoroethyl)sebacate were the substrates. Another study carried out from the same authors, tested other solvent systems apart from veratrole and diphenyl ether, such as dodecane, hexyl ether, isoamyl ether and trietylene

glycol dimethyl ether. Diphenyl ether continued giving the best effectiveness among all the solvents.[111]

Recently,[94] various solvent systems have been studied for the synthesis of PBS at 90 ºC from BDO and DES using immobilized CALB at different pressures. As expected, regardless of the solved employed, lowering the pressure during the synthesis helped to increase the M_n values of the polymers. From all the solvents tested, only three of them led to relatively high M_n products (>10000 g mol^{-1}): phenetole, diphenyl ether and anisole, being anisole the solvent that produced the highest molecular weight (13800 g mol^{-1}), followed by diphenyl ether (12200 g mol^{-1}) and phenetole (10900 g mol^{-1}). Regarding the other solvents studied, n-dibutyl ether and xylene led to the precipitation of PBS after 24 h, while CALB showed low affinity to dodecane, being too hydrophobic (log *P* = 6.1), and affecting the 3D structure of the enzyme, producing a low degree of reaction.

Something similar happened in the study carried out by Debuissy *et al.*[112] in which different solvents for the synthesis of copolymer poly(3-hydroxybutyrate-*co*-butylene succinate) were tested (see **Scheme 9**). Authors analyzed the evolution of the molecular weight (M_n) of the copolyesters under reduced pressure in diphenyl ether, dibenzyl ether and anisole. Diphenyl ether produced the highest molecular weights, while anisole did so when the chain of poly(3-hydroxybutyrate)-diol oligomers was longer.



Scheme 9. Enzymatic synthesis of poly(3-hydroxybutyrate-*co*-butylene succinate) by a two-step process in different solvent systems.

Mahapatro *et al.*[91] examined four solvents (diphenyl ether, xylene, tetraethylene glycol dimethyl ether and 2-methoxyethyl ether) for the enzymatic reaction of 1,8-octanediol and adipic acid, and as in the studies discussed before, diphenyl ether gave the product with the highest molar mass.

Mixed solvent systems have been also studied for enzymatic polyester production. For example, Juais *et al.*[43] examined the synthesis of aliphatic isosorbide polyesters in bulk and in a 6:1 mixture of cyclohexane:benzene obtaining a M_w of 3800 g mol^{-1} and 40000 g mol^{-1}, respectively. Solvent dosage effect over enzymatic polymerization reaction was also studied.

Jiang *et al.*[40] investigated the effect of diphenyl ether dosage on the reaction yield and M_n during the enzymatic production of poly(butylene succinate-*co*-itaconate) (PBSI). To that end, the amount of diphenyl ether was varied between 50 wt% and 400 wt% and authors observed

that the M_n increased from 3852 to 4554 g mol^{-1} when solvent amount was increased from 50 wt% to 150 wt%. Further increase of the solvent dose from 150% to 400% significantly lowered the M_n presumably due to an increase in the amount of by-products (water and residual alcohol) together with a decrease in the polymerization rate in a very dilute reaction.

As deduced from the analyzed literature, commonly a decrease in the activity of the biocatalyst occurs when the log *P* of the solvent decreases.[113] This is due to the interaction of solvent molecules with the catalytic site of enzyme distorting its structure or extracting the essential water molecules that stabilize it. However, during the enzymatic polymerization of glycerol and sebacic acid, Perin *et al.*[88] observed that a decrease in the activity of the biocatalyst was not accompanied with a decrease in log *P* of the solvents used: tetrahydrofuran, *tert*-butanol, acetone and acetonitrile. They obtained an increase in the molecular weight following the solvent order: acetone > tetrahydrofuran > acetonitrile > *tert*-butanol, whose log *P* values are: -0.23, 0.49, -0.33 and 0.35, respectively. By using acetone as a solvent at 40 ºC not only higher M_n (9400 g mol^{-1}) was achieved but also higher degree of branching (41 %), while the reactions performed in tetrahydrofuran at 40 ºC resulted in lower M_n and branching degree, 3200 g mol^{-1} and 38%, respectively. By comparing the results with other authors, Perin *et al*. concluded that the effect of the solvent over the enzyme activity and structure was probably not the main factor behind the different polymer molecular weights obtained. They attributed this phenomenon to solvent-polymer interactions that can favor/impede the availability of reactive groups during polymer chain growth. Nevertheless, the study demonstrates the feasibility of using low hazard solvent system, such as acetone, and mild reaction conditions to prepare polyesters.

Ionic liquids (IL) are arising as a "green" alternative solvents for enzymatic polymerizations.[114–116] Enzymes have shown exceptional thermal and operational stability in anhydrous ILs due to their conformational stiffness in the dehydrated state. CALB, both, in its free and immobilized forms, has exhibited significantly greater stability in hydrophobic ILs

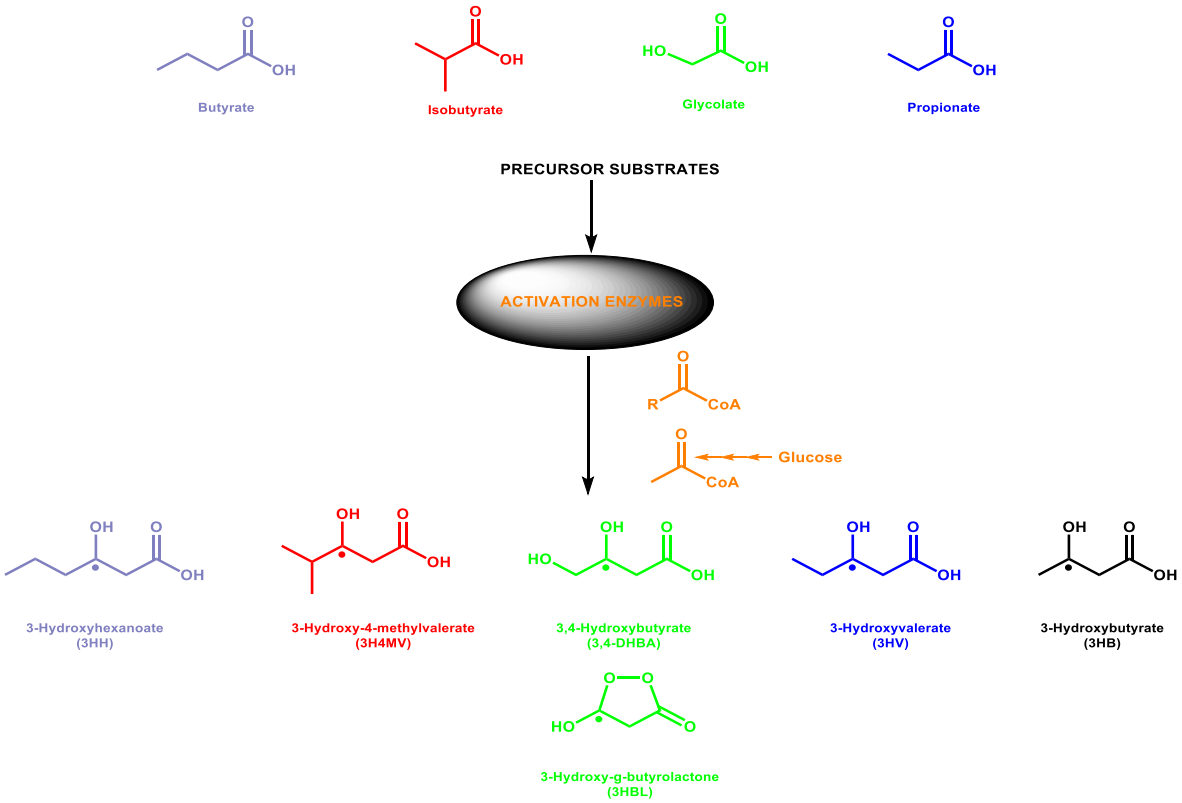
compared to common organic solvents used in polymerization reactions.[12,117–123] However, not all ILs have this effect, for example ILs with anions, tetrafluoroborate [BF_4], hexafluorophosphate [PF_6], and bis(trifluoromethane)sulfonimide [NTf_2], were reported to cause partial inactivation of the enzyme.[119,124–126] Gorke *et al.*[123] reported the enzymatic polymerization of ε-caprolactone in the presence of immobilized CALB, in three different ILs ([C_4mim][BF_4], [C_4mim][PF_6] and [C_4mim][NTf_2], being [C_4mim] 1-butyl-3-methylimidazolium). After 24 h of reaction, the highest M_n (12700 g mol^{-1}) was obtained when the polymerization proceeded in [C_4mim][BF_4]. Yoshizawa-Fujita *et al.*[127] investigated the enzymatic polymerization of L-lactide in the same four ILs as Gorke *et al.* analyzing the conversion, molecular weight, and yield of the poly(L-lactide) (PLLA) obtained and comparing the results with the ones from reactions performed in toluene and in bulk. Their results suggested that the anion species of ILs had significant effect over conversion, molecular weight and yield. Interestingly, molecular weights of PLLAs, mainly from [C_4mim][BF_4] and [C_4mim][NTf_2] were higher than those obtained in bulk and in toluene, whereas the yields were lower than those of bulk polymerization. The low yield of the polymers was attributed to the high solubility of PLLAs in ILs, what complicates the extraction process to separate the obtained polymer from the solvent.

From the reviewed literature it is evident that the choice of solvent is indeed an important factor for the enzymatic syntheses of polyesters. Therefore, there is an increasing amount of studies that evaluate the molar mass, yields and more recently the transesterification rates of enzymatic synthesis in different solvent systems.

2. Enzymatic polyester synthesis

2.1. Saturated Polyesters

Polyhydroxyalkanoates (PHAs) are one of the most important families of biodegradable and biocompatible polyesters, which are mainly composed of 3-hydroxyacids. Martin *et al.*[128] developed a pathway to enzymatically obtain 3-hydroxyacids, including 3,4-dihydroxybutyric acid (3,4-DHBA), 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV). The carbon sources used in the system were glucose and one precursor substrate, which give rise to the corresponding 3-hydroxyalkonoic acid products. . Feeding of butyrate, isobutyrate and glycolate lead to the production of 3-hydroxyhexanoate, 3-hydroxy-4-methylvalerate and 3,4-DHBA+3-hydroxy-γ-butyrolactone, respectively (see **Scheme 10**).



Scheme 10. Schematic representation of the 3-hydroxyacid synthetic pathway.

The main drawback of these biological syntheses is that PHA-synthase needs an expensive coenzyme-A functionalized precursor.[129–131] The advantage in comparison with lipase catalyzed polymerizations is the high molecular weight reached.[132] Nowadays, the main studies are focused on the development on engineering bacteria for enhanced PHA biosynthesis and diversity.[133] High polymer yield, more than 25% (g/g) on the synthesis of medium-long chain PHA homopolymer of 3-hydroxydodecanoic acids, with M_w of 316000 g mol^{-1} and copolymer made of 3-hydroxy octanoic, decanoic, dodecanoic and tetradecanoic acids with a M_w of 128000 g mol^{-1} were obtained by engineered strains of *Yarrowia lipolytica*.[134]

In addition to the great family of PHAs, another distinguished biobased polymer is poly(lactic acid) (PLA). In spite of the tremendous success, there are few works of enzymatic synthesis of PLA since it was successfully described by Matsumura group[135] in 1997 using lipase from *Candida cylindracea*, porcine pancreatic lipase and *Pseudomonas cepacia* lipase PS. This is mainly because its low yields in spite of their high weight average molecular weight (M_w) ranging from 8000 to 270000 g mol^{-1}. *Candida antarctica* Lipase B (CALB) was not effective until Moeller group[136] used mild reaction conditions (50-70 °C) in toluene solution to obtain poly(D-lactic acid) (PDLA). In this case, enzyme deactivation occurs by the water removal from the enzyme. However, by choosing the right conditions PDLA with M_n of 12000 g mol^{-1} and polydispersity index (PDI) of 1.1 was obtained. Modifications/mutations of CALB were also made to improve the propagation rate, the yield as well as the M_n on the D,D-lactide ring open polymerization (ROP).[137] Copolymerization was also used to obtain lactate-bearing copolyesters via CALB enzymatic reaction[76] with L-lactide (LLA), diesters (DEA or DED), and HDO or DBO. The yields obtained were good between 75-80% and the copolyesters produced M_w around 20000 g mol^{-1} with PDIs near to 2. In other studies, the immobilization of CALB in clay was compared with that in acrylic resin in the polymerization of lactide.[138] With the latter enzymatic system, PDLA with a M_n of 2600 g mol^{-1} was produced, whereas the clay-immobilized and free forms of CALB exhibited slower kinetics and produced oligomers.

Moreover, random copolymers with ε-caprolactone (ε-CL) with D-lactide in presence of clay-immobilized CALB were also successfully obtained giving rise to hybrid materials.

Recently, L-lactide and D,L-lactide were also successfully copolymerized with DEA, HDO and poly(ethylene glycol) methyl ether (MeO-PEG5K-OH), dimethyl.[139] In this case, the obtained polymers reached high M_w values from 15800 to 30000 g mol^{-1} with low PDIs ~1.8. These amphiphilic block copolymers can self-assemble to form micelles in water with sizes between 110-170 nm.

The other main polyester, polycaprolactone (PCL), is difficult to obtain by enzymatic ROP because its slow rate of polymerization.[140] CALB-catalyzed copolymerization was used to obtain polyesters based on ε-CL and β-lactam[141] with alternating or random microstructure distribution. The yields were near to 50% and with low M_w from 1773 to 5242 g mol^{-1} and PDI around 3. This lipase was also used in the copolymerization of ε-CL with ε-thiocaprolactone (ε-TCL).[142] The incorporation of ε-TCL produced a decrease on the M_n and on the thermal properties. In this work, the copolymers presented domains with block structure and with two different melting temperatures when using two-steps strategy and PCL oligomers of 8000 g mol^{-1}.

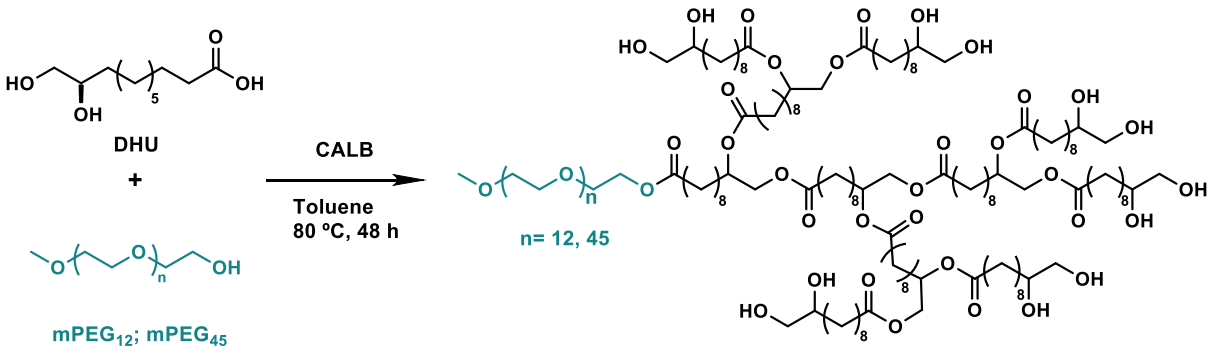
Wu's group developed CALB-catalyzed Baeyer–Villiger oxidation (BVO)-ROP tandem process for the preparation of substituted polyesters starting from obtained ketones.[143] In this case, the reactions have low yields with high M_w polyesters, while high yields up to 60% were obtained for low M_w polymers.[142] They used the cooperation between CALB and alkaline protease from *Bacillus subtilis* (BSP) in the copolymerization of bulky ibuprofen-containing hydroxyacid methyl ester (HAEP) and ε-CL. [144] The M_n varies from 3130 g mol^{-1} when catalyzed by CALB, 720 g mol^{-1} by BPS up to 9200 g mol^{-1} when two enzyme feeding sequence was used. This group also used the chemo-enzymatic pathway to synthesize chiral polyesters from customize (R)- and (S)-lactones, such as ω-substituted-δ-valerolactone [145] or 4-substituted-ε-CL.[146] More recently, they applied the substrate engineering strategy, i.e.,

modifying D-aspartic acid diester structure to hide the chiral centers and, thus chiral polyesters of D-aspartic acid with M_n up to 39500 g mol^{-1} were successfully prepared.[147]

Aliphatic polyesters were also successfully produced via CALB-catalyzed polycondensation of DBO and various diacid ethyl esters, using a two-stage method in diphenyl ether.[95] The synthetic aliphatic polyesters reached high M_w values up to 94000 g mol^{-1}. The studies on the effect of diacid ethyl esters revealed that CALB prefers diacid ethyl esters having a chain length of more than 2 (n >2, n is the number of methylene groups between the two carbonyl groups); and CALB showed the highest specificity for DEA among all the tested diacid ethyl esters (n =2-10).

Recently, Muñoz-Guerra's group [148] obtained poly(ethylene succinate) (PES) by enzymatic ring opening polymerization of cyclic oligo(ethylene succinate)s, which were prepared by CALB-catalyzed cyclocondensation of dimethyl succinate (DMS) and ethylene glycol. Polyesters presented high M_w above 60000 g mol^{-1}. This cyclic formation was also used for the synthesis of high molecular weight copolyesters containing butylene succinate (BS), ε-hydroxycaproate or L-lactate units with a random distribution. They also prepared cyclic oligomers of hexamethylene furanoate and hexamethylene terephthalate using CALB and further used for ROP enzymatically catalyzed synthesis of aromatic-aliphatic polyesters.[149] This group also prepared poly(BS-*ran*-ε-CL) copolyesters by CALB-enzymatic ROP.[150] Depending on the composition, the copolymers M_w range from 4100 to 13500 g mol^{-1}, being lower values than the corresponding homopolymers. Moreover, these copolyesters were able to crystallize in the entire composition range and displayed a pseudo-eutectic region.[148,150–152] They also utilized CALB to obtain copolyesters containing ammonium groups by reaction of DMS, L-glutamic acid dimethyl ester hydrochloride (Ga) and 1,4-BD.[153] The incorporation of Ga in the PBS decreases the M_n from 25500 to 4500 g mol^{-1} when the ratio DMS/Ga turns from 100/0 to 50/50.

A variety of copolyesters with PDL has been also synthesized. Copolyesters of ethyl glycolate and PDL by combination of ROP and polycondensation reactions using CALB as enzymatic catalyst were synthesized.[154] These copolymers presented high M_w around 20000 g mol^{-1} with tendency to the alternating microstructure and high crystallinity. Enzymatic copolymerization of PDL with DBO and dimethyl 3,3′-dithiodipropionate (DMD) was also described.[77] These copolymers possessed M_w values in the range between 18000 and 23000 g mol^{-1}, and PDIs between 1.8 and 1.9. In this work, it was pointed out that lipase is highly tolerant of DMD disulfide functional groups. PDL was also CALB-enzymatically block copolymerized with a block copolymer of metoxy-PEG and δ-decalactone (DL) to obtain triblock copolymers of metoxy-PEG-*b*-PDL-*b*-PPDL.[155] Metoxy-PEG with different lengths were also used to enzymatically synthesize block copolymers with 10,11-dihydroxy undecanoic acid (DHU) via polycondensation.[156] The resulting amphiphilic polymers were hyperbranched (see **Scheme 11**) and with tendency to aggregate in micellar structures with sizes between 150 and 300 nm.



Scheme 11: Synthesis of amphiphilic polymers by copolymerization of DHU in presence of methoxy-PEG with different lengths. Representative of mPEG-*b*-PDHU structure.

Moreover, PDL in the presence of triethylene glycol monomethyl ether was enzymatically grew from poly(4-benzyl formate piperidine lactone) (PNPIL) to obtain block copolymers.[157] These copolymers were acidic hydrolyzed to obtain cationic polymers for biomedical applications as

antimicrobial systems as described below in the section 4 of this review. The copolymerization rendered mainly homopolymer of PDL, only a small amount is copolymerized, from an initial 50/50 ratio of PNPIL/PDL only 3/48 is the final polymer with a M_n around 12000 g mol^{-1} and a PDI of 1.3.

Avérous and col.[93] also analyzed the effect of the comonomer length on the CALB enzymatic synthesis of poly(propylene succinate) (PPS), PBS, poly(propylene adipate) (PPA) and poly(butylene adipate) (PBA) as well as poly(BS-*ran*-BA) and poly(PA-*ran*-BS) copolyesters. The incorporation of PDO instead of BDO produced a diminishment on M_w and crystallinity. Poly(BS-*ran*-BA) presented also isodimorphic co-crystallization behavior, exhibiting pseudo-eutectic region. They also studied the effect on CALB enzymatic transesterification by the ratio between 1,4-BD and 2,3-BD in the random copolymerization with DES.[94] The copolyesters were obtained with high yields and their M_w decreased with increasing 2,3-BD content from 26200 to 5800 g mol^{-1}. All copolyesters showed an excellent thermal stability with initial degradation temperatures above 250 °C. They analyzed the effect of transesterification also in the random copolymerization of poly(3HB-*co*-BS).[112] The M_w values varied from 10800 to 34000 g mol^{-1} and PDI data from 1.67 to 2.81.

Gross's group[29] synthesized poly(oleic diacid-*co*-glycerol) copolymers with CALB and the results were compared with those obtained with dibutyl tin oxide (DBTO) catalyst. Oleic diacid (1,18-cis-9-octanedecenedioic acid, OD), was obtained from convert oleic acid to oleic diacid using *Candida tropicalis*. Different structures were attained by changing the stoichiometry of oleic diacid to glycerol, having linear and hyperbranched polymers avoiding the crosslinking reactions frequently obtained with chemical catalyst. Very recently, El Fray and col.[158] compared the enzymatic synthesis of poly(butylene succinate-*co*-dilinoleic succinate)][20] with that using titanium dioxide/silicone dioxide catalyst. The M_w obtained by enzymatic synthesis was lower than using inorganic catalyst; however presented higher crystallinity degree.

Vouyiouka *et al.*[159] described the synthesis of polyesters from CALB-catalyzed polycondensation of two biobased diacids, 1,12-dodecanedioic acid and 1,14-tetradecanedioic acid, with 1,8-octanediol. Thermal properties were found mainly dependent on the diacid length. In fact, an increase in the length of the diacid provides a polyester conformational characteristic nearer to that of polyethylene. However, low-molecular-weight polymers were obtained with intrinsic viscosity values of 0.115–0.189 dL g^{-1}. This group also prepared poly(butylene succinate) PE4.4, poly(octylene sebacate) PE8.10, poly(octylene dodecanate) PE8.12, and poly(octylene tetradecanate) PE8.14 from diols (1,4-BD and 1,8-octanediol) and diacids or their derivatives (DES, sebacic acid, 1,12-dodecanedioic acid, and 1,14-tetradecanedioic acid) with molecular weight ranging from 3700 to 8000 g mol^{-1} and high yields.[87] Poly(glycerol-1,8-octanediol sebacate) and poly(sorbitol-1,8-octanediol-sebacate)[99] were recently developed by enzymatic polycondensation as analogues in biomedical uses of poly(glycerol-sebacate).[160]

Sugar-derived aliphatic diols with rigid and chiral structures, 1,4:3,6-dianhydrohexitols (DAHs), are good candidates to obtain polymers with high glass transition temperature (T_g) and good optical properties;[110] however, they are quite expensive. In contrast, glycerol and D-sorbitol are abundant and inexpensive biobased aliphatic polyols and can be a good alternative. Glucose and sucrose, and sugar alcohols such as erythritol, xylitol and sorbitol, are polyols with multiple hydroxyl groups. Recently, two series of copolyesters based on bicyclic units derived from D-glucose were incorporated in poly(butylene sebacate) (PBSe), replacing either the butylene or the sebacate units. Mixtures of BDO, DESe, and the bicyclic di-O-methylene diacetal of either D-glucitol or dimethyl D-glucarate were polycondensated in the melt using CALB.[161] These copolyesters with M_w ranging from 8800 to 13100 g mol^{-1} presented higher T_g than PBSe and lower crystallinity and melting temperature.

Poly(glycerol adipate) (PGA) was obtained by CALB-catalyzed reaction of divinyl adipate with glycerol.[107] However, these polyesters possessed low M_n and large PDIs. Our group has recently studied different enzymatic synthesis conditions aiming to control the end-group functionality in a serial of glycerol-derived macromers. The judicious selection of catalyst amount and monomer ratio, allowed the control of their terminal structure, containing mainly vinyl, hydroxyl or hydroxyl/vinyl moieties, in a single-step. Additionally, further enzymatic functionalization resulted in end-capped macromers with methacrylate or thiazol moieties. These macromers retained secondary hydroxyl moieties and are of practical importance to develope UV curable materials and/or antimicrobial.

Loos and col. [106,162] used BHMF as building block on CALB-enzymatic polycondensation with diacid ethyl esters. Polymers with low M_n (~ 2000 g mol^{-1}) were obtained because of etherification side reaction. For this reason, the enzymatic polymerization did not change with diacid ethyl esters having different chain length. Recently, this group also studied[163] the possibility of furan-based copolyesters by analyzing the effect of aromatic units of dimethyl 2,5-furandicarboxylate (DMFDCA) in the reaction with 2,5-BHMF, diols and diacid ethyl esters. The furan monomers with aliphatic diols produced polymers with high M_w of 35000 g mol^{-1}; however, with diacid ethyl esters the M_w significantly decreased. They also copolymerized DMFDCA with 2,3-butanediol, isosorbide, D-sorbitol, glycerol and diethylene glycol (DEG), but polymers instead of oligomers were only found with DEG and with long-chain aliphatic diols.[44] Recently, they also copolymerized with 1,4-cyclohexanedimethanol (1,4-CHDM)[164] and proved the recyclability of lipase although the yield diminished after reuse.

Newly, aromatic-aliphatic polyesters based on the aliphatic diesters DMS, dimethyl adipate and dimethyl sebacate and the aromatic diols 2,5-BHMF, 3,4-BHMF and 2,6-pyridinedimethanol (2,5-BHMP) (see **Scheme 12**) were also obtained using CALB.[165] All the diols reacted, and

the yields were between acceptable to good, 44 and 93%, whereas low M_n ranging from 500 to 4200 g mol^{-1} were found.

Scheme 12. Monomers used for the enzymatic polycondensation reactions. Furan-based (in blue) and pyridine based (in red) monomers with the related polymers.

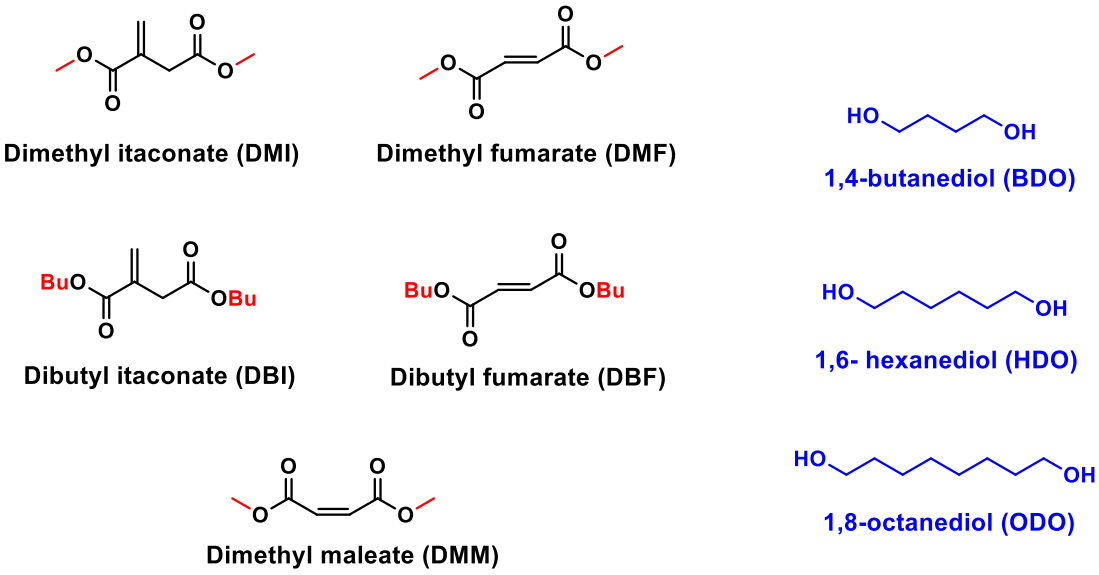
2.2. Unsaturated Polyesters

The unsaturated polyesters have unreacted double bonds and are susceptible to be modified or crosslinked as it will be described in the next section.

Gross and col.[30] prepared crosslinkable unsaturated polyol-polyesters by a one-pot CALB-catalyzed copolymerization of crude linoleic acid (LA), glycerol and oleic diacid, maintaining the ratio oleic diacid/glycerol constant and varying the amount of LA in the reaction. In this case, the incorporation of LA decreased the M_n from 11000 to 6000 g mol^{-1} while the number of trisubstituted G units increased.

The group of Loos reported that CALB-catalyzed polycondensations of succinic acid, itaconic acid, and 1,4-BD only yield oligomers.[166] By replacing the unactivated dicarboxylic acids with the alkyl diesters, poly(BS-*co*-itaconate) with various chemical compositions were successfully obtained.[40] They also synthesized itaconate-based unsaturated aliphatic polyesters via CALB-catalyzed polycondensation of dimethyl itaconate and various diacid ethyl esters, using a two-stage method in diphenyl ether.[95] The synthetic unsaturated polyesters reached also high M_w values up to 57900 g mol^{-1}. These materials have the ability to crosslink and depending on the amount of itaconate incorporated the resulting resins become more rigid and brittle. Contemporaneously, Pellis *et al*.[167] also obtained copolyesters by using CALB and itaconates,

fumarates and maleates monomers and diols with carbon numbers from 4 to 8 (see **Scheme 13**). They also analyzed the restrictions of enzymatic polycondensation of itaconates with diethyl itaconate. The experimental and computational analyses also revealed that BDO was unsuitable to copolymerize by polycondesation with itaconate (i.e. dimethyl itaconate) whereas other comonomers such as cyclic and rigid 1,4-cyclohexanedimethanol promotes the elongation of the oligomers.[3]



Scheme 13. Structures of itaconates, fumarates and maleates monomers and diols.

Recently, the group of Loos [168] also copolymerized muconic acid and its diester-modified muconic acid isomers (*cis*,*cis*-muconate, *cis,trans*-muconate) via CALB-enzymatic synthesis with diols of various carbon lengths, between 4 and 12, and also with polyoxyalkylenes with carbon numbers of 6 and 8. In this case, the M_w are lower than those compared with saturated polyesters, and are higher in the case of *cis,trans*-muconate because of lower steric hindrance than *cis*,*cis*-muconate.

Unsaturated polyesters were also synthesized using itaconic anhydride (IAn), succinic anhydride (SAn) or glutaric anhydride (GAn) and diols, i.e. BDO, HDO, 1,8-octanediol and 1,10-decanediol.[169] Although the reaction between IAn and diol did not occur, when using

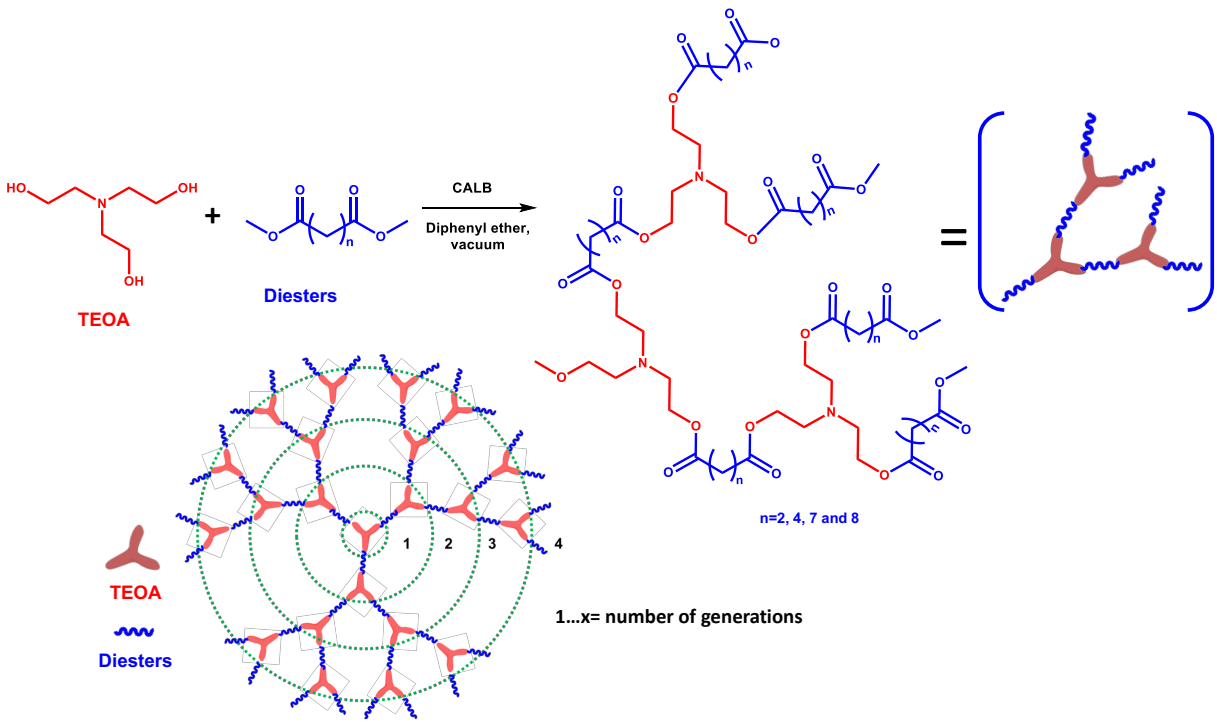
three monomer components, IAn, SAn or GAn, and diol, polyesters in good yields can be produced. However, low M_n values of 560 to 3690 g mol^{-1}, are typically found.

The synthesis of isosorbide or isomannide copolyesters with DEA and unsaturated diesters like itaconate, fumarate, *trans*-glutaconate, or *trans*-*β*-hydromuconate was performed with CALB to render unsaturated polymers with M_w values in the range of 4000-16000 g mol^{-1} when fumarate or glutaconate esters were added in 5 mol% ratio against adipate.[170]

Unsaturated polyesters were also obtained from glycerol, pentaerythritol, azelaic acid, and tall oil fatty acid (TOFA) using CALB.[171] The M_w of these polyesters varied from 20000 to 40000 g mol^{-1} when increases the ratio between azelaic acid/TOFA maintaining glycerol constant. In these polymers, as M_w increases the blanching degree also does.

2.3. Other polyesters

In this section is collected the copolymerization of esters with other groups, mainly amides but also amine or urethane. A series of poly(amide-*co*-ester)s with M_n up to 17550 g mol^{-1} were produced by CALB-catalyzed polycondensation reaction between different aliphatic (oligo)esters and diamines in a three step synthetic protocol.[172] The group of Wu also synthesized multifunctional hyperbranched poly(amine-ester)s by reaction with CALB between triethanolamine (TEOA) and diesters (dimethyl succinate, dimethyl adipate, dimethyl azelate and dimethyl sebacate) (see **Scheme 14**).[173]



Scheme 14. Synthesis of hyperbranched poly(amine-ester)s with ester terminals.

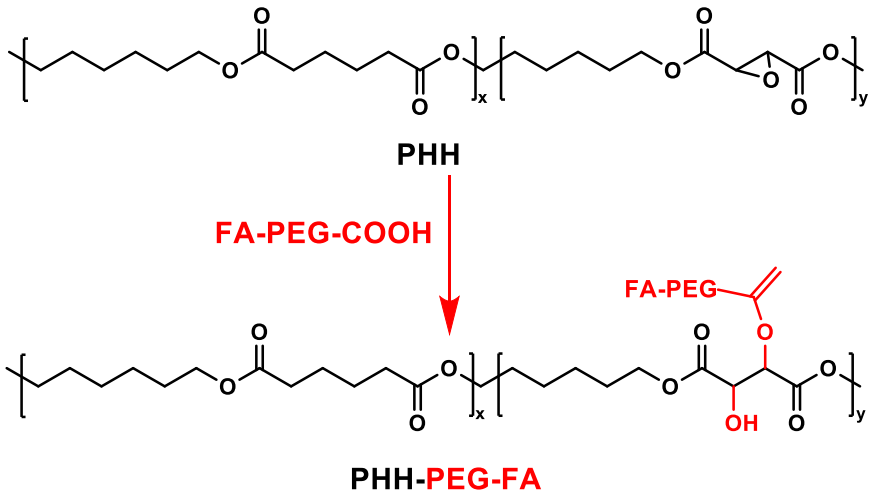
They also applied the tandem combination of CALB and Pd/C-catalyzed dynamic kinetic resolution (DKR) and subsequent CALB-catalyzed polycondensation, as an efficient protocol to prepare poly(ethylene glycol)-functionalized poly(amine-*co*-ester)s containing (R)-mexiletine, an antiarrhythmic agent.[174] Using also CALB, they prepared amphiphilic diblock methoxy PEG-*b*-poly(amide-*co*-esters) copolymers containing different profens such as ketoprofen, naproxen and ibuprofen.[175]

3. Functionalization via enzymatic catalysis

In this section the functionalization using enzymes to modified polyesters or polymers through polyesters is described. This process presents advantages in comparison to the chemical methods such as the milder reaction conditions, high specificity/selectivity, and less damages. In most of the cases, the enzyme is used to hydrolyze the polymer creating hydroxyl and carboxyl groups that are reactive.[176]

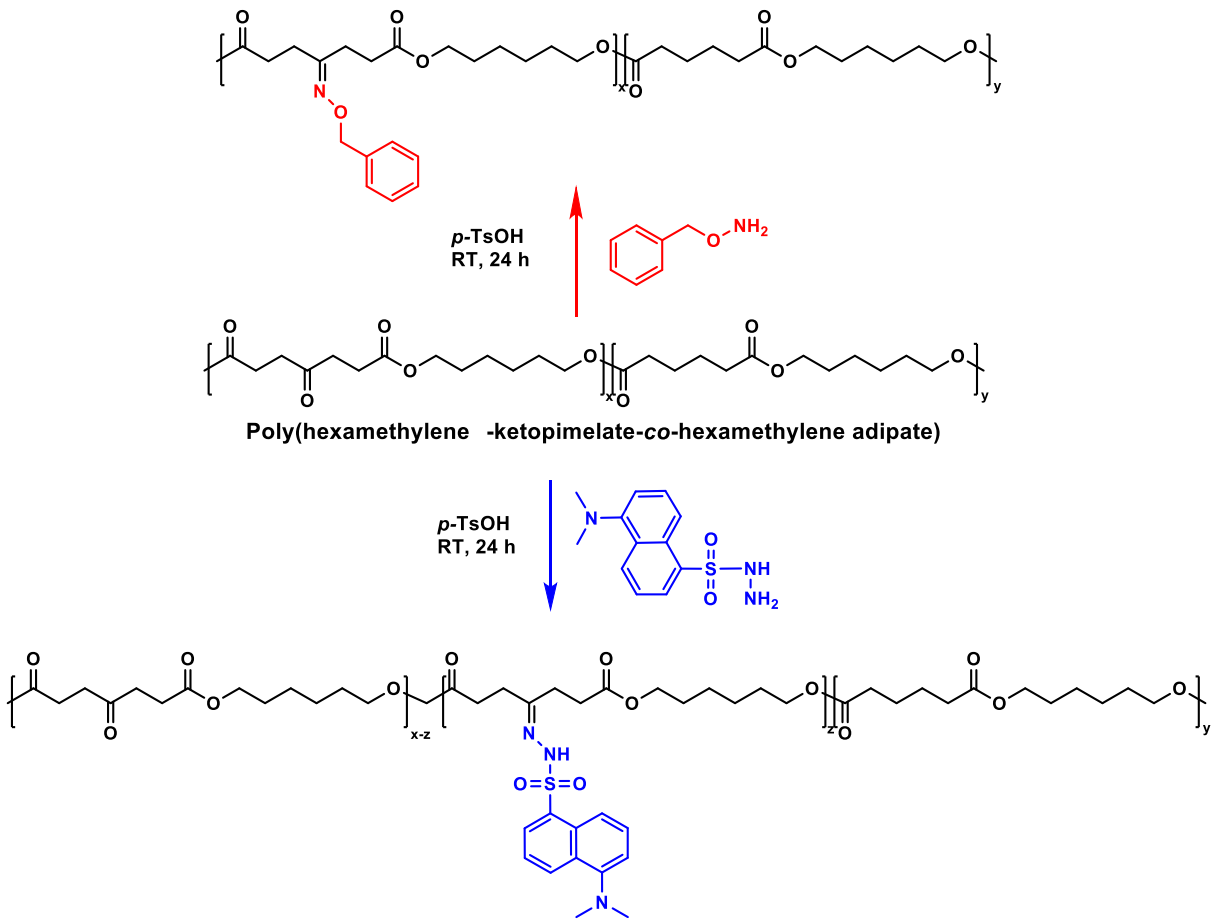
3.1. Modification of polyesters

In the case of saturated polyesters, the modification comes from the incorporation of reactive groups on the polymers. Typically, functional groups are introduced in the comonomers for a subsequently enzymatic polycondesation, followed by chemical synthetic procedures. This is the case of polycondensation of divinyl adipate (DVA) with 2-(bromomethyl)-2-methylpropane-1,3-diol instead of linear diol.[177] Then, the bromo is changed to azide group, which enables the incorporation of polymers such as polyethylene glycol by click chemistry. In another example, poly(hexamethylene adipate-*co*-hexamethylene 2,3-epoxy succinate) (PHH) was conjugated with a folate-poly(ethylene glycol)-carboxylate (FA-PEG-COOH) through epoxy group (see **Scheme 15**).[139]



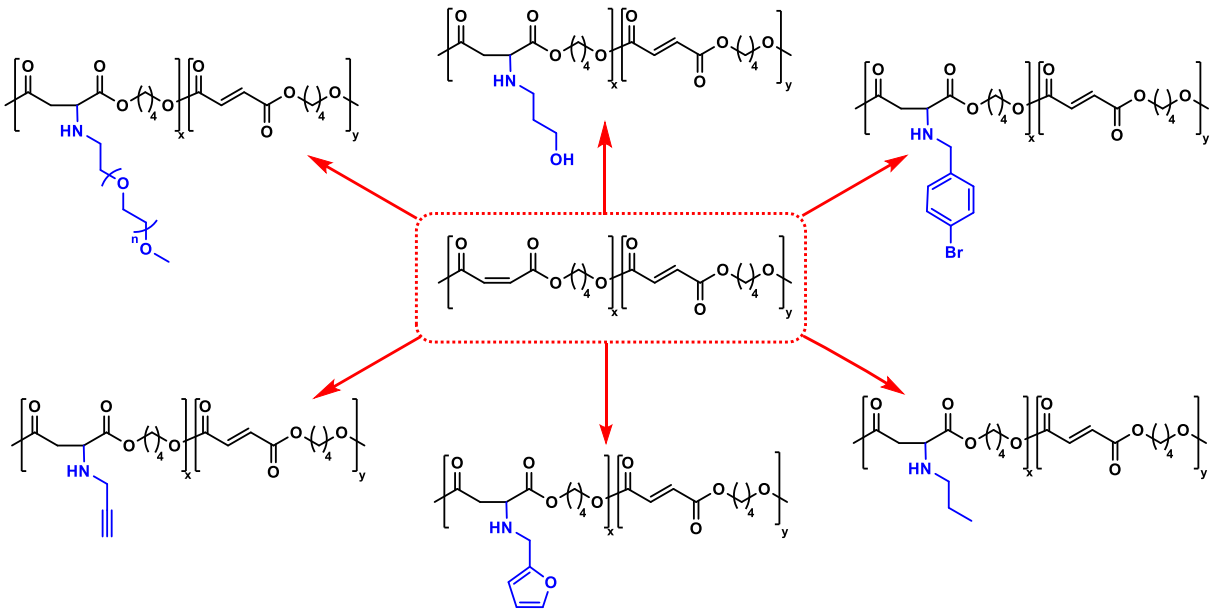
Scheme 15. Preparation of PHH-FA-PEG conjugate by linking with FA-PEG-COOH.

PGA that has one hydroxyl group as end group, was also modified by acylation with fatty acids.[178,179] Moreover, in polyketoesters (PKE), ketone-containing aliphatic polyesters, such as poly(hexamethylene γ-ketopimelate) and poly(hexamethylene γ-ketopimelate-*co*-hexamethylene adipate)[180], their modification was performed *via* oxime click chemistry (see **Scheme 16**). In spite of the variety of chemical procedures used such functionalizations of polyesters, the enzymatic method was not usually applied to modify them.



Scheme 16. Post-polymerization modification of poly(hexamethylene γ-ketopimelate-*co*-hexamethylene adipate) using an aminooxy or hydrazide agent.

On the other hand and as mentioned before, the unsaturated polyesters are able to undergo modifications by their double bonds. Likewise, most of these modifications are mainly performed by chemical methods rather than through enzymatic approaches. The incorporation of several primary amines (see **Scheme 17**) was performed in different unsaturated polyesters by aza-Michael addition. [181] These reactions are mainly made to give an amphiphilic character, which allows their self-assembly in physiological conditions and their applications as drug carriers, tissue engineering and others as it will be further commented.[167,182–184] Yan *et al.*[185] recently modified PBS with bromine, which was incorporated into the double bond. Then, it was used to react with tertiary amines to render quaternized polyesters.

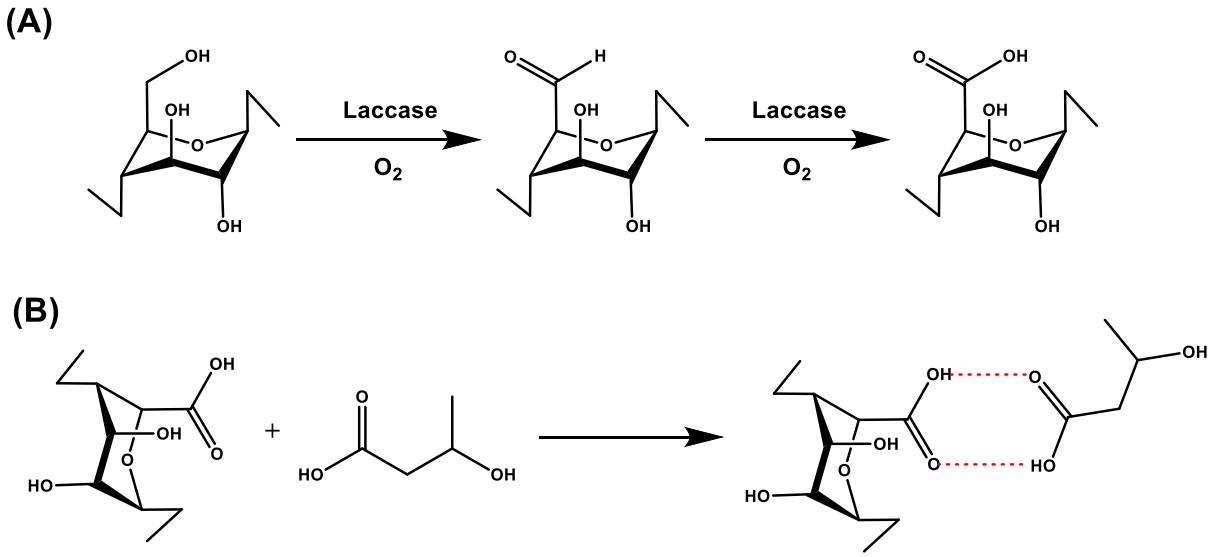


Scheme 17. Structures of various derivatives prepared using aza-Michael addition onto the polymer obtained from polycondensation of maleic acid with 1,4-butanediol at 180 ºC treated with a variety of primary amines, namely NH_2-PEG 2000, 3-amino-1-propanol, 4-bromobenzylamine, propylamine, furfurylamine, propargylamine.

3.2. Polyesters grafting from/onto polymers

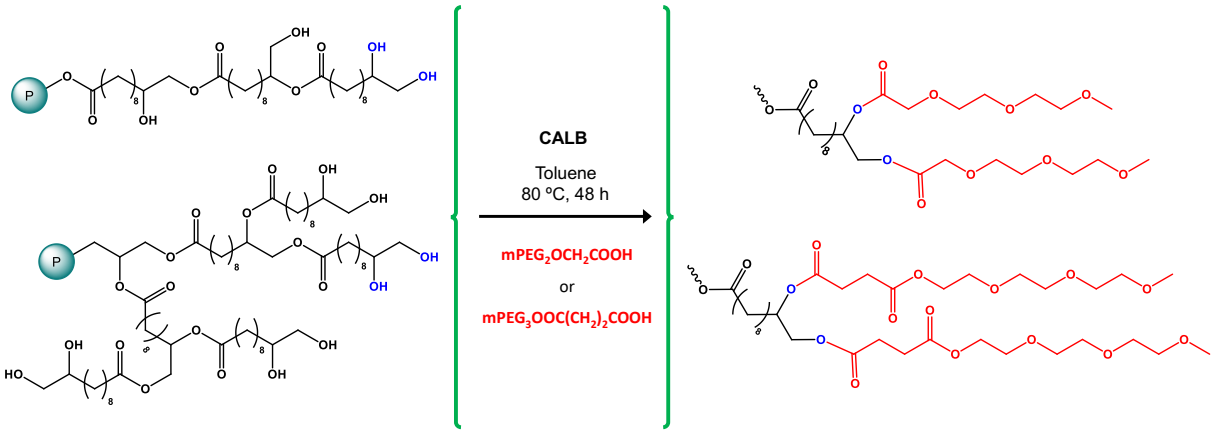
In a first attempt, the group of Iqbal grafted poly(3-hydroxybutyrate) (P3HB) onto the ethyl cellulose (EC) by using laccase (an oxidative enzyme) (see **Scheme 18**).[186] The mechanical surface properties of resulting polymer were compared with the corresponding P3HP and EC homopolymers and one P3HB-*g*-EC obtained without laccase. Although the tensile strength and Young's modulus decreased with respect to EC the elongation at break increased. Moreover, the hydrophilic property of the P3HB-*g*-EC was much better than that of pure P3HB. Later, this group was able to graft natural phenols such as *p*-4-hydroxybenzoic acid, ferulic acid, gallic acid, thymol and caffeic acid onto P3HB-*g*-EC by using also laccase enzyme.[187–189] These biocomposites displayed an excellent antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* bacterial strains among with good biocompatibility with human keratinocytes-like HaCaT.

Recently, they used laccase supplemented with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) to perform the grafting of P3HB onto EC and they observed homogeneous distribution of polyester on the surface compare to that obtained with pure laccase.[190]



Scheme 18. Representation of (A) laccase-assisted cellulose surface functionalization to form a C6-carboxylate group via a C6-aldehyde group; (B) proposed mechanism of graft formation through hydrogen bonding between P(3HB) and functionalized cellulose.

Amphiphilic polyesters were also prepared by grafting carboxyl functionalized di and tri-ethylene glycols (2-(2-(2-methoxyethoxy)ethoxy)acetic acid ($mPEG_2OCH_2COOH$) and 2-(2-(2-methoxyethoxy)ethoxy)ethyl monosuccinate ($mPEG_3OOC(CH_2)_2COOH$)) onto poly(10,11-dihydroxy undecanoic acid) (PDHU) and poly(10,11-epoxy undecanoic acid) (PEUA). [156] In the latter, the transesterification takes place leading to a highly branched structure (see **Scheme 19**). The grafting degree was higher in the case of $mPEG_2OCH_2COOH$ than $mPEG_3OOC(CH_2)_2COOH$, due to higher reactivity of α-alkoxy carboxylic acid.



Scheme 19. CALB modification of poly(10,11-epoxy undecanoic acid) (PEUA) and poly(10,11-dihydroxy undecanoic acid) (PDHU) with $mPEG_2OCH_2COOH$ and $mPEG_3OOC(CH_2)_2COOH$.

As mentioned above, Avérous's group enzymatically synthetized poly(ε-thiocaprolactone) and copolymers of ε-caprolactone with ε-thiocaprolactone. These polymers were then, coupled to chitosan backbone using hexamethylene diisocyanate as grafting/coupling agent.[191] Another approach using unsaturated polyesters is their use as macromonomers for radical polymerization. This is the case of rigid poly(diisopropyl fumarate), which was reacted with ethyl acrylate, given branch flexibility.[192]

4. Bioapplications

Nowadays, enzymes and enzymatic synthesis have been used in different areas, including the food industry, the textile industry, pharmacology and medical science. But perhaps, thanks to their inherent benefits, the use of these catalysts will become more relevant in biomedical applications or in applications that have some implication in health. There are many advantages associated with enzymatic synthesis, such as (a) mild reaction conditions, (b) catalysts with low toxicity, high and tunable activity which are often recyclable, (c) the avoidance of toxic heavy metal catalysts, (d) often good linearity of products due to steric hindrance at the enzyme active

site, (e) few by-products, and (f) less need for protection and deprotection steps.[193] Most of these advantages are crucial in order to develop safe materials for biomedical applications. In this section of the review, we cover biomedical applications where enzymatically synthesized polyesters play a relevant role such as (a) controlled drug delivery systems and (b) gene delivery, (c) tissue regeneration and (d) polymers with antimicrobial activity.

4.1. Enzymatic polyesters for drug delivery applications

Advances in new technologies have made possible the development of more powerful and effective drugs. However, these advanced compounds present some problems such as low solubility in physiological conditions or large size, since many of them are based on proteins or peptides.[194,195] These problems have a direct effect on the effectiveness of the drug, which could end up in unsuccessful treatments. In this sense, investigations on more effective drug delivery approaches as well as new modes of action are the major driving forces in polymer therapeutics. Polymer based drug delivery systems have evolved from systems relying on classical release mechanisms to intelligent delivery systems, which incorporate responsiveness to physiological environments, with better targeting skills, biocompatibility and low or absence of toxicity. Most studies on drug delivery applications are about polyesters, as most polyesters possess biodegradability, and what is more important, they can further protect the drugs inside from degradation, prolong drug circulation in blood and sustain their release. [196,197]

In this subsection, we will describe examples of enzymatic polyesters used for the development of polyester-drug conjugates, drug carriers and stimuli responsive drug delivery systems.

4.1.1. Polyester-drug conjugates

In the line of polymer-drug conjugates, Stebbings *et al.*[198] designed three enzymatic polyesters, based on ibuprofen modified monomer, the dicarboxylic acid sugar L-malic acid, which is naturally found in fruits such as apples, grapes and berries. In this case, instead of conjugating polymer and the drug, the strategy consisted first, in the modification of the L-malic acid monomer by attaching ibuprofen as pendant group and the subsequent enzymatic

polymerization using as comonomers three aliphatic diols with different length chains (PDO, 1,5-pentanediol, or 1,8-octanediol). It is worth mentioning that NMR analysis demonstrated that ibuprofen structure was not affected during the polymerization process. Interestingly, the length of the aliphatic diols resulted in a direct effect on the ibuprofen release; aliphatic diol with shorter chain, and thus more water-soluble resulted in a faster release. Finally, the cytocompatibility studies performed using fibroblasts demonstrated biocompatibility of the polyesters.

Similarly, enzymatic PGA was also applied for development of drug delivery systems through the formation of polymer-drug conjugates. In this case, the strategy consisted in the delivery of drug onto the host as biodegradation of the PGA material itself occurred *in vivo*. Probably, the major advantage of this approach could be the possibility to avoid critical processing steps for the correct encapsulation, targeting and delivery of drugs. An example of this approach can be found in the work of Wersig *et al.* with the conjugation of PGA with indomethacin.[199] They took advantage of the OH groups of PGA that remains intact after the enzymatic synthesis, and esterified them up to different degrees with the anti-inflammatory drug indomethacin so that a pro-drug was created. Conjugates with different indomethacin loads were prepared, and further release experiments carried out *in vitro*, in different neutral and slightly acidic conditions to evaluate the release kinetic.

Likewise, Qian *et al.* enzymatically synthesized biodegradable polyester pro-drugs via CALB catalyst, to obtain poly(amide-*co*-ester) backbone conjugated with three kinds of nonsteroidal anti-inflammatory drugs: naproxen, ibuprofen and ketoprofen.[200] The resultant enzymatic polyester-drug conjugate demonstrated to have relatively high drug-loading contents of 44.7–59.7 wt% since every repeat unit contained one drug molecule. The release of the drug under physiological conditions was also confirmed.[200]

4.1.2. Enzymatic polyesters as drug carriers

For the development of drug carriers, polymeric systems in the form of nanoparticles, micelles, nanogels and dendritic structures have been widely studied. These structures provide protection to drugs to plausible degradation in physiological conditions and also lead to a decrease in toxicity. In the case of chemotherapeutics, the use of polymers as carriers also improves drugs solubility. In addition to this, nanotechnology and advances in polymer synthesis such as the use of enzymes as catalyst for the preparation of polyesters, allow the production of polymer carriers with desired and defined structures to fit the treatment of diverse diseases.

For instance, the enzymatically synthesized PGA has been widely studied as it has demonstrated to be a biocompatible and biodegradable polymer. One of the advantages of using enzymes as catalyst for the synthesis of PGA is that the hydroxyl group of the main chain remains available for potential functionalization without the need of protection. In addition to this, the amphiphilic balance of PGA within the repetitive unit, may cause the self-assembly into NPs in water by simple nanoprecipitation, without the need of additional stabilizers.[201] Based on this background, several groups have worked on the development of PGA based nanoparticles where model drugs and proteins have been successfully encapsulated. For instance, Weiss *et al.* exploited the self-assembly ability of PGA by preparing fatty acid modified PGA self-stabilizing nanoparticles with various shapes, defined sizes and narrow size distribution. Such nanoparticles were also reported as not cytotoxic on *in vitro* studies, which enabled them for their use on *in vivo* models. [178,202,203]

Salem *et al.*[204–209] also worked on the preparation of PGA based copolymer, by the incorporation of PDL in the reaction, to finally produced PGA-*co*-PDL nanoparticles. These nanoparticles were successfully used for the encapsulation, targeting and delivering of active molecules (drugs) as ibuprofen, sodium diclofenac and indomethacin, and proteins among other active compounds. The developed materials were designed with particular focus in their use as pulmonary drug delivery systems taking advantage of the spray-dry technique for the production of nanoparticles as well as microparticles.

Doxorubicin (DOX) is a commercial drug used in the treatment of various cancers, like leukemia, lymphomas, breast carcinoma, and many other solid tumors. However, one of the drawback of this anticancer drug is its toxicity that can cause irreversible damage in the body.[210] Consequently, research efforts have been focused on the development of polymeric drug delivery systems to protect DOX and provide a controlled and sustained release. Due to the advantage that enzymatic synthesis offers over synthetic one, as already mentioned along the review, several investigations have been carried out for the development of enzymatic polyesters as carriers and further delivery of DOX. For instance, Liu *et al.* evaluated enzymatically synthesized poly(PCL-*co*-*p*-dioxanone) (poly(PDL-*co*-DO)) copolymers as new materials for biomedical application, and in particular for DOX controlled release.[211] These nanoparticles were prepared with different dioxane content in order to evaluate its effect in final nanoparticle size and drug uptake/release. The obtained DOX-loaded nanoparticles demonstrated controlled and continuous release of drugs over 20–60 days.[211] Yang *et al.* synthesized nanoparticles based on poly(butylene-*co*-sebacate-*co*-glycolate) (PBSG) copolymers by a single emulsification-solvent evaporation process, being PBSG polyester previously synthesized via CALB catalyzed polycondensation of BDO, ethyl glycolate (EGA), and DESe.[212] The obtained nanoparticles were able to release DOX over extender period of approximately 60 days, caused by the gradual hydrolytic degradation. Additionally, they exhibited a efficient internalization to HeLa cell, and low cytotoxicity compared to free DOX.[212]

4.1.3. Stimuli-responsive drug delivery systems

Within the field of polymer based controlled drug delivery platforms, stimuli responsive polymer systems able to respond to temperature, pH and redox changes among others, have been widely studied due to their potential biomedical applications especially in the development

of anticancer treatments. In this sense, the use of enzymatic polyesters has been also extended for the development of advanced stimuli responsive systems. Regarding thermoresponsive enzymatic polyesters with potential application as drug delivery systems, Wu *et al.* developed polyesters based on the malic acid-drug (ketoprofen) conjugate and linear PEG catalyzed by lipase under solvent-free conditions.[213] It is worth mentioning that PEG-based amphiphilic block copolymers have the potential to be used in drug delivery applications due to its biocompatibility, besides of providing colloidal stability in micelles formation and more important, offers resistance to both protein adsorption and cellular adhesion. The obtained enzymatic polyester is formed by hydrophilic PEG segments and hydrophobic drug segments, which exhibits a lower critical solution temperature (LCST) at 10–12 °C as identified by turbidity alteration measured by the change of the solution transmittance using a UV-Vis spectrometer. The traditional polymerization methods are usually not versatile enough to allow incorporation of diverse comonomers and to modify crucial properties like hydrophobicity of polyester products, therefore, in a recent work Rao *et al.*[214] took advantage of the versatility provided by the enzymatic synthesis and prepared for the first time temperature responsive amphiphilic poly(PEG400-*co*-succinic acid)-*co*-(diol(3EG)-*co*-succinic acid) (PPSDS) alternating copolyesters with PEG segments on both backbones and side chains. They could fine-tune the cloud point (T_{cp}), which is the critical temperature, with the proportion of PEG400 within the copolymer. As the ratio of PEG400 increases, T_{cp} is shifted from 26 °C up to 41 °C with a faster temperature response. In addition to this, the temperature responsive copolymer showed the ability to self-assemble at low temperatures and form nanovesicles. Depending on the PEG content different temperature-controlled vesicle sizes could be obtained. As authors concluded, these controlled “expansion and contraction” of nanovesicles could contribute to enhance loading rates, thus showing potential application as stimuli-responsive drug delivery systems.[214]

Liu *et al.*[139,215–219] designed a series of multifunctional stimuli responsive nanoparticle drug delivery systems with potential application for anticancer treatment. Their research was based in the development of pH or/and redox responsive nanoparticles obtained from enzymatically synthesized poly(amine-*co*-ester) block copolymers self-assembly. For the development of pH responsive nanoparticles, Liu *et al.* synthesized PEGylated poly(amine-*co*-ester) terpolymers in one step *via* lipase catalyzed copolymerization of PDL, DESe, and N- methyldiethanolamine (MDEA) comonomers in the presence of poly(ethylene glycol) methyl ether as a chain-terminating agent.[215] The obtained block copolymer, PEG-*b*-poly(PDL-*co*-MDEA-*co*-sebacate) (PEG-*b*-PPMS), comprised a PEG chain segments, which is hydrophilic and the hydrophobic part corresponding to the random PPMS chain segments. Therefore, block copolymers were able to self-assemble to form stable, nanoscopic and pH responsive micelles, which swell upon decreasing of the pH from physiological to 5.0 because of the PPMS amino group protonation in the core of the micelle. These micelles were proven as drug delivery systems by the encapsulation and further controlled release of a representative anticancer drug, docetaxel (DTX).[215] Similarly, PEG-poly(PCL-*co*-butylene-*co*-3,3′-dithiodipropionate) (PEG-PPBD) copolymers bearing disulfide (–S–S–) functional groups in the main chain were also prepared.[216] This enzymatic copolymer self-assembles in aqueous medium to form nanosized micelles with redox responsiveness (ROS) for anticancer drug delivery. The obtained nanomicelles (loaded with doxorubicin DOX) demonstrate to have drug delivery ability when a reductant was added to the nanomicelles dispersion. The presence of the reductant caused the swelling of the micelle since it reduces and cleaves disulfide bonds *via* reversible thiol–disulfide exchange reactions, which ultimately triggered the fast release of the drug. Liu *et al*. refined and evolved these systems toward the design of nanoparticle for anticancer treatment with responsiveness to tumor-relevant pH and intracellular reduction potential.[217,218] In a recent research, pH- and ROS- responsive biodegradable block copolymers, PEG-*b*-poly(PCL-*co*-N-methyl-diethyleneamine sebacate-*co*-2,2'-thiodiethylene sebacate) PEG-*b*-PMT, were

synthesized through one-step enzymatic polymerization. The self-assembly of these copolymers into micelles resulted in the formation of pH and ROS responsive nanoparticles, which can be used for the delivery of docetaxel (DTX) with potential application for the treatment of solid tumors (see **Figure 1** for illustrative representation).

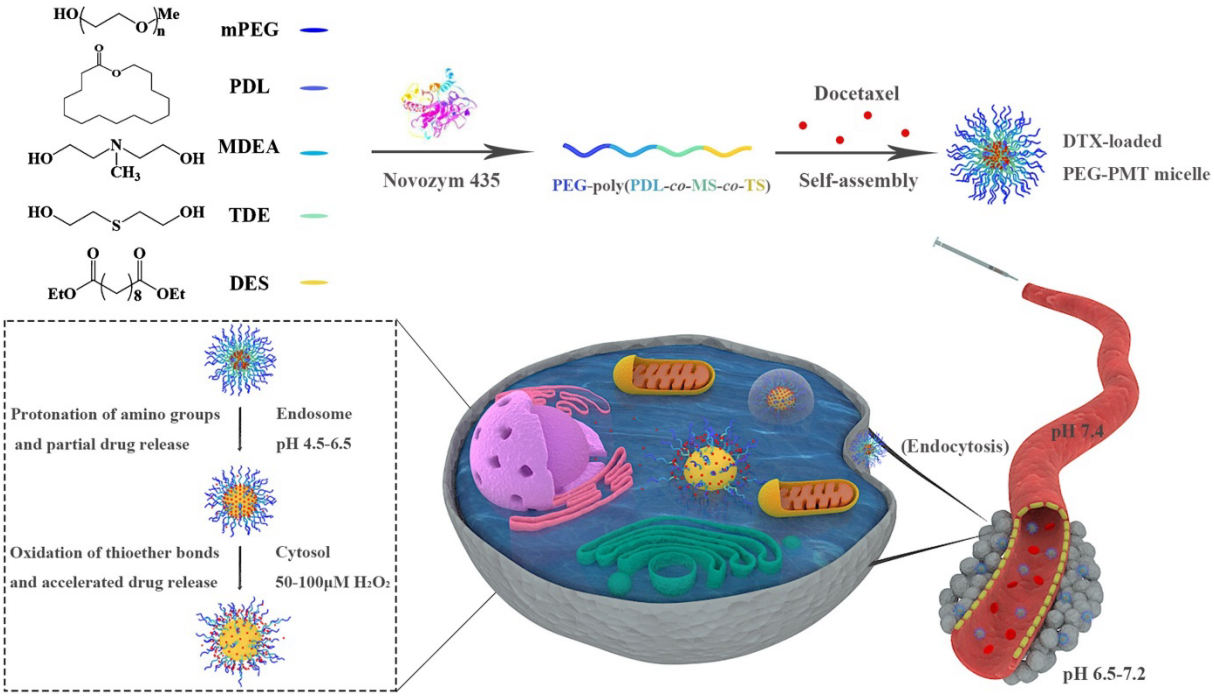


Figure 1: Scheme and illustration of PEG-*b*-PMT block copolymer self-assembly toward micelle formation with simultaneous encapsulation of docetaxel (DTX) and further release mechanism in response to pH and redox changes within the tumor cell. Reproduced with permission.[219] Copyright 2020, Elsevier

4.2. Enzymatic Polyesters for Gene therapy

Gene therapy is a new and efficient form of medical treatment. It has emerged as a new strategy against diseases that are currently incurable.[220–222] However, the therapy effect is dependent on the tool responsible for the delivery. The success of the treatment, therefore, depends on the successful design of the delivery tool or vectors. These gene vehicles can be categorized into two major types: viral and non-viral vectors. The use of viral vectors has raised considerable safety concerns, related to immune and inflammatory responses, besides of toxicity

problems;[223] therefore, non-viral strategy has become more attractive. Various types of non-viral vectors have been employed for gene-delivery applications: inorganic materials, lipids and cationic polymers, from which cationic polymers have become more relevant. These polymers form complexes with negatively charged DNA by means of electrostatic interactions. These complexes, also known as polyplexes, provide protection to DNA from degradation besides of favoring the cellular uptake, among other advantages.

Since the work of Langer *et al.*[224] on biodegradable and non-toxic poly(amino-ester) based systems, biodegradable polyesters containing cationic amino groups have demonstrated enormous potential for gene delivery applications.[225,226] However, the classical synthetic method to obtain poly(amino-ester) resulted in low molecular weight polyesters, factor that could directly affect the gene transfection efficiency.[227] In this case, the enzymatic synthesis of polyesters containing cationic amine groups was a successful strategy to obtain higher molecular weight polymers, which improved the gene transfection efficacy.[228,229] Another important factor that plays a crucial role in gene delivery is the hydrophobicity. This factor contributes to improve the interactions with DNA through cooperative binding, besides of promoting the interaction with cell membrane and further endocytosis, but also facilitates the DNA release from polycation carriers.[230–233]

Bearing in mind the importance of having a polymer with high molecular weight and hydrophobicity for a successful gene therapy, Zhou *et al.*[229] designed a terpolymer via CALB catalyzed enzymatic synthesis. The strategy consisted in the copolymerization of lactone, DESe and N-methyldiethanolamine (MDEA). Authors varied the lactone content through the selection of lactone comonomers of specific ring size, so that hydrophobicity of the final polymer could be adjusted, besides of obtaining a high molecular weight polyester. The gene delivery ability of the enzymatic terpolymers was evaluated, *in vivo* and *in vitro*, for luciferase gene transfection for lung cancer tumor treatment. The results revealed that gene transfection efficiency of lactone-DES-MDEA terpolymers could be improved by using a large lactone and

by adjusting lactone content in the polymers. As a major results, they could demonstrated a significant inhibition of tumor growth with the targeted delivery of gene by the enzymatic terpolymer, with negligible toxicity both *in vitro* and *in vivo*.[229]

Another drawback that can affect the performance of enzymatic polyesters-DNA complex (polyplex) for effective gene delivery is the lack of sufficient stability *in vivo*. In order to overcome this problem, one of the strategies consisted in the conjugation of enzymatic cationic polyesters with poly(ethylene glycol), or what is also known as PEG-ylation.[234,235] This modification contributed to increase water-solubility as well as colloidal stability of the polyplex, which enhance in vivo survival capability to reach and transfect. Following this approach, Zhang *et al.*[234] prepared nanomicelles from amphiphilic (PEG-poly(ω-pentadecalactone-*co*-N-methyldiethyleneamine-*co*-sebacate) (PEG-PMS). From this polyester, luciferase-encoding plasmid DNA (LucDNA)-loaded micelles were prepared and its colloidal stability, cytotoxicity and hemolysis activity further evaluated. The PEG-ylation strategy resulted in highly stable polyplexes with reduced cytotoxicity and hemolysis activity.[234]

The significance of this research on enzymatic polyesters for gene delivery applications is clearly evidenced in the recent research of Zhou *et al.*[236], which took advantage of enzymatically synthesized PEGylated poly(lactone-*co*-amino ester) to develop nanoparticles conjugated with small molecules (AMD3100) known to be highly expressed in breast cancer brain metastases (BCBM). These nanoparticles served to deliver an artificially designed gen, proMel, able to release melittin, an antitumor agent, which kills surrounding tumor cells. The study performed in mice demonstrated the capacity of PEGylated poly(lactone-*co*-amino ester) nanoparticles to effectively inhibit the progression of breast cancer metastases in the brain, besides of prolonging the survival.[236]

4.3. Enzymatic polyesters for tissue regeneration.

In the area of tissue engineering, where substitutes capable of imitating normal functions of damaged tissues are developed, polymers play a prominent role. Polymers are the materials used in the development of scaffolds, one of the three pillars of tissue engineering (scaffolds, cells and signs). The function that polymeric scaffolds needs to accomplish is mimicking the chemical components, physical structures and biological functions of the natural extracellular matrix (ECM) to function as structural support and guide cell growth and anchoring. For this purpose, polymers need to meet some requirements such as being porous and permeable, so that nutrients could enter cells, possess appropriate structural surfaces for cellular adhesion, as well as mechanical properties similar to the tissue to be regenerated. In addition to this, scaffolds need to be non-toxic, biocompatible and biodegradable.

There are several polyesters that fulfill some of the mentioned requirements for the construction of scaffold for tissue engineering applications: PEG, polycaprolactone (PCL) [237–239], PBS and PBSe based systems [240–242], polyglycerol based polymers (synthesized from combination of glycerol and diacids) [243–245], among others, which have been proven to be ideal materials for their application in skin, nerve and cardiac tissue regeneration, besides others. However, in general all these polymers have been prepared *via* synthetic routes requiring of high reaction temperatures and the use of organic metals as catalyst, which may drag problems in their final applications. In particular, the use of organic metals as catalyst might bring some toxicity problems to the material, since the purification process is difficult and there is a risk of remaining residual metal.

Although the positive impact of green methods to develop non-toxic, biocompatible and biodegradable polymers, yet the works referred to enzymatic polyesters with potential application in tissue regeneration are scarce. For instance, Sonseca *et al.* [20] developed for the first time in the literature high molecular weight poly(butylene succinate-*co*-dilinoleic succinate) (PBS-*co*-DLS) copolyester from DES, BDO and dimer linoleic diol, via two step method using CALB as catalyst. The obtained thermoplastic elastomer was processed by means

of electrospinning technique giving rise to homogeneous electrospun mats without defects with potential application for cardiac tissue engineering.[246,247]

Similarly, Lang *et al.*[248] took advantage of the enzymatic synthesis to prepare high-molecular-weight PGSe from 1,8-octanediol, glycerol, and sebacic acid using CALB as catalyst. The obtained polymers showed tunable molecular weights, better thermal properties, and excellent electrospinnability. This work also put in relevance the potential and versatility of enzymatic PGSe for the development of biomaterials for tissue engineering applications.[248]

4.4. Enzymatic polyesters with antimicrobial properties.

In the last decade, much effort has been put in order to design and develop new antimicrobial polymeric materials as a solution for the increased resistance of microorganisms against antibiotics. Among them, cationic polymers are the ones that have arouse most interest in recent years. They are characterized by having positive charges incorporated in their main or side chain as well as an amphiphilic character. Precisely, they owe their antimicrobial properties to their dual cationic and amphiphilic character. Polymers with antimicrobial activity can be applied in biomedical applications, such as stents, prostheses, probes, sutures, dressings, masks, etc., as well as in other areas as packaging, textiles and paints, among others. In the case of some biomedical applications, these polymers should be biodegradable in physiological conditions. But, it is also important to mention that many of these applications are of single-use, such as protective masks, wound dressings, etc. This is devastating for the environment due to the generated waste and CO_2 emission when producing or incinerating them, which contribute negatively to the climate emergency that the society is facing as most of the synthetic cationic polymers reported are non-biodegradable. Therefore, the synthesis of biodegradable polymers containing quaternary ammonium groups with antibacterial properties has important research significance. In this respect, biodegradable polyesters combined of functionalized with quaternary ammonium groups are considered as promising polymers for the development of antimicrobial materials, and several examples of their potential can be found in the

literature.[185,249,250] A recent example can be found in the work of Yang *et al.*[185] through the synthesis of random copolyesters of poly(butylene succinate-*co*-butylene fumarate) (PBSF) followed by quaternization reaction giving rise to quaternary ammonium cationic copolyesters with excellent antibacterial performance against both *S. aureus* and *E. coli*. All these biodegradable polyester-based antimicrobial materials are prepared following a synthetic route. Still, the research on the enzymatic synthesis of polyesters with antimicrobial activity is scarce. For instance, Bautista *et al.*[153] focused their work in the functionalization of PBS, motivated by the fact that the lack of functionalities in PBS has become a shortcoming that prevents the expansion of its use in advanced biomedical applications. In particular, they aimed to incorporate cationic units in order to provide antimicrobial activity. Interestingly, in a previous approach devoted to the synthesis of PBS terpolyesters containing minor amounts of quaternized glutamic acid, they found out that the high temperatures required for melt polycondensation resulted in the decomposition of the ammonium units which hampered the polymer chain growth.[251] Taking this into account, they changed the strategy and successfully synthesized cationic PBS copolyesters bearing respectively ammonium and tributylphosphonium side groups via enzymatic route using CALB as catalyst.[153] The presence of both ammonium and phosphonium groups provided PBS with remarkable antimicrobial activity against gram-positive (*S. aureus)* and gram-negative (*E. coli)* bacteria (see **Figure 2**).

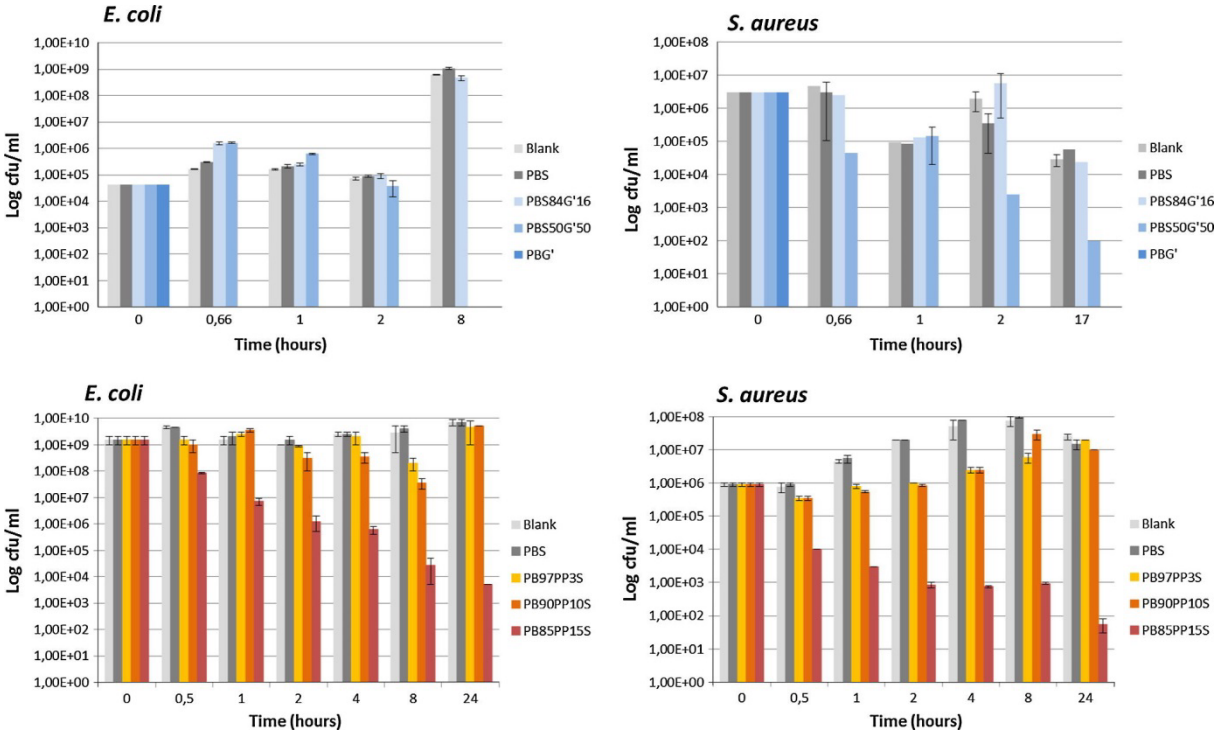


Figure 2. Microbial colony counts for *E. coli* and *S. aureus* in contact with PBS and PBS copolyesters containing ammonium (top) and phosphonium units (bottom). Reproduced with permission.[153] Copyright 2016, Elsevier.

Another example of enzymatic synthesis of cationic polyesters is the work of Xiao *et al.* on the preparation of poly(4-benzyl formate piperidine lactone-*b*-PDL) (PNPIL-*b*-PPDL) block copolymers via enzymatic ring opening polymerization.[157] In a second step, acidic hydrolysis of the copolymer gave rise to cationic poly(4-piperidine lactone-*b*-PDL) (PPIL-*b*-PPDL) with pendant secondary amino groups anchored to the backbone, which provided high antibacterial activity against gram-positive (*S. aureus)* and gram-negative (*E. coli)* bacteria.

5. Conclusions

There are many interesting works related to the enzymatic synthesis of polyesters, both through polycondensation and ring opening polymerization as has been presented in this review. However, although the use of enzymes as catalysts provides the opportunity of carry out

polymerization reactions under mild conditions, of importance when working with sensitive moieties and monomers, to achieve high molecular weights needed for industrialization, what will result in polymers with good mechanical stability is still a pendant task. Most of the research done in the enzymatic polymerization field is devoted to study the possibilities of application in polymer science, while efforts related to the industrial implementation of the enzymatic polymerization processes are limited. Despite these drawbacks, enzymatic synthesis is a very powerful tool to use in the modification of polymers, since it allows obtaining systems that can hardly be obtained with other methodologies, especially if they are natural polymers. In this sense, enzymatic synthesis can contribute to the production of new functional polymers based on effective selective modification. Enzymatic polymerization processes, often needs optimization of synthesis conditions for specific situations, therefore, the integration of enzymatic and chemoenzymatic syntheses is a key strategy to open a wide range of possibilities to develop materials with applications in biomedicine or nanoscience among others. Therefore, more investigations in enzyme technology, biotechnology, polymeric synthesis and product development are needed.

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