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Review

The Closure of the Cycle: Enzymatic Synthesis and Functionalization of Bio-Based Polyesters

Q1 Alessandro Pellis,[1] Enrique Herrero Acero,[2] Valerio Ferrario,[3] Doris Ribitsch,[2] Georg M. Guebitz,[1,2] and Lucia Gardossi[3,*]

The polymer industry is under pressure to mitigate the environmental cost of petrol-based plastics. Biotechnologies contribute to the gradual replacement of petrol-based chemistry and the development of new renewable products, leading to the closure of carbon circle. An array of bio-based building blocks is already available on an industrial scale and is boosting the development of new generations of sustainable and functionally competitive polymers, **such as polylactide acid (PLA)**. Biocatalysts add higher value to bio-based polymers by catalyzing not only their selective modification, but also their synthesis under mild and controlled conditions. The ultimate aim is the introduction of chemical functionalities on the surface of the polymer while retaining its bulk properties, thus enlarging the spectrum of advanced applications.

Introduction

For several decades, the demand for polymers and plastics derived from fossil fuels has grown at a faster rate than for any other group of bulk materials, and expectations are that this trend will continue until 2020 [1]. With the worldwide increase in demand, the amount of plastic material released to the environment has become a significant problem because such material does not biodegrade easily or quickly, if at all, leading to ecological problems such as the formation of plastic patches in the ocean or in rivers [2]. The UN Environmental Programme estimated that the overall natural capital cost of plastic use in the consumer goods sector each year is US$75 billion, representing financial impacts resulting from issues such as pollution of the marine environment or air pollution caused by incinerating plastic. Of the natural capital costs of plastic, 30% are due to greenhouse gas emissions from raw material extraction and processing [3].

Biotechnology research has responded to the need to mitigate the environmental impact of plastics with research and technological innovations that now enable the biotechnological production of bio-based monomers from **renewable** carbon (see Glossary) on an industrial scale [4].

Among polymers, polyesters are a widely used class with applications ranging from clothing to food packaging and from the car industry to biomedical applications. The possibility to synthesize polyesters from bio-based monomers is demonstrated by PLA, currently the most important bio-based polyester in terms of volume, with a capacity of approximately 180 000 tons/y. Renewable polyesters can be also biosynthesized by microorganisms through complex

Trends

Different integrated biotechnological advances are gradually replacing petrol-based chemistry and contribute to the development of new chemicals and plastics. Some biobased polymers, such as PLA, are chemically synthesized and are already available on an industrial scale.

A long-term contribution to the production of renewable building-blocks and monomers is expected from biotechnology research on the bioconversion of CO_2 and microbial electrocatalysis.

Advanced applications of polymers are obtainable by introducing chemical functionalities on the surface of the polymer while retaining its bulk properties. Such modifications can change the superficial hydrophobicity as well as introduce a 'pendant' as anchoring point or for successively chemical modifications. These possibilities are of key importance, especially for biomedical applications.

Biocatalyzed polymerization is not yet economically competitive. The conventional process configurations and reactors used in chemical synthesis do not respond to the complexity of the biocatalytic systems. Thus, the need to improve mass transfer while preserving the integrity of the biocatalyst still requires a specific tailored solution.

Robust enzyme immobilization, as well as thin film conditions or ionic liquids, are some of the solutions proposed for overcoming such limitations.

regulatory pathways responding to external stimuli, including poly(hydroxyalkanoate)s (PHAs), which are **biodegradable** microbial polyesters commercially produced via fermentation, although we do not discuss them here [5].

Research aiming at developing the next generation of bio-based polyesters must not only address their sustainability, but also pursue their competitiveness in terms of their superior technological and functional properties. Biocatalysis goes a step further by enabling the synthesis of structured, functionalized, and biodegradable polyesters through highly selective and benign synthetic processes. Moreover, biocatalysts enable targeted hydrolyses and modifications of polyesters that are not possible with conventional chemical strategies [6,7]. Here, we illustrate how polymer chemistry is already benefitting from a range of biotechnological advances that enable the environmentally sustainable production of high-quality polyesters with new functional properties. Innovations in the field of bio-based polyesters are a paradigm of the increasingly intimate integration between biotechnologies and sustainable chemistry, which responds to the pressing challenges of a circular economy [8].

Bio-based Monomers and Polyesters

The percentage of chemical production based on biotechnology is estimated to increase from less than 2% in 2005 to approximately a quarter of all chemical production by 2025 [9,10]. The largest contribution will come from the conversion of renewable carbon into chemicals via biotechnological routes. Factors boosting the integration between biotechnology and chemistry include the expected decrease in petroleum production and concerns about CO_2 emissions; however, there is also the need to improve public confidence in the chemical industry. Although only 7% of worldwide petroleum consumption is currently used for chemical production, bio-based processes leading to platforms of chemical building blocks will create higher added-value compared with current biofuel production processes [4].

By 2030, the market value of bio-based building blocks is expected to reach €3.2 billion, whereas the demand for fermentation-based chemical building blocks was less than €700 million in 2013[i].

Such building blocks could either be produced from renewable carbon through green chemical conversion routes or via microbial conversions. The incorporation of fermentative production of basic building blocks as unit operations in integrated **biorefineries** [4] is dependent on intense research activities ranging from microbial strain development and engineering to fermentation and down-stream processing optimization. Different critical analyses of research advances for enhancing the commercial potential of specific building blocks have been extensively reviewed elsewhere [11,12].

Strong research efforts are also currently directed towards the bioconversion of CO_2 into chemical building blocks by designing artificial metabolic routes or through microbial electro-catalysis. However, intense fundamental research is still needed before consumers will benefit from the practical applications of such technologies [12].

One of the key end uses for bio-based building blocks is expected to be in the production of bio-based polymers, with a projected market value for renewable plastic of €5.2 billion by 2030 [10]. Recent industrial analysis (November 2015) suggested that worldwide production capacity will triple from 5.7 million tons in 2014 to nearly 17 million tons in 2020[ii]. The most relevant monomers already available for use in fermentation technologies or in chemical processes and that are already tested in the synthesis of polyesters are detailed in Table 1.

[1]University of Natural Resources and Life Sciences Vienna, Department for Agrobiotechnology IFA-Tulln, Institute for Environmental Biotechnology, Konrad Lorenz Strasse 20, A-3430 Tulln an der Donau, Austria
[2]Austrian Centre of Industrial Biotechnology, Konrad Lorenz Strasse 20, A-3430 Tulln an der Donau, Austria
[3]Laboratory of Applied and Computational Biocatalysis, Dipartimento di Scienze Chimiche e Farmaceutiche, Università degli Studi di Trieste, Piazzale Europa 1, 34127, Trieste, Italy

*Correspondence: gardossi@units.it (L. Gardossi).

Table 1. Overview of the Biotechnological Routes and Status of the Industrially Most Relevant Bio-Based Monomers that can be Used in the Synthesis of Bio-Based Polyesters

Monomer	Biotechnological Route	Refs	Company	Status	Application of the Corresponding Bio-Based Polyesters	Refs
Sorbitol	Fermentation + hydrogenation	[14]	Roquette, ADM	Market	Functional polyesters; coatings	[10,11, 64,65]
Isosorbide	Sorbitol dehydration	[17]	Roquette	Market	Thermosetting resins	
Ethylene glycol	Ethanol dehydration	[15]	India Glycols Ltd, Greencol Taiwan	Market	PET; PEF	
1,3-propanediol	Fermentation	[18]	Du Pont, Tate & Lyle, Metabolic Explorer	Market	PTT; fibers; elastomers; polyester-urethanes	
1,4-butanediol	Fermentation, succinic acid hydrogenation	[19]	BioAmber, Genomatica, Mitsubishi	Market	PBAT; PBS; PBT	
Adipic acid	Fermentation + hydrogenation	[20]	Celexion LLC, BioAmber, Rennovia, Verdezyme	Market	Resins; polyester-amines; polyester-urethanes	
Itaconic acid	Fermentation	[21]	Qingdao Kehai Biochemistry, Itaconix	Market	Photocurable precursors; plasticizers	
Lactic acid	Fermentation	[22]	Nature Works, BASF, Purac, Cargill, BBCA, Galactic	Market	PLA	
Succinic acid	Fermentation	[23]	BioAmber, Myriant, Reverdia, BASF, Purac, Succinity	Market	Textiles; coatings; PBS; PBT	
Terephthalic acid	Isobutylene oxidation, fermentation	[24]	Virient, Annellotech, Genomatica	Pilot plant	PET; coatings	
Levulinic acid	Fermentation, acid treatment of C6 sugars	[25]	GFBiochemicals, Bio-on, Biofine Renewables	Market	Coatings, hyperbranched dendrimeric polyesters	[66]
Malic acid	Fermentation	[27]	Novozymes	Pilot plant	Functionalized chiral polyesters	[52]
2,5-furandicarboxylic acid	Fermentation + dehydration + oxidation	[29]	Avantium	Pilot plant	PEF; polyester-urethanes	[13]

Glossary

Biodegradable: property of a material that enables it to be chemically dissolved by bacteria, fungi, or enzymes.

Biorefinery: the process that entails refining of biomass in a commercial context for the production of fuels, chemicals, polymers, materials, food, feed, and value-added ingredients.

Cutinases: (E.C. 3.1.1.74) hydrolytic enzymes that catalyze the hydrolysis of the ester bonds of cutin, a natural polyester of the plant cuticle.

Immobilized biocatalysts: enzymes formulated to become insoluble and enabling recycling. The enzymatic proteins can be either anchored on solid matrixes, cross-linked, or entrapped in porous materials.

Lipases: (E.C. 3.1.1.3) hydrolytic enzymes that catalyze the hydrolysis of triglyceride ester bonds.

Prodrug: an inactive compound that is metabolized (i.e., converted within the body) into a pharmacologically active drug.

Renewable resource: organic natural resource that can replenish to overcome usage and consumption, either through biological reproduction or other naturally recurring processes.

Stirred tank reactor: a particular type of chemical reactor, usually considered the simplest type of reactor, where mixing is provided by a mechanical stirrer system, such as a turbine wing or a propeller

Q21

Polyesters are generally produced through chemical processes such as ring opening polymeri-zation (ROP) or via polycondensation of di- and trifunctional polyols with dicarboxylic acids or their diesters and/or anhydrides. The resulting bio-based polyesters can be endowed with various properties, and are classified as a function of their sustainable qualities as renewable,

biocompatible, and/or biodegradable. Therefore, they are not biologically degradable per se since their monomers might be linked together via chemical bonds recalcitrant to enzymatic hydrolysis [13].

Polyesters synthetized via enzymatic catalysis are also biodegradable because the enzymes used for catalyzing the synthesis of ester bonds are also able to catalyze their hydrolysis. Nevertheless, some chemically synthesized ester bonds can be also hydrolyzed enzymatically: although PLA is produced via conventional chemical ROP starting from bio-based lactic acid, it is formed by enzymatically hydrolyzable ester linkages. However, it is classified as bio-based biodegradable polyester [14]. Further information on the environmental classification and certification of plastics is available from European Bioplastics[iii].

Bio-based chemicals and polymers suffer severe economic competition from cheaper products synthesized by conventional routes from hydrocarbons that were optimized more than 100 years ago. PLA-based products had already been developed by the 1940s and 1950s, but their production became economically viable only 70 years later. This demonstrates the importance of optimizing the productivity and robustness of bioconversions to achieve cost-effective production.

The success of bio-based polyesters does not rely solely on their capacity to replace fossil-based polymers while being economically competitive. Rather, the next generation of bio-based polyesters should bring entirely new advanced chemical and functional properties to the polymer scenario. This challenge has been addressed over the past decade by exploiting the unique selectivity and efficiency of biocatalysts (Table 2); here, we analyze the potential and limitations of the enzymatic approach for the hydrolysis, modification, and functionalization of bio-based polyesters [15–17].

Enzymatic Functionalization and Modification of Polyesters

Polymers with a heterogeneous atom backbone composition, such as polyesters, polyamides, and polyurethanes, can be degraded by microorganisms [18]. Since 1997, several isolated **lipases** have been reported to catalyze the hydrolysis of aliphatic polyesters, while only a few hydrolases are active on aromatic-aliphatic polyesters [18]. These polyester-degrading enzymes represent a milder and selective alternative to chemical or physical treatments aiming at introducing chemical functionalities to the surface of polymers while retaining their bulk properties. One of the major advantages of enzymatic polyester modification is the possibility of fine tuning and controlling the degree of functionalization by operating under mild conditions [17,19]. Here, we describe the major advances in enzyme-catalyzed hydrolysis of bio-based polyesters achieved over the past few years, focusing on the two most industrially relevant polymers, PLA and PET.

Enzymatic Functionalization of PLA

PLA is a fascinating polymer: it can be obtained from renewable agricultural sources and its production consumes CO_2 while providing consistent energy savings. Due to its biocompatibility and processability properties, PLA is used for biomedical applications exploiting the fact that it can be absorbed by humans. It is also widely used as a packaging material, taking advantage of its ability to biodegrade [20].

Partial hydrolysis of the outer layer of the polymer for the generation of either carboxyl or hydroxyl groups can not only enhance its biocompatibility [21], but also promote the coupling of molecules carrying various functionalities. Compared with poly(ethylene terephthalate) (PET), PLA is more sensitive to alkaline hydrolysis and nonwoven PLA undergoes a 100% weight loss after only a 30-min incubation with 10–20% NaOH. Controlled enzymatic hydrolysis was shown to be an alternative to alkaline treatments to avoid material damage. Different lipases from

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Table 2. Most Recently Developed Enzymatic Processes for Synthesis and Functionalization and/or Hydrolysis of Bio-Based Polyesters

Biocatalyst	Material class	Goal	Refs
CaLB	Lactic acid-based	ROP of D,D-lactide	[46,57]
	Terephthalic acid-based; ethylene glycol-based; 2,5-furandicarboxylic acid-based	Synthesis of aromatic/aliphatic polyesters	[67,68]
	Sorbitol-based	Synthesis of hydroxy-functional polyesters	[51]
	Adipic acid-based; 1,4-butanediol-based; Isosorbide-based	Synthesis of aliphatic polyesters	[16,63]
	Itaconic acid-based; 1,4-butanediol-based	Synthesis of vinyl-functional polyesters	[17]
	Glycerol-based	Branched-controlled polyesters, epoxide-containing polyesters	[17,69]
	Itaconic acid-based		
	Malic acid-based	Copolymers of L-malic acid, adipic acid, and 1,8-octanediol	[52]
	Adipic acid-based		
HiC	Adipic acid-based; 1,4-butanediol-based	Synthesis of aliphatic polyesters	[21]
	Terephthalic acid-based; ethylene glycol-based	PET hydrolysis	[27]
	Lactic-acid based	Surface functionalization of PLA films	[60,70]
Thermobifida cellulosilytica cutinase 1	Terephthalic acid-based; ethylene glycol-based	Surface functionalization of PET films	[33,71]
Thermobifida fusca cutinase	Terephthalic acid-based; ethylene glycol-based	Degradation of PET nanoparticles	[23,72]
Thermobifida halotolerans esterase	Terephthalic acid-based; ethylene glycol-based	PET hydrolysis	[18]
	Lactic-acid based	PLA hydrolysis	
Thielavia terrestris cutinase	Succinic acid-based; 1,4-butanediol-based	PBS hydrolysis	[19]
	Terephthalic acid-based; ethylene glycol-based	PET hydrolysis	

Aspergillus niger, *Candida cylindracea*, and *Candida rugosa* were tested on nonwoven PLA and optimum surface hydrolysis conditions were reported, enabling the preservation of their mechanical or structural properties [22]. One of the most-studied enzymes for the hydrolysis of PLA is proteinase K, which catalyzes the hydrolysis of PLA by acting on amorphous regions between the crystalline regions, rather than on the folding chains. Tsuji and co-authors reported how proteinase K hydrolyzes PLA via both endo- and exo-chain scission.

Cutinases have also been investigated for their ability to hydrolyze PLA films and/or fibers and are gaining increasing attention for applications in polymer surface functionalization [22,23]. Ribitsch *et al.* reported that cutinase from *Thermobifida halotolerans* hydrolyzes PLA films, leading to the release of lactic acid monomers. The resulting polar groups on the PLA surface cause a decrease in the water contact angle (from approximately 76° to the complete spread of the water drop on the polymer surface) [23].

The enzymatic functionalization of PLA has biomedical application relevance due to the possibility of increasing its biocompatibility while leaving its bulk properties unaltered. Nyanhongo *et al.*

reported the activation of the surface of PLA membranes via hydrolysis catalyzed by cutinase from *Humicola insolens* (HiC), followed by the coupling of unfolded human serum albumin to the newly generated superficial carboxylic groups. The hydrophilic groups of the grafted human serum albumin were important to promote the interaction between the PLA membrane and cells, resulting in up to 2.4 times higher cytocompatibility [21].

Enzymes were also used for the selective excavation of the PLA-based core of block copolymer micelle assemblies and their shell cross-linked nanoparticle analogs. The hydrolysis rate of PLA from the cores of the block copolymer micelles was significantly higher than for bulk PLA [24].

Enzymatic Modification of PET

PET is currently the most produced polyester worldwide, with applications as a synthetic textile fiber, film, medical device, and packaging material. It is synthesized from ethylene glycol (EG) and terephthalic acid (TA). The latter is currently produced via catalytic oxidation of petrochemical *p*-xylene and has an estimated global market of 50 000 kton/y.

The most recent advances towards bio-based PET rely on the strong integration of chemistry and biotechnology. Bio-based *p*-xylene can be produced directly from isobutanol obtained by fermentation of C5/C6 sugars, which is then chemically converted to p-xylene [25]. Over the past few years, the lab-scale production of bio-based TA has been reported via the cycloaddition of acrylic acid and isoprene (both bio-based) to obtain fully bio-based TA with a 94% yield [26]. Moreover, bio-based TA was obtained from furfural, which is chemically produced from inedible cellulosic biomass. In 2009, the Coca-Cola Company announced the production of a bio-based PET bottle as a result of the use of 30% bio-based EG and, more recently, a 100% bio-based PET bottle was developed in partnership with the US company Virent. The latter produces bio-based *p*-xylene via aqueous phase chemical reforming of C_5/C_6 sugars [25].

Despite the optimal mechanical properties, the main drawback of PET is its highly hydrophobic nature, showing a poorly wettable surface that is difficult to functionalize. Industrial applications frequently require an activated or reactive surface to graft molecules, such as flame retardants or water-soluble dyes. Several methods, including plasma or chemical treatments, caused aging effects (which decrease the resulting surface hydrophilicity) or a consistent reduction of the polymer weight and strength [27]. Enzymatic hydrolysis showed the potential to overcome such limitations and provide surface functionalization without affecting the bulk properties of the polymer [19]. Several polyesterases were reported to be active in the hydrolysis of PET [23,28]; in particular, cutinases from *Thermobifida* species were demonstrated to be active either on PET or on small PET model substrates [23,29–32]. A correlation was found between the kinetic parameters of soluble substrates, the release of hydrolysis products, and the final degree of PET hydrophilization. Interestingly, the hydrolysis efficiency of two highly homologous cutinases from *Thermobifida cellulosilytica* was found to be influenced by the electrostatic and hydrophobic properties of the enzyme surface in proximity to the active site [27]. Several PET-based substrates ranging from films [19,27] and fibers [30] to nanoparticles [33] were enzymatically hydrolyzed using cutinases as biocatalysts and, in the case of cutinase from *Thermobifida fusca*, the hydrolysis products were observed to cause inhibition of the enzyme [33].

Cutinases from *Fusarium solani*, *Pseudomonas mendocina*, and *H. insolens* were also reported to be active on PET [34]. Of these, HiC was the most active due to its stability after prolonged incubation at 70 °C, which corresponds to an optimal hydrolysis temperature, being very close to the T_g of PET. At this temperature, the enzyme benefits from higher mobility of the polyester chains in the amorphous phase, thus increasing the accessibility of HiC to the ester bonds [34].

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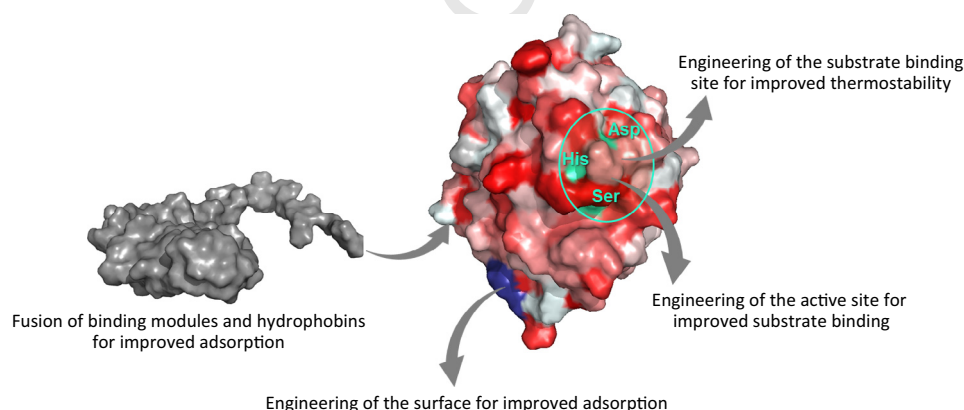> **Box 1. Engineering of Cutinases for PET Hydrolysis**
>
> **Q23** Although cutinases are the most active enzyme class in terms of PET hydrolysis, the need for more efficient hydrolytic processes has pushed the development of rationale enzyme-engineering strategies to enhance the hydrolytic activity of cutinases. The rationale behind most engineering strategies applied so far was to enlarge the active site space and increase its hydrophobicity, resulting in the better binding of bulky substrates [73]. Introduction of mutations Leu81Ala and Leu182Ala in the cutinase from *Fusarium solani pisi* increased four- and fivefold, respectively, the activity towards PET fibers. Not only the active site architecture is important; although less understood, the polarity of the enzyme surface is also a key factor. In the case of *Thermobifida fusca* cutinase, mutation outside of the active site, such as the introduction of a nonpolar alanine in the mutant Ile218Ala and double mutant Gln132Ala/Thr101Ala, improves significantly the hydrolysis of PET [74]. In the case of *Thermobifida cellulosilytica* cutinase 2, exchange of Arg29 and Ala30, located outside the active site, by Asp and Val, respectively, increased the hydrolysis activity up to threefold [75].
>
> Fusion of different binding modules to catalytic domains and, thus, increasing the surface catalytic activity at the interface, has been an evolutionary strategy developed by nature to improve degradation of complex polymers [76]. This approach inspired the fusion of binding modules to the C terminal of a PET-hydrolyzing enzyme to drive enzyme adsorption on the polymer surface and increase its hydrolytic activity [77]. The hydrophobicity of the C terminal-fused binding domain affects enzyme absorption on PET: cutinases equipped with hydrophobic binding modules adsorb to a higher extent compared with those carrying hydrophilic binding modules, leading to almost four times higher activity compared with the native biocatalysts. Recently, hydrophobins were successfully fused to the same cutinase, increasing its PET hydrolytic activity [70]. Engineering the hydrophobicity of the binding domain also led to a better polymer–enzyme interaction. In the case of a *T. fusca* cutinase binding module construct, mutations Trp68Leu or Trp68Tyr on the binding module resulted in a 1.5-fold improvement towards PET [78].
>
> Increasing thermostability also enhances polymer hydrolysis. Since temperature increases the mobility of the polymer chains and eases the enzymatic action [79], the improvement of enzyme thermal stability represents the aim of another rational engineering strategy. In cutinase from *Saccharomonospora viridis* AHK190 [80], mutations Ser226Pro and Arg228Ser led to a 6 °C increase in the enzyme melting temperature and a 1.5 increase in PET hydrolytic activity.

198　As already mentioned, PET is largely used for the manufacturing of plastic bottles, which uses a
199　low crystallinity polymer to achieve high bottle transparency. This particular form of PET is an
200　optimal substrate for enzymatic hydrolysis. Therefore, cutinases were suggested for PET
201　recycling [35] in a process that could overcome the quality limitations of current recycling
202　strategies based on blending. The enzyme hydrolyzed the polymer to its constituent monomers
203　(terephthalic acid and ethylene glycol) so that the freshly produced monomers could then be
204　repolymerized after separation of dyes and contaminants. The hydrolytic properties of cutinases
205　are also exploited in detergent formulations, where enzymes act as effective antipilling agents by
206　partially hydrolyzing fuzz from polyester fibers [36,37] (Box 1).

207　**Q2**　Recent developments in the field of biocatalyzed hydrolysis (Figure 1) of PET are focused mainly
208　on improvement of the activity of enzymes via approaches of enzyme engineering, targeting both
209　the active site and sorption properties (Box 2, Figure 2).

Figure 1. Biocatalyst Engineering Strategies for Enhanced Hydrolysis of Aromatic-Aliphatic Polyesters.

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Box 2. New Perspectives for Making Enzymatic Polycondensation Scalable
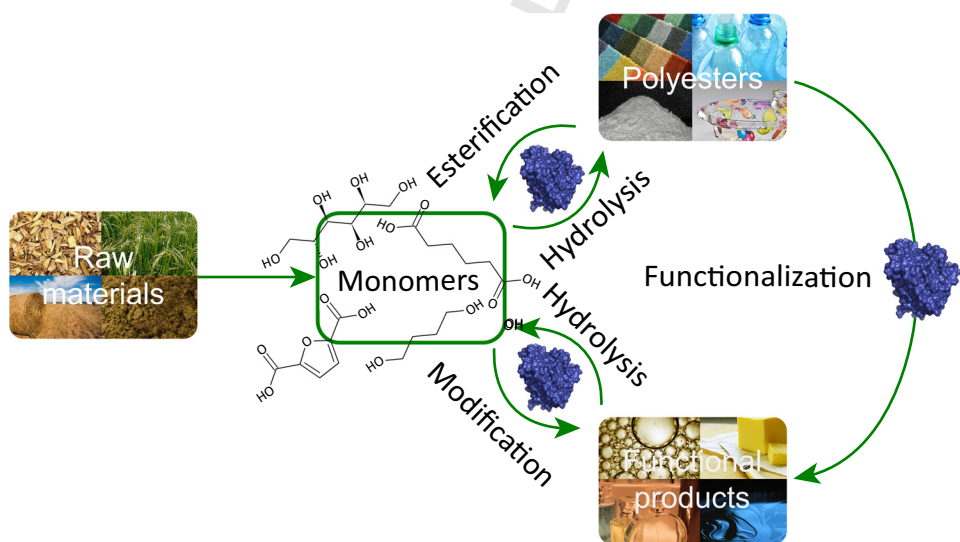
In 2010, Gross reviewed the wide array of different polyesters synthesized via enzymatic catalysis on a laboratory scale and identified the main factors hampering the exploitation of this wealth of knowledge on an industrial scale [41]. Since then, research advances have resulted from Gross' call for improved activity and robustness of biocatalysts under manufacturing systems (e.g., stir tank reactors). Recent studies proposed alternative integrated solutions for enzymatic polycondensation since they pointed out the inadequacy of batch and stirred tank reactors for these highly viscous systems [46,68]. Processes were configured by combining robust covalently immobilized lipases with thin-film conditions. The advantages of working under thin-film conditions are several, including: (i) the integrity of the biocatalyst is preserved because no mechanical stirring is required; (ii) mass and heat transfer are highly efficient; and (iii) thin-film processes provide an easy route for fast removal of volatile components and water under reduced pressure.

Q24 The concept was experimentally validated first on the laboratory scale (~10-g monomers) in the synthesis of BDO with AA and DMI and ensured the integrity of the biocatalyst over eight reuse cycles. On a pilot scale (~10-kg monomers) the thin-film system was operated continuously within turboreactors [48]. Interestingly, it was demonstrated that the polycondensation of AA with different polyols can be conveniently carried out in two steps: after an initial biocatalyzed oligomerization, the enzyme can be removed and the reaction can be thermodynamically driven thanks to the removal of co-product (i.e., water). This solution allows reduction of the exposure of the biocatalyst to stressing factors.

Regarding the improvement of enzyme activity, monomolecular dispersion of the native enzyme would lead to the highest reaction rate, but contamination of the product with the enzyme must be avoided. As an alternative solution, it was demonstrated that distributing the catalyst on the widest carrier surface facilitated the enzyme–substrate approach and promoted polycondensation. CaLB covalently immobilized on carriers with low protein loading was shown to be a practical and economical solution to the problem, although further improvement might come from the development of novel cheaper, renewable, and efficient carriers. This conclusion is applicable more generally not only to solvent-free enzymatic polycondensations, but also to any biocatalyzed process hampered by mass transfer limitations and viscosity.

Key Figure

Q22 Graphical Representation of the Enzymatic Circle of Bio-Based Polyesters

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Figure 2.

Biocatalyzed Synthesis of Functionalized Polyesters

Greener Synthesis under Challenging Reaction Conditions

Polycondensation of dicarboxylic acids with polyols is the primary route by which commercial polyesters, such as PET, PPT, and poly(butylene adipate-co-terephthalate) (PBAT) are currently synthetized [11]. Classically, the synthesis is conducted using a wide array of toxic metal catalysts at temperatures that may exceed 150–250 °C [38,39]. These conditions are optimal for polymerization because they reduce the system viscosity, although they also cause unde- sired side reactions of chemical unstable substrates, such as epoxy and vinyl moieties [6]. In these cases, the selectivity and extraordinary activity of enzymes under mild conditions repre- sent a solution for circumventing the limitations of conventional chemical polycondensations. Moreover, enzymes make the use of toxic and unselective metal catalysts unnecessary.

Enzymatic polycondensation is made possible by esterases, in particular lipases, which are active in low-water media and can catalyze esterification and transesterification in organic solvents or in solvent-free bulk systems. Enzymatic processes can synthesize aliphatic and, to a lesser extent, also aromatic polyesters [40,41].

On the way towards greener processes, Takamoto *et al.* investigated the enzymatic synthesis of polyesters in scCO$_2$ via both polycondensation (divinyl adipate and 1,4-BDO) and ROP (of ε-CL) [42]; however, in both cases, work up of the reaction involving dissolution of the product in organic solvent and filtration of the biocatalyst was necessary. An alternative solution was the introduction of ionic liquids as green reaction solvents, which are able to solubilize a range of monomers and are characterized by high thermal stability. This approach is particularly interesting in the case of high melting point substrates, which cannot be polymerized in bulk [43]

Immobilization of the biocatalyst is mandatory in these synthetic processes, first to avoid the aggregation of the hydrophilic enzyme molecules, second, to recycle the expensive enzyme and, finally, to prevent the contamination of product by the enzyme protein [44]. Currently, more than 90% of the academic work on enzymatic polyesters synthesis uses Novozym® 435, a formula- tion of lipase B from *Candida antarctica* (CaLB), immobilized via adsorption on methacrylic resins that works efficiently also in solvent-free systems at temperatures of 70–90 °C [6].

However, the pioneering work of Binns [45] demonstrated that during one single cycle of polycondensation between AA and BDO, 10% of the protein detaches from the Novozym® 435 carrier and contaminates the product. This is mainly the consequence of the weak anchoring of the lipase on the carrier through physical adsorption on the methacrylic resin. Recently, the problem was overcome by applying covalently immobilized CaLB [46] (Box 2).

Temperatures ranging from 60 °C to 90 °C are used in solvent-free systems to reduce viscosity and vigorous mixing is applied to improve mass transfer [47], but this combination causes considerable stress on the biocatalyst, affecting severely the economic viability of the process. In these reaction conditions, the integrity of the biocatalyst is a major problem. This fact was underlined by Korupp *et al.*, who described the scale-up of a lipase-catalyzed polyester synthesis using the commercially available Novozym® 435 in bulk systems. The authors managed to obtain bio-based polyesters from GLC and AA with a molecular weight of 2–3 kDa, but noticed that a protein content of 0.48 g kg^{-1} was present in the final product. This implies a 45% destruction of the immobilized preparation during the reaction [47]. The most recent studies indicate that classical **stirred tank reactors** are inappropriate for efficient enzymatic polycondensation, whereas processes on thin films [15] and in turboreactors [48] assure good mass transfer while causing low stress to the enzymatic preparations (Box 2).

Functionalized Polyesters from Biocatalysis

The use of enzymes in polymer synthesis is primarily directed to the preparation of highly structured polyesters characterized by a regular presence of functional groups, which are of great interest especially in drug formulations. Hydroxyl-, thiol-, or carboxyl-functional pendant groups along the macromolecular chains facilitate covalent anchorage of **prodrugs** [49].

By changing the monomer ratios of copolymers or the chemical structures of the pendant groups, the hydrophobic–hydrophilic balance and the degradation rate of polymers can be adjusted to the needs of different applications. Furthermore, by post modification of pendant functional groups, novel comb, graft, or network polymers can be prepared. In this case, enzymatic catalysis represents an appropriate way to obtain functional polyesters containing sensitive groups. The bio-based monomer itaconic acid, for example, was polymerized with several polyols to give side-chain functionalized polyesters where the vinyl moiety was pre-served. Molecular weights obtainable through lipase catalysis are relatively modest (1–2.5 kDa) due to the scarce inherent reactivity of itaconic acid. However, the vinyl group is prone to further functionalizations and/or cross-linking of the polymer [15,16,50]. Computational studies guided 'substrate engineering' approaches for selecting diol structures favorable to chain elongation in the polycondensation of dimethyl itaconate [16].

Similar polyesters containing a lateral epoxy moiety have also been reported [51]. Malic acid represents another interesting bio-based dicarboxylic because it carries a hydroxy group and it is also chiral. Yao *et al.* used (L)-malic acid and adipic acid in different ratios together with 1,8-octanediol in reactions catalyzed by CaLB that led to polyesters with a molecular weight of up to 10 kDa [52]. The products demonstrated that CaLB accepts only the primary hydroxyl group of the diol for the polycondensation, whereas the secondary hydroxyl group of malic acid remains available for further modifications.

In addition to the monomers reported in Table 1, glycerol has a major role as renewable monomer since it is largely available on the market as an inexpensive co-product of the transesterification of triglycerides in biodiesel synthesis or from bioethanol production [4]. The chemical synthesis of polyesters with multifunctional monomers, such as glycerol, needs strict synthetic control to prevent branching and formation of gels [53]. For example, CaLB demonstrated regioselectivity towards 1,3-OH groups in the polycondensation of glycerol with esters of itaconic acid [16] and epoxide monomers [6,50,51].

A future challenge for enzymatic polycondensations lies in the incorporation of aromatic dicarboxylic esters to produce PET-like aromatic-aliphatic polyesters. The attempts to poly-merize such compounds reported so far [54,55] demonstrated a modest monomer conversion rate. A recent study reported the synthesis of furan-based polyesters starting from the bio-based 2,5-bis(hydroxymethyl)furan, but the obtained molecular weights were below 3.0 kDa [56]. Comparable results (M_n of 23.7 kDa) were obtained by combining the dimethyl ester of the furandicarboxylic acid with 1,10-decanediol [57].

The difficulty of obtaining high-molecular-weight polyesters by enzyme catalysis has been recognized as one of the major limitations towards the industrial exploitation of this environ-mentally benign methodology. However, this problem can be circumvented by the synthesis of oligomers, or telechelic prepolymers, with functional ends. The synthesis of oligomers also prevents the precipitation of polymers during the synthesis [58,59]. The functional ends of the telechelics can be used in a second chemical step after the removal of the biocatalyst and films **Q6** with different properties are obtained by Currently there is a strong need for enzymes with novel selectivities due to an increasing range of bio-based building blocks with potential for the synthesis of structurally different specialty polymers and products for the pharmaceutical or food

industry [6]. Although this problem might be approached by enzyme engineering, the only study addressing the mutation of enzymes for polyester synthetic purposes is related to a CaLB mutant that showed 90-fold increased activity for the ROP of D-lactic acid [60].

Cutinase enzymes have emerged as interesting alternative to lipase biocatalysts because of their wide substrate specificity. HiC was found to have high activity at 70 °C and to catalyze polycondensation and ROP reactions of an array of substrates [61,62], with a selectivity preference towards long dicarboxylic acids (C_{10}-C_{13}) [63].

Concluding Remarks

Biotechnological conversion of renewable carbon into chemical building blocks for polymers and other chemicals is paving the way for the sustainable innovation of chemistry. Although industrial projections indicate that, by 2025, sustainable biotechnologies will contribute to the production of a quarter of chemicals and polymers, their success will depend not only on technological factors. Availability of renewable feedstock not competing with food is one of the issues, together with the capacity of new products to penetrate the market. Concerning the first issues, there is much excitement around the bioconversion of CO_2 by chemo-enzymatic routes or through microbial electrocatalysis, but strong research efforts are still required in this field. In the future, algal technologies and other nonconventional sustainable sources of biomass (e.g., insects) may also contribute to relieve the pressure on soil exploitation.

In the meantime, research must be directed not only towards the replacement of existing petrol-based polymers, but also for conferring higher value and competitive functions to bio-based polymers. Biocatalysis already contributes not only to the selective modification and degradation of bio-based polymers, enabling the closure of the carbon circle, but also to the *in vitro* synthesis of advanced polymers, which are not accessible by chemical routes. Despite major advances achieved using modern genetic tools, efforts are still needed to not only enlarge the portfolio of enzymes endowed with necessary selectivities, but also for the optimization of biocatalyzed processes moving away from conventional chemical-engineering paradigms. These integrated efforts are necessary for the success of the new generation of bio-based polymers, which must compete with well-established products, optimized through decades of chemistry research and innovation practices.

Q8 Uncited references

[81–91].

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Outstanding Questions

How long will it take to develop a range of different bio-based monomers available on an industrial scale? **Q7**

How long will it take to integrate bio-based building block production together with biocatalyzed polymerization in integrated biorefineries?

What engineering or screening approach would be most appropriate for obtaining enzymes able to convert efficiently aromatic monomers in polycondensation processes?

How can we design enzymes and processes that enable the biocatalyzed synthesis of polymers with higher molecular weight?

How 'sustainable' and 'renewable' are biocatalyzed processes making use of petrol-based resins for enzyme immobilization? In addition, what would be the most promising renewable biomass or material for replacing organic resins as an immobilization matrix?

Will biotechnology succeed in the development of appropriate CO_2 conversion routes to obtain high-value products and chemicals?

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