Review

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Renewable building blocks for sustainable polyesters: new biotechnological routes for greener plastics

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Abstract

The next generation of plastics are expected to contribute to a massive reduction in the carbon footprint by the exploitation, in industrial productive processes, of renewable monomers such as polyols and dicarboxylic acids obtainable via biotechnological production. More specifically, there is a rising demand for advanced polyesters displaying new functional properties while meeting higher sustainability criteria. Polyesters are part of everyday life with applications in clothing, food packaging, car manufacturing and biomedical devices. This review is intended to provide an overview of the array of renewable building blocks already available for synthetic purposes and exploitable in the production of polyesters. Moreover, new greener routes for more environmentally friendly polyester production and processing are discussed, pointing out the major technological challenges.
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Keywords: renewable plastics; green chemistry; polyesters; biotechnological production of building blocks; industrial biotechnology

INTRODUCTION

In the chemical industry, together with petrochemicals and fine chemicals, polymers are some of the major products accounting, only in Europe, for more than 60 000 companies employing over 1.45 million people.[1] Almost a quarter of worldwide polymer production capacities are located in Europe; just considering EU-15 countries, the actual petrochemical polymer production is estimated at 15.4 million tons per year. The substitution of petrochemical polymers with bio-based alternatives is actually considered as the necessary answer to the unacceptable environmental and social costs of petroleum-based and non-degradable plastics.[2]

The production and commercialization of renewable bio-based polymers are expected to continuously grow by 2020, thus representing a real alternative to fossil carbon source-derived polymeric products. Industrial analysis and projections report that in 2030 there will be both biodegradable and non-biodegradable bio-based plastics on the market.[3] Biodegradable plastics will be widely used in disposable products whereas non-biodegradable bioplastics will be aimed at durable applications and recycling. The bioplastics market value is expected to reach *ca* €5.2 billion in 2030.[4]

The term 'bio-based plastics' generally refers to all those polymers obtainable by processing synthetic polymeric materials based on building blocks obtained after fermentation of natural feedstock, such as, for instance, poly(lactic acid) (PLA) which currently represents the most important biopolymer in terms of production volume with a capacity of about 180 000 tons per year.[5] Notably, bio-based plastics differ from bioplastics, namely plastic items made by Nature but include also plastics obtained by direct processing of naturally occurring polymers (biopolymers), such as thermoplastic starch, polyhydroxylalkanoates and rayon.[5] Bio-based polymers are not automatically classifiable as biologically degradable, since in most cases they are chemically synthesized through the formation of covalent bonds that might be recalcitrant to biological degradation. Ideally, complete polymer hydrolysis to the corresponding monomers would lead to valuable building blocks not accessible with conventional recycling strategies (Fig. 1).[6–8]

In Nature, enzymes catalyse the breakdown of naturally occurring polymers such as lignocellulose and also polyesters such as cutin. Scientific advances in the field of biocatalysis have led to the development of suitable enzymes and reaction conditions for the synthesis and functionalization of polymers and polyesters in particular.[9–14] Due to their remarkable catalytic efficiency and recyclability, enzymes are attractive and sustainable alternatives to toxic catalysts used in polyester production, such as metal catalysts and tin in particular.[15]

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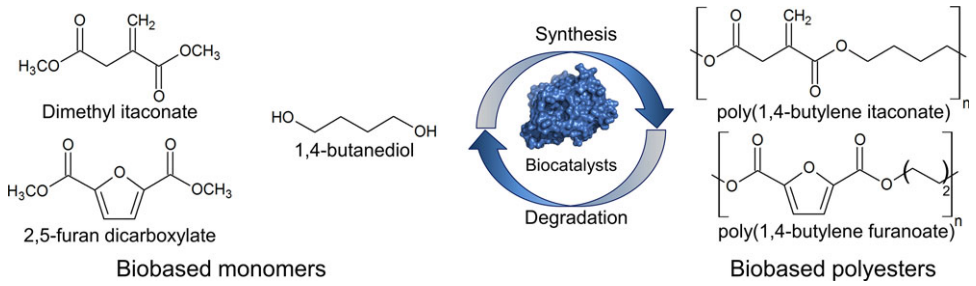


Figure 1. Biocatalysed synthesis and recycling of aliphatic and aromatic renewable polyesters: the use of enzymes as catalysts confers biodegradability to the produced polymers.

Experts and stakeholders generally agree that one of the crucial factors that will affect the success of renewable polyesters is the productivity and robustness of bioconversions, which should be greatly improved to become cost-effective. This review focuses attention on the chemical building blocks already available for the production of the next generation of polyesters that aims at the massive reduction in the carbon footprint by addressing the biotechnological production of the most relevant bio-based monomers but also the recent biotechnological advances aiming at the sustainable production and processing of renewable and biodegradable polyesters.[16–18]

BIO-BASED MONOMERS

The possibility of synthesizing polyesters from bio-based monomers has attracted considerable industrial interest in the last decade. The following sections analyse and discuss the biotechnological strategies for the production of the most relevant monomers available for polyester synthesis (Scheme 1).

Bio-based dicarboxylic acids

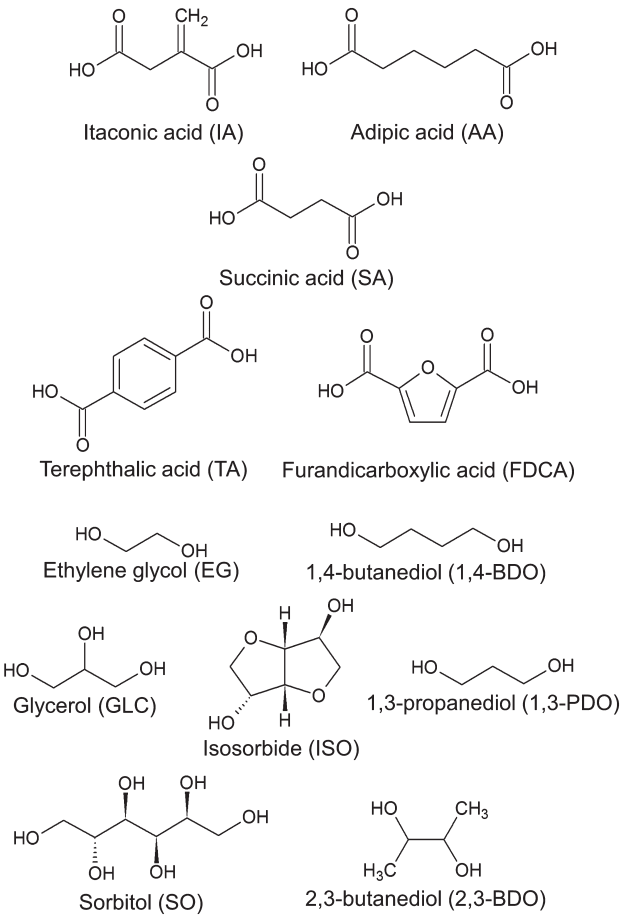
Itaconic acid (IA)

IA has been known since 1837 when Baup first described the thermal decomposition of citric acid, leading to IA.[19] Chemical routes to produce IA are the Blatt method or the Montecatini method. Neither thermal decomposition nor alternative chemical methods are used for commercial production since fermentation by fungi is economically more profitable. Biosynthesis of IA was first described by Kinoshita in 1932 who isolated the product from cultivation media of the osmophile eukaryotic *Aspergillus itaconicus*.[20] Later on various *Aspergillus terreus* strains were found more suitable for the fermentation process.[21]

The IA fermentation process works optimally under PO_4^{3-}-limited growth conditions at sugar concentrations between 100 and 150 g L^{-1}. The best yields of IA production were achieved using glucose or sucrose as substrates, but for the economic sustainability of the process, complex carbon sources like starch, molasses and hydrolysates of corn syrup or wood were also tested and found to be suitable.

During the fermentation process, the pH drops below 2 and IA becomes the main fermentation product. For an optimal reaction setup the temperature is usually maintained at around 37 °C. An adequate oxygen supply is essential since anaerobic conditions will irreversibly kill the cells.[22] The common product recovery leading to industrial-grade IA involves a first filtration step for the removal of mycelium and solids, followed by evaporation at sufficiently acidic conditions, cooling and finally crystallization. Higher grade IA is achieved by treating the hot evaporate with activated carbon and filtering. Although nowadays *A. terreus* is the mostly frequently used commercial producer of IA, several attempts have been made to identify alternative microorganisms. The use of yeasts or filamentous fungi strains from *Ustilago zeae* improves the fermentative process by reducing the sensitivity to substrate impurities or determining an easiest downstream of the fermentation broth.[22] Economically speaking, the most productive process was established by Pfizer which involves a submerged fermentation process using suspended *A. terreus* biomass, inoculated as spores on pretreated molasses.[23]

IA is currently used in paper-coating and carpet-backing, which are the primary consumers at the industrial scale. Some IA derivatives are used in medicines, cosmetics, lubricants and herbicides.[22,24]



Scheme 1. Most important bio-based dicarboxylic acids and polyols currently available for the enzymatic synthesis of polyesters.

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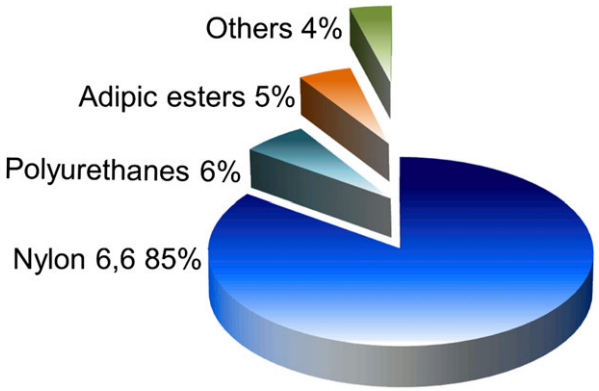

Figure 2. Most common commercial polymeric products derived from the bio-based monomer adipic acid.

Adipic acid (AA)

The global demand of AA has been estimated to be 2.6 million tons per year with a growth rate of 3–5% per year. The major producers of AA worldwide include Invista, Ascend, Honeywell, BASF, Radici, China Shenma and PetroChina. High-purity fibre-grade AA is used to produce nylon 66 (Fig. 2), while low-purity AA is used to produce polyurethanes.

In more than 90% of the industrial plants currently operating, production of AA involves the oxidation of cyclohexane or other petroleum-based feedstock. AA is commonly found in Nature in the juice of sugar and red beets.[25] In cellular metabolism, this dicarboxylic acid is an intermediate in the degradation pathways of cyclohexane, cyclohexanol and cyclohexanone.[25] Metabolic pathways for AA biosynthesis have not been described yet in the literature. However, AA can be obtained by chemo-catalytic conversion of the bio-based precursors *cis*,*cis*-muconic acid or glucaric acid by a hydrogenation process using Pt on carbon or nanoparticles of Ru10Pt2 as catalysts.[26]

The synthesis of AA from renewable carbon sources such as glucose derived from starch or cellulose is a promising alternative route to this important commodity chemical. Biotechnological conversion of D-glucose into *cis*,*cis*-muconic acid was first reported by Draths and Frost using engineered *Escherichia coli* cells.[27] Starting from this fermentative step, Niu and co-workers performed a chemo-catalytic hydrogenation achieving a two-step process with a total AA production yield of 97% (mol/mol) from D-glucose.[28] As an alternative, the bio-based production of AA can also be achieved via the α-aminoadipate pathway or starting from long-chain carbon substrates.[25]

Several start-up companies such as Rennovia and Verdezyne have developed bio-based routes to produce AA with the final aim to create 100% bio-based nylon; such approaches were demonstrated to be cost-competitive with the conventional cyclohexane oxidation process.[29] The lower production costs and the need for implementing industrial sustainability are the two main drivers to consider alternative 'green' feedstock sources for bio-based AA production.

Succinic acid (SA)

Since 2008, various companies (such as DSM, BASF and Purac) have shown an interest in the production of bio-based SA at an industrial scale.[30] As for the bio-based monomers, mentioned above, also for SA the most important production process from renewable feedstock is microbial fermentation of various glucose sources by a variety of microorganisms such as genetically engineered *Escherichia coli*, *Actinobacillus succiniproducens* and *Anaerobiospirillum succiniproducens*.[31] The processes actually are in use by two companies: the Myriant SA biorefinery in Lake Providence (Louisiana, USA) that employs grain sorghum grits as its saccharificable starting material[32] and the Reverdia process (used by DSM + Roquette) where ethanol and SA are co-produced through glucose fermentation. Both processes run with genetically modified anaerobic bacteria, in such a way that alcoholic fermentation sustains the SA production.[33]

Theoretical calculations performed by Pinazo *et al.* concluded that, despite having a lower material efficiency, fermentative SA production is attracting attention due to its very competitive cost and market position close to competitiveness with an important petrochemical feedstock such as maleic anhydride.[30] In addition, SA production costs might be reduced by the evolution of crop growing and entirely crop usages (from first to third biomass generation).[31]

The high interest in SA is because of the fact that this dicarboxylic acid is a key component/intermediate in the production of several solvents, adhesives, printing inks, magnetic tapes, coating resins, plasticizers, emulsifiers, deicing compounds and chemical and pharmaceutical intermediates.[34] In addition, SA can be hydrogenated to obtain 1,4-butanediol (that can be in turn carbonylated to obtain AA) (Fig. 3).

Terephthalic acid (TA)

TA is one of the most important monomers for polyester synthesis since it is one of the starting monomers (together with ethylene glycol) for the production of poly(ethylene terephthalate) (PET). TA is currently produced via catalytic oxidation of petrochemical *p*-xylene and has an estimated global market of 50 million tons per year.[35] Nevertheless, three routes already exist enabling the synthesis of bio-based *p*-xylene: (i) pyrolysis of biomass; (ii) yeast fermentation of sugars into isobutanol; and (iii) chemical conversion of carbohydrates.[36] Cycloaddition of acrylic acid and isoprene (both bio-based) to obtain fully bio-based TA has been also reported at the laboratory scale. The method can be adapted also for the synthesis of bio-based isophthalic acid.[37] An interesting alternative is represented by a the synthesis of bio-based TA starting from furfural, which is industrially produced from inedible cellulosic biomass.[38] Scheme 2 illustrates the most important routes for the production of bio-based TA. Notably, the announced production of bio-based 'plant bottle' by Coca Cola starting from bio-sourced PET is expected to have a major impact on the PET industry. At the moment, Coca Cola in partnership with Gevo uses bio-based TA derived from isobutanol as previously described. The bottles on the market are nowadays composed of up to 30% of bio-based monomers derived from sugar cane.[39]

Furandicarboxylic acid (FDCA)

Dehydration of sugars available within biorefineries can lead to a family of products, including dehydrosugars, furans and levulinic acid. FDCA is a member of the furan family, and is usually synthesized by oxidative dehydration of glucose using oxygen, or electrochemistry.[35] The conversion can also be carried out by oxidation of 5-hydroxymethylfurfural. FDCA has been suggested as an important renewable building block because it can substitute TA in the production of various polyesters due to comparable properties of the final material (e.g. PET and poly(butylene terephthalate)).[40] The versatility of this compound is also evident

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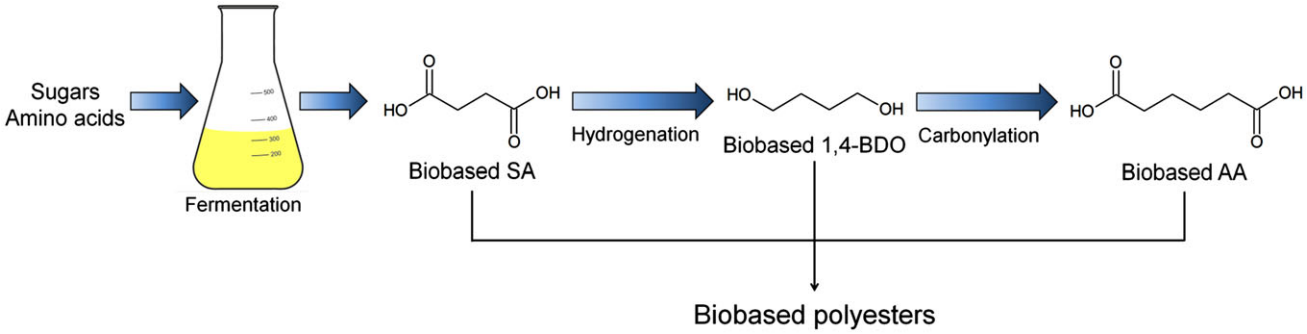
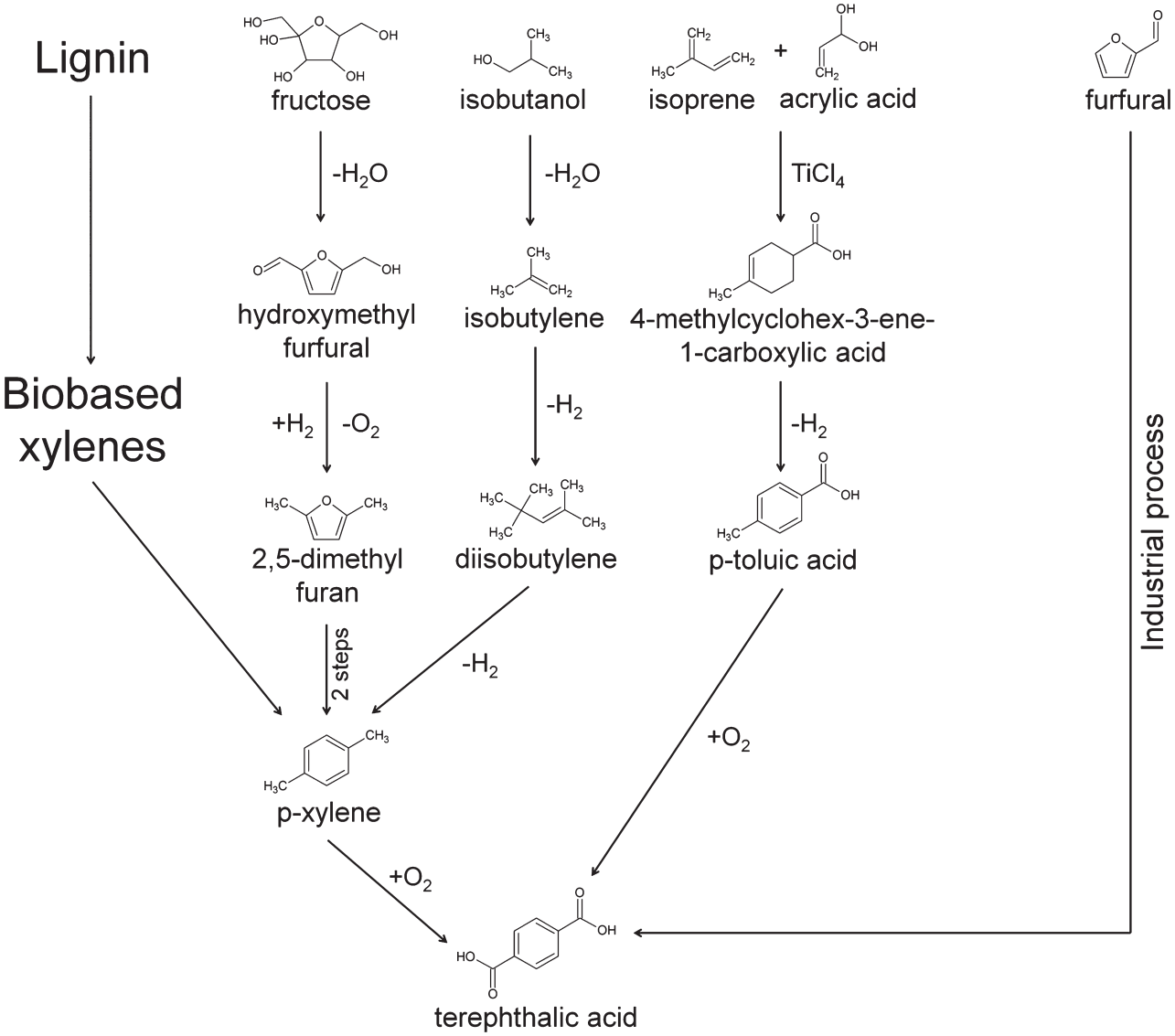


Figure 3. Biotechnological process for the production of bio-based succinic acid (SA) and its derivatives 1,4-butanediol (1,4-BDO) and adipic acid (AA).



Scheme 2. Most important routes for the production of bio-based TA.

when considering various FDCA-based derivatives accessible via relatively simple chemical reactions.[41]

The primary technical barriers for the production of FDCA from renewable materials include the development of effective and selective sugar dehydration, which is currently an uncontrolled process. In addition, the control of FDCA reactivity makes it difficult to develop efficient esterification reaction for FDCA-based polyesters synthesis.[42] Recently, Avantium (Geleen, The Netherlands) has announced a new technology that involves a highly reactive catalyst together with an efficient separation technology, which would result in economically feasible production of FDCA starting from 2016. The company is currently running a pilot plant

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with a 40 tons per year capacity. The planned industrial production capacity is estimated to be between 30 000 and 50 000 tons per year (http://www.avantium.com/).

The use of FDCA in the production of bio-based alternatives to PET is expected to account for over 60% of global FDCA production by 2020. Since PET is widely used in the food and packaging industries, there is a strong interest in developing bio-based alternative polymers. In particular, a combination of FDCA with ethylene glycol will lead to 2,5-furandicarboxylate; such a polymer is expected to be commercialized in 2018 with a production range of about 300 000 tons per year.[43] Another important application of FDCA is expected to be the production of aliphatic–aromatic polyamides.

Bio-based polyols

1,3-Propanediol (1,3-PDO)

The microbial production of 1,3-PDO is one of the oldest processes reported in the literature. This diol has a wide range of possible applications, e.g. composites, adhesives, solvents, monomers for aliphatic polyesters, and as an anti-freezing agent.[44] In addition, 1,3-PDO is used for the production of poly(trimethylene terephthalate), a polymer with remarkable 'stretch–recovery' properties that is used in apparel, upholstery, specialty resins and other applications. Various bacteria including *Klebsiella pneumoniae*, *Enterobacter agglomerans*, *Lactobacillus brevis* and *Clostridium butyricum* have been reported to produce 1,3-PDO during anaerobic growth on glycerol.[45] The highest concentration of 1,3-PDO was obtained using a *K. pneumonia* strain that led to a concentration of 73.3 g L^{-1}. In a continuous fermentation, the productivity was reported to be 3.5 times higher than in fed-batch mode.[46] In addition, DuPont is currently working on the development of a recombinant *Escherichia coli* strain for the fermentative production of 1,3-PDO where the necessary metabolic pathways, missing in wild type *E. coli*, have been introduced from a natural glycerol and 1,3-PDO producing *Saccharomyces cerevisiae*. Such engineered *E. coli* strain was successfully able to produce the desired diol up to a concentration of 135 g L^{-1} with a productivity of 3.5 g L^{-1} h^{-1}.[47]

2,3-Butanediol (2,3-BDO)

The industrial production of 2,3-butanediol (2,3-BDO) by fermentative process has been known since the twentieth Century. Microorganisms considered of industrial relevance are *Klebsiella* sp. and *Paenibacillus polymyxa* and their potential for 2,3-BDO production were extensively investigated by Ji *et al.*[48] The fermentation carbon source (commonly starch or sugar substrates) represents the main 2,3-BDO production cost; thus cheaper biomass-derived sugars like non-cellulosic (food industry residues, hexose-rich plants and glycerol) and lignocellulosic (corn cob, molasses and wood) might represent more profitable alternatives.

After the carbon source, the economic cost of downstream processing represents another important issue for the commercialization of microbially produced 2,3-BDO. Recovery of 2,3-BDO from fermented broth is especially difficult due to its high boiling point, great affinity for water and the presence of dissolved and solid constituents of the fermentation mash.[48] Efficient processes for 2,3-BDO recovery include aqueous two-phase extraction, *in situ* recovery and integrated solvent extraction and pervaporation.[49] These processes improve considerably the separation efficiency and represent the key for a successful and feasible 2,3-BDO fermentation in the near future.

The strong interest in implementing this bioprocess is driven by the large number of industrial applications of 2,3-BDO and its derivatives (e.g. printing inks, perfumes, foods, fumigants, softening agents, plasticizers and pharmaceutical products). Moreover, the microbial production will increase the independence of oil supply and price for the production of platform chemicals.[50]

1,4-Butanediol (1,4-BDO)

1,4-BDO is an important chemical that is used for the manufacture of over 2.5 million tons of polymers annually. Nowadays its production is almost entirely based on fossil carbon resources (production via the Reppe process in which acetylene is reacted with formaldehyde) with the exception of BASF and Bioamber that started production via hydrogenation of SA which is accessible from biogenic sources as described above.[51]

A promising laboratory-scale alternative for direct 1,4-BDO production from fermentation is reported by Yim *et al.* 1,4-BDO is a non-natural compound that is not normally synthesized by living organisms, so the metabolic pathway for its production is totally absent in such microorganisms.[52] An algorithm was used in order to predict which pathways are needed for the synthesis of 1,4-BDO using *E. coli* as host. An optimized pathway, using glucose as carbon source, was achieved and resulted in a productivity of 18 g L^{-1} of 1,4-BDO.[52]

1,4-BDO and its derivatives represent a market ripe for the introduction of a competitive bio-based route. An economic analysis of 1,4-BDO production has shown that biologically derived SA has the potential to dramatically decrease production costs of 1,4-BDO and therefore of its derivatives: tetrahydrofuran (important reaction solvent and also widely used in the manufacture of various polymers), γ-butyrolactone (currently manufactured from either 1,4-BDO or maleic anhydride), *N*-methylpyrrolidone and 2-pyrrolidone.[34] Recently, the successful production of bio-based 1,4-BDO on a commercial scale was achieved from the partnership between Genomatica and DuPont Tate & Lyle. Five million pounds were produced in the Tennessee Biochemical Plant by direct fermentation using conventional sugars as feedstock.

Glycerol (GLC)

Microbial production of GLC has been known for more than 100 years and it was already commercially produced during World War I. After this period, GLC biotechnological production declined since it was unable to compete with chemical synthesis from petrochemical feedstocks, especially because of low GLC fermentation productivity and difficulties in extraction and purification from fermentation broths.[21] As the cost of propylene has increased and its availability has decreased, especially in developing countries, GLC has become an attractive feedstock for production of various chemicals; GLC microbial production has become attractive again as an alternative route.[22] The production of this polyol by *Saccharomyces cerevisiae*, in anaerobic conditions, processes sugars into ethanol in a redox-neutral process which leads to GLC as by-product. GLC can also be produced by bacteria and algae. Bacterial production is known for the slow fermentation rate and relatively low yields and therefore has received limited attention in the past decades. Only processes based on *Lactobacillus lycopersici* and *Bacillus subtilis* have shown promising yields of up to 30%.[53] Interestingly, it is possible to use directly CO_2 and light for the autotrophic production of GLC using green algae species *Dunaliella tertiolecta* and *D. bardawil*. Preliminary studies led to up to 5 g L^{-1} of GLC in hypersaline medium.[53]

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GLC is a simple polyalcohol with many uses in the cosmetic, paint, automotive, food, tobacco, pharmaceutical, pulp and paper, leather and textile industries or as a feedstock for the production of various chemicals. GLC has also been considered as a feedstock for new industrial fermentations in the future. For example, GLC can be fermented to 1,3-PDO.[44] The present GLC production reaches volumes of about 60 000 tons per year. Nowadays, approximately 25% of world GLC production occurs via the oxidation or chlorination of propylene, but this route has declined in importance partially because of environmental concerns.[53]

Sorbitol (SO) and isosorbide (ISO)

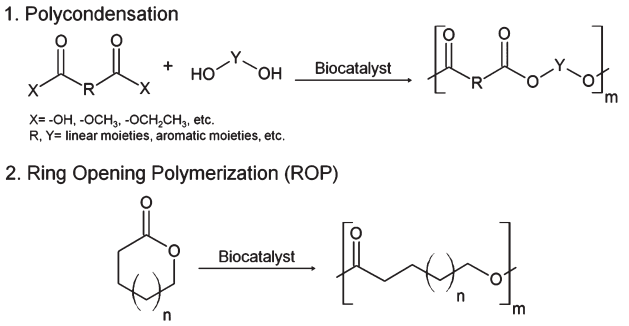
Despite the fact that chemical reduction of glucose is well established and leads to the production of over 50 000 tons of SO per year, a number of promising biochemical routes for SO production have been investigated. All of the commercial processes for SO production are based on batch processes using Raney nickel as catalyst which ensure the complete conversion of glucose.[52]

The main applications for SO are related to the food industry and involve very strict regulations in terms of purity. Relevant SO non-food applications are as polyol component in polyurethanes, as components of heat stabilizers and alkyl resins in varnishes, paints and inks.[54] Fermentations of sucrose or glucose–fructose mixtures by *Zymomonas mobilis* were reported to produce quantitative yields of SO and gluconic acid as co-products.[55] Recently an efficient conversion of glucose to SO using resting cells of an engineered *Lactobacillus plantarum* (with a theoretical yield of 97%) has been reported.[56] Despite the biochemical routes described above, the most common way to obtain SO is the enzymatic hydrolysis of cereal-extracted starch and mannans followed by production of D-sorbitol and D-mannitol via hydrogenation.

ISO, another important bio-based monomer for polyester synthesis, can be obtained via dehydration of SO. ISO is currently produced at an industrial scale and is commercially available, but its insufficient purity and high cost have limited its exploitation in the polymer field. Nowadays the improvement of purification techniques together with the lowering of the production costs has renewed the interest in ISO as a polyester building block.[57] ISO has been demonstrated to be a very effective monomer for increasing the glass transition temperature of polymers. Currently, ISO is used as copolymer with PET in rigid bottle production and for hot fill applications (glass replacement).[42]

Ethylene glycol (EG)

EG is also an important diol for the polymer industry since it is one of the two components of PET. The huge increase of biodiesel production over the last few years where GLC represents a by-product has led to a dramatic price reduction, making this monomer a very attractive starting material for the production of EG and 1,2-propylene glycol. The conversion of GLC into a family of derivatives (that includes EG, 1,2- and 1,3-propylene glycol) is obtained via a catalytic hydrogenolysis.[54] Petrochemical EG is currently being produced on an industrial scale starting from the oxidation of ethylene followed by water addition. Since it is possible to produce bio-based ethylene from ethanol it is also possible to produce bio-based EG; however, even if such a bio-based pathway is already technically feasible, the process turns to be not efficient in terms of carbon and oxygen yields. Processes for EG production via xylitol, SOR and GLC are more sustainable, although still require further development. Nowadays the ethylene oxidation process is the most widely used for EG production. Leading soft drink manufacturers use nowadays up to 30% of EG from renewable origin, in this specific case sugar cane, in the production of PET bottles. Another sustainable process for the production of EG from lignocellulose feedstock is still in an initial phase.[35]



Scheme 3. Routes for the enzymatic synthesis of bio-based polyesters. (1) Polycondensation reaction of diacids (or their diesters) with polyols. (2) Ring-opening polymerization of lactones.

SUSTAINABLE BIOCATALYTIC METHODS FOR POLYESTER SYNTHESIS

After the development of inorganic and Ziegler–Natta polymerization catalysis, an alternative strategy arose during the 1980s involving enzymes as biocatalyst for polymer synthesis. Esterases, and in particular lipases, catalyse the hydrolysis of fatty acid esters in aqueous environments.[12] Some of these hydrolases were found to be stable in organic solvents where they are able to catalyse reverse reactions, namely esterification and transesterification.[58] Hence, these enzymes were studied regarding synthesis of aliphatic – and to a lesser extent of aromatic – polyesters.[59]

Hydrolases for polyester production are relatively stable, commercially available and easily produced. Among lipases, undoubtedly the most widely used biocatalyst for polyester synthesis is lipase B from *Candida antarctica* (CaLB), due to its commercial availability as a free and an immobilized catalyst.

Polycondensation *versus* ring-opening polymerization (ROP)

Polyester synthesis can be accomplished via polycondensation of dicarboxylic acids with polyols (Scheme 3). Early reports on enzymatic polycondensation of dicarboxylic acids and polyols indicate the formation of only low-molecular-weight products.[60] In order to produce polyesters of high molecular weight, it is necessary to remove the by-products (water or alcohol in the case of diesters) formed during the reaction in order to shift the equilibrium to the polymerization reaction.[61] Equilibrium can be shifted towards polyesterification by using diesters instead of dicarboxylic acids, since the volatility of the by-product (alcohol) is higher than that of water (by-product from dicarboxylic acids).[60] Likewise, vinyl esters of dicarboxylic acids have been reported to be very effective monomers for enzyme-catalysed polymerizations since the vinyl alcohol (polycondensation leaving group) is irreversibly tautomerized to acetaldehyde (boiling point of 20.2 °C) leading to the desired polyester in high yields, even if high acetaldehyde concentration might lead to a decrease of enzyme activity, clearly affecting biocatalyst reuse. An effective and commonly used method

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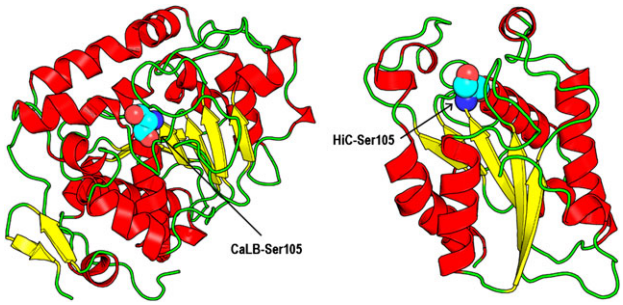

Figure 4. Structure of the most used enzyme for the biocatalysed synthesis of polyesters nowadays, CaLB (on the left), compared with the emerging cutinase from *Humicola insolens* (HiC) on the right (structures retrieved from Protein Data Bank, codes 1TCA and 4OYY, respectively). Catalytic serine for each enzyme is highlighted in sphere representation.

to increase the polymer molecular weight and the reaction yield is to perform the reaction under vacuum conditions to remove by-products and boost thermodynamic equilibrium towards the synthesis.[62]

In the 1990s the first enzymatic ROPs of lactones to give the corresponding polyesters were described (Scheme 3). ROP of lactones and carbonates does not produce a leaving group during the course of the reaction.[63] Unsubstituted lactones with a ring size of 4–17 carbon atoms were polymerized using various lipases both in bulk and in a variety of solvents.[64] A representative example of ROP is given by the synthesis of polycaprolactone starting from ε-caprolactone. Toluene has been selected as solvent for CaLB-catalysed ROP, since it is able to solubilize substrates and products while retaining the activity of the biocatalyst.[65] Water plays a major role in ROP reactions and it is important to ensure its removal from the reaction system to achieve good conversions and high-molecular-weight products. Gross and co-workers recently reported the synthesis of polyesters with M_w of 163 kDa after only 15 min of reaction, starting from ω-pentadecalactone and using a reactive extrusion technique.[66]

Biocatalysts for polyester synthesis

In 1984 Okumura and co-workers published the first study where a lipase from *Aspergillus niger* was able to catalyse the polycondensation of several dicarboxylic acids and polyols to afford short oligomers.[67] Following this study, lipases gained interest as catalysts for polyester synthesis and several members of this family were investigated to explore their potentialities in this field.[63] In the 1990s Linko and co-workers conducted a wide range of studies where they compared various lipases. From these experimental evidences, the most promising biocatalysts for polyester synthesis turned out to be the lipase from *Mucor miehei*.[63] Using this enzyme, polymers of AA and 1,4-BDO were obtained with a molecular weight of over 40 kg mol^{-1}.[64]

After these studies carried out between 1980 and the 1990s, CaLB (Fig. 4) arose as the biocatalyst of choice and it still remains as the most commonly used enzyme for synthetic applications.[68–71]

The most used preparation of CaLB is the commercially available Novozym 435®, consisting of the lipase adsorbed on a macroporous acrylic resin.[12] The biocatalyst displays different activity and selectivity according to the medium used for the reaction, so that supercritical carbon dioxide was also tested as a reaction solvent as an alternative to the most commonly used organic solvents or bulk reactions.[72,73]

The need to enlarge substrate specificity for the synthesis of new polyesters has boosted the study of various hydrolases, especially belonging to the cutinase family. Cutinase from *Humicola insolens* (Fig. 4) was studied by Gross and co-workers in the ROP and polycondensation reactions starting from a wide range of substrates.[74,75] Improvement of catalytic activity of CaLB towards ROP of D-lactic acid[76] has also been achieved through protein engineering approaches. Recently also the cutinase 1 from *Thermobifida cellulosilytica* was investigated for bio-based polyester synthesis with a combined experimental and modelling approach.[77]

Challenges and advantages of biocatalytic routes

It must be noted that suitable activity and specificity of an enzymatic protein is not sufficient to guarantee its efficient application to polyester synthesis. The enzymes must be immobilized[78–83] to allow recovery and reuse and that implies the necessity of tailored immobilization protocols preventing the detachment of the protein from the support and the contamination of the product. Moreover, the reaction configuration must preserve the integrity of the biocatalysts, which easily undergo fragmentation under mechanical and magnetic stirring conditions.[58,83–87] This is even more relevant in the case of solvent-free polymerizations, where the viscosity of the reaction system requires vigorous mixing.[88] New covalent immobilization methods and reactor configurations exploiting thin-film systems have been recently developed to overcome these technological limitations.[12,89] Finally, downstream processing still represents a major challenge in enzymatic synthesis of polyesters. Supercritical carbon dioxide[90] and ionic liquids[91] were explored as possible greener solutions to limit the use of conventional organic solvents.

These scientific and technological challenges still prevent the biocatalysed production of polyesters at an industrial scale. Nevertheless the research in this field is motivated by the necessity of replacing classical chemical routes[92] employing toxic catalysts (e.g. Zn, Mg and Co acetates, Sb and Ti oxides). Moreover, temperatures required by chemical polycondensation (generally >200 °C)[93,94] cause undesired side reactions (e.g. dehydration of polyols or β-scissions of polyesters to acid and alkene terminal groups) and degradation of chemically unstable monomers. For example, siloxane, epoxy and vinyl moieties (Scheme 4) represent unsuitable functional groups which react in an uncontrolled way;[95] on the other hand, such functional polyesters are of extreme interest in the biomedical field due to their biodegradability and lower toxicity. In this case enzymatic catalysis represents an appropriate way to obtain functional polyesters containing sensitive groups. For example, IA (or its derivatives) was polymerized with several polyols to give side-chain-functionalized polyesters where the vinyl moiety was preserved after the reaction and could therefore be used for further functionalizations/crosslinking of the polymer.[59,96] In particular, IA was successfully polymerized with 1,6-hexanediol leading to a mixture of products with M_w of around 30 kDa.[97]

Similar polyesters containing a lateral epoxy moiety were also reported after a polycondensation carried out using CaLB as catalyst.[98]

A future challenge for enzymatic polycondensations certainly comprises the incorporation of aromatic dicarboxylic esters as substrates in order to produce PET-like aromatic–aliphatic polyesters. Several attempts to polymerize such compounds were reported[99,100] and they led to products with modest molecular weight.[101]

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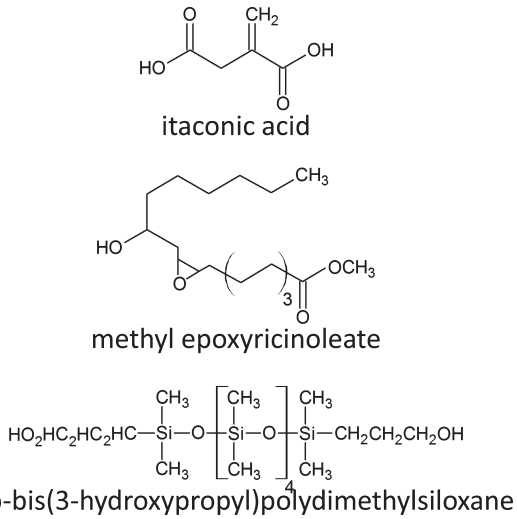


Scheme 4. Monomers carrying functional groups which can react in an uncontrolled way with classical chemical polymerization methods.

ENZYMATIC DEGRADATION AND TARGETED MODIFICATION OF POLYESTERS

Ever since plastics were introduced into human daily life, a significant improvement of stability and durability has been achieved, producing materials strongly resistant to environmental conditions (e.g. polyethylene bags). With an increase of the worldwide demand over the years, the amount of plastic materials started to be an environmental problem due to their durability in the environment after disposal, leading to ecological problems like the formation of the plastic ocean patches or their presence in rivers.[102]

Nevertheless, polymers with a heterogeneous atom backbone composition such as polyesters, polyamides and polyurethanes can be degraded by microorganisms and isolated enzymes.[103,104] Most importantly, mild enzymatic reactions allow one to finely tune and control the degree of hydrolysis and functionalization of a polymeric surface, which can be exploited for further functionalization and advanced applications (Fig. 5).[105,106] In that respect, modification and functionalization of PLA have been extensively studied and the topic has been reviewed by Belgacem and Gandini.[5]

Regarding PET, because of its highly hydrophobic nature, studies have been focused on the increase of its wettability to enable the grafting of molecules like flame retardants or water-soluble dyes.[107] Chemical treatments of PET cause unselective hydrolysis and severe reduction of molecular weight,[13,15,108] whereas the enzymatic hydrolysis catalysed by cutinases from several *Thermobifida* species (and not only) enables the tuning of surface functionalization without affecting the bulk properties of the polymer (Scheme 5).[106,109–112] Cutinases from other organisms such as *Fusarium solani*, *Pseudomonas mendocina* and *Humicola insolens* were also reported to be active on PET.[15,113,114]

Enzymatic hydrolysis is not only important for surface functionalization of PET as mentioned above. Currently, the majority of PET produced is used in the plastic bottle manufacturing industry, which uses low-crystallinity PET to achieve high bottle transparency. This material is an optimal substrate for enzymatic hydrolysis; therefore cutinases were suggested for PET recycling[13,115] in a process which could overcome the quality limitation of current recycling strategies based on blending, by hydrolysing the polymer to its constituent monomers, TA and EG.

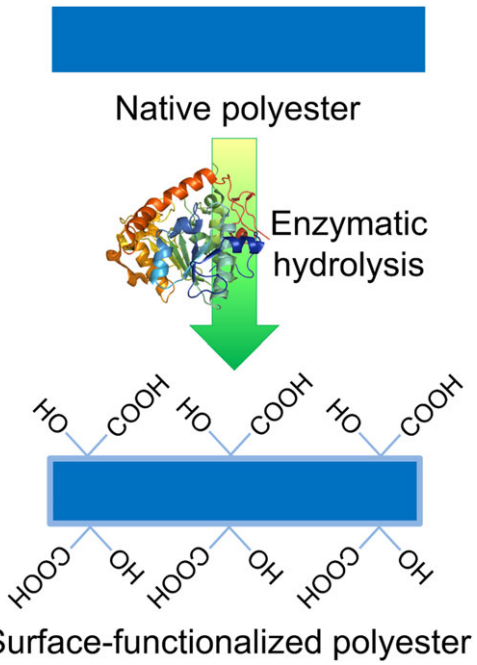
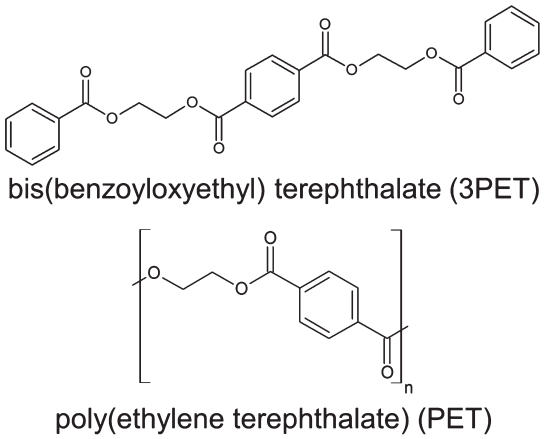


Figure 5. Enzymatic functionalization of polyesters. The biocatalyst hydrolyses only the surface chains of the polymer, leaving the bulk properties unaltered.



Scheme 5. Structure of bis(benzoyloxyethyl) terephthalate model substrate (top) and of poly(ethylene terephthalate) aromatic–aliphatic polyester.

The resulting monomers are potentially reusable as substrates for polymerization after separation of dyes and contaminants. Protein engineering approaches were used to enable the practical applicability of enzymes in PET degradation and, more specifically, to increase the thermal stability,[116] the activity[13] and the absorption[11] of the enzyme on PET substrates.

The study of enzymatic degradation and modification of polyesters also involves poly(1,4-butylene succinate) (PBS) and similar copolymers such as poly(1,4-butylene adipate), poly[(1,4-butylene succinate)-*co*-adipate] (PBSA) and poly[(1,4-butylene succinate)-*co*-(ethylene succinate)] (PBSE). Their biodegradability was assessed both in seawater and in soil but the degradation mechanism is still a subject of research. Lee and co-workers describe the degradation mechanism of PBS using a lipase from *Pseudomonas cepacia* as biocatalyst. This enzyme

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was found to be highly active in the degradation of this aliphatic polyester and its copolymers. An exo-type hydrolysis mechanism was proposed since the terminal chain of PBS possesses a conformational similarity to the ordinary di- and triglycerides which are the natural lipase substrates.[117]

Honda *et al.* investigated also the hydrolysis of a co-polymer of PBS and PLA, namely poly[(1,4-butylene succinate)-*co*-(L-lactate)]. In their work they describe how the enzymatic hydrolytic process is influenced not only by the crystallinity but also by the orientation of the polymer chains of the film.[118]

Lipase from *Candida cylindracea* was used for the hydrolysis of PBSA, a tri-component mixture of 1,4-BDO, AA and SA: functionalization was achieved while no significant decrease of molecular weight was observed. Enzymatic degradation of these polymer types varied depending on both thermal properties and degree of crystallinity.[119]

PBSE was investigated by Mochizuki and co-workers, who studied the degradation catalysed by various lipases. Lipase from *P. nitens* turned out to be the most efficient and this was ascribed to the high hydrophobicity of the lipase binding domains. Additionally, cutinase from *Thielavia terrestris* (25 mmol L^{-1} citrate buffer, pH = 4, 50 °C for 24 h)[120] and two cutinase-like enzymes from *Cryptococcus* sp. strain S-2 and from *Pseudozyma antarctica* were reported to be active in the hydrolysis of PBS and other polyesters.[121]

OUTLOOK

Biotechnological production of renewable monomers is already contributing to the replacement of petroleum-based plastics with novel bio-based polymeric products. Improvement of the efficiency of fermentation processes remains a major challenge for enlarging the array of monomers available at an industrial scale, thus following the successful example of PLA. In the future, polymer chemistry is expected not only to introduce bio-based monomers within existing productive processes, but also to create new options for the plastic market starting from the most promising building blocks. In that respect, the possibility to tune polymer functional properties and biodegradability by means of optimized biocatalysts will guide the development of new effective polymers. Significant issues that are so far unresolved and which require further research are the development of an agreed methodology for the evaluation of emissions from direct and indirect land use change and the quantification of the impacts of biomass production on regional biodiversity. Such assessments should be complemented by further approaches like environmental risk assessment, or certification approaches for good and sustainable agricultural practices like eco-management and audit schemes. Currently, no single approach gives a complete and balanced picture.

Author contributions

The article was written through contributions of all authors. All authors have given approval to the final version of the article.

NOTES

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