Chapter 1

Enzymatic Synthesis of Biobased Polyesters and Polyamides

Abstract: Enzymatic polymerization of biobased polymers is an appealing topic both in academia and industry areas, as it provides a great opportunity to address the current pollution problems in the polymer industry and to reduce the dependence of depleting fossil resources. The scope of this research is to establish a biocatalytic approach towards various biobased polyesters and polyamides, including saturated aliphatic polyesters, unsaturated aliphatic polyesters, furan polyesters (semi-aromatic polyester alternatives) and furan polyamides (semi-aromatic polyamide analogues). In addition, the structure-properties relationships are established for the synthesized biobased polyesters and polyamides, to explore their potential applications. In this chapter, a general introduction about polyesters and polyamides is presented; and the biobased monomers used in this research for biocatalytic polyester and polyamide synthesis are outlined. Moreover, lipases and lipase-catalyzed polymerization of polyesters and polyamides are briefly introduced. Furthermore, the recent developments in the field of the enzymatic synthesis of biobased polyesters and polyamides are discussed in details.
1.1 Polymers: from Petrol-Based to Biobased and Beyond

Polymers are one of the most important materials that are being exploited and developed by mankind, which play an essential and ubiquitous role in our modern life. They are large molecules or macromolecules that are composed of many small molecular fragments known as repeating units. They are in widespread use as plastics, rubbers, fibers, coatings, adhesives, foams and specialty polymers.\(^1\)

According to their origin, polymers can be classified as natural polymers or synthetic polymers. Natural polymers occur in nature via \textit{in vivo} reactions, where biocatalysts, normally enzymes, are inevitably involved. Natural polymers can be found in all living organisms: plants, animals and human beings. Examples of natural polymers include lignocellulose, starch, protein, DNA, RNA and polyhydroxyalkanoates (PHAs), just to name a few. Normally the structures of natural polymers are well-defined, with some exceptions like lignocellulose.

Synthetic polymers are commonly produced via polymerization of petrol-based chemicals having simple structures. Chemical catalysts, especially metal catalysts, are normally used in the production of synthetic polymers. As the booming of petrochemical industry and the concomitant availability of cheap petroleum oils, as well as, the well establishment and advancement of polymerization techniques, numerous synthetic polymers are developed, for example, phenol-formaldehyde resins, polyolefins, polyvinyl chloride, polystyrene, polyesters and polyamides, and so on. Synthetic polymers which include the large group known as plastics, became prominent since the early 20\textsuperscript{th} century; and plastics are widely used as bottles, bags, boxes, textile fibers, films, and so on. In contrast with natural polymers, synthetic polymers usually possess much more simple and random structures.

Currently, there is a huge demand for polymers. The global production of plastics increased from 225 million tons in 2004 to 311 million tons in 2014 (Scheme 1.1);\(^2\) and the global polymer production is expected to reach 400 million tons in 2020.\(^3\) This huge polymer consumption leads to a massive demand for fossil resources for the polymer industry, which however brings some severe problems. On the one hand, fossil resources are depleting resources with limited storage; and their formation requires millions of years. There is a great concern that fossil resources will be exhausted fast within several hundred years. On the other hand, hazardous waste and emissions are generated along with the consumption of fossil resources, which induce severe environmental problems such as global warming and pollutions like smog and haze which are breaking out frequently for instance in China nowadays. Driven by the growing environmental concerns, it is necessary and appealing to
develop sustainable polymers for reducing the current dependence on fossil resources and decreasing the production of pollutants. As a matter of fact, laws have been approved by the European Union to reduce the usage of environmentally abusive materials, and to trigger more efforts to find eco-friendly materials based on renewable resources.\textsuperscript{4, 5}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{Global production of plastics from 2004 - 2014.\textsuperscript{2}}
\end{figure}

Biobased polymers are pointed out to be the most promising alternatives,\textsuperscript{5-16} which are defined as “sustainable materials for which at least a portion of the polymer consists of materials that are produced from renewable raw materials”.\textsuperscript{17} Generally speaking, biobased polymers can be produced via three routes.\textsuperscript{8, 11} (1) pristine natural polymers, or chemical or physical modifications of natural polymers; (2) manufactured biobased polymers from a mixture of biobased molecules with similar functionalities that are converted from biomass feedstocks; and (3) synthesis of biobased polymers via polymerization of biobased monomers with tailored chemical structures.

Some natural polymers such as natural rubber, cotton, starch and PHAs, are useful materials; however, they are limited in variety, and their properties and applications are also limited as they are determined by their chemical structure. Considering the rich abundance of biomass feedstocks in nature, it is of great interest to produce biobased polymeric materials by chemical or physical modifications of natural polymers, or from biobased molecules that are converted from biomass feedstocks. Actually the former approach was already used by human beings long time ago during the 1800s. Many commercially important polymers are obtained via this approach, for example, vulcanized natural rubber, gun cotton (nitrocellulose), cellulose esters and cellulose ethers. However, chemical and physical modifications of natural polymers are often subject to the poor solubility and process difficulty of natural polymers, as well as, unwanted impurities within the network of natural
polymers which are hard to remove. On the other hand, conversion of biomass feedstocks to end-products is a promising pathway for the production of high tonnage consumer polymeric products such as paper, paints, resins and foams.\textsuperscript{11} For instance, oleochemicals can be converted from vegetable oils and fats, which are biobased building blocks for the production of thermoset resins and polyurethanes. However, the obtained biobased polymeric materials often possess diverse chemical structures; and it is nearly impossible to produce biobased polymers with identical structures as the petrol-based counterparts, due to the use of biomolecule mixtures. Besides, some unwanted structures or impurities might be inherited from the biomolecule mixtures, which might greatly influence the properties and applications of the final polymeric materials.

Utilization of biobased monomers with tailored structures in polymer synthesis is the most promising approach towards biobased polymers, which can result in not only sustainable alternatives to petrol-based counterparts with similar or identical structures, but also in novel green polymers that cannot be produced from petrol-based monomers.\textsuperscript{5, 8, 9, 14-16} However, this is also the most expensive approach of all three as aforementioned.

Benefiting from solar energy, numerous biobased monomers can be produced from yearly-based biomass feedstocks via biocatalytic or chemo-catalytic processes, which provide a great opportunity for the synthesis of various kinds of biobased polymers.\textsuperscript{5, 7-11, 14-16, 18-26} Meanwhile, more and more biobased monomers are already or will become commercially available in the market due to the fast development of biotechnologies and their price will be competitive with that of the petrol-based chemicals.\textsuperscript{26-33}

Enzymatic polymerization is an emerging alternative approach for the production of polymeric materials, which can compete with conventional chemical synthesis and physical modification techniques.\textsuperscript{34-43} Enzymatic polymerization also provides a great opportunity for the synthesis of novel macromolecules that are not accessible via conventional approaches. Moreover, with mild synthetic conditions and renewable non-toxic enzyme catalysts, enzymatic polymerization is considered as an effective way to reduce the dependence of fossil resources and to address the high material consumption and pollution problems in the polymer industry.

At present, petrol-based monomers are still predominately used in enzymatic polymerizations. By combining biobased monomers and enzymatic polymerizations in polymer synthesis, not only the research field of enzymatic polymerization could be greatly accelerated but also the utilization of renewable resources will be
promoted. This will provide an essential contribution for achieving sustainability for
the polymer industry, which will eventually play an important role for realizing and
maintaining a sustainable society.

In this research, we focus on the enzymatic synthesis of sustainable polyesters and
polyamides from biobased monomers, using lipases as the biocatalysts. In the
following section of this chapter, some background information about polyesters,
polyamides, biobased monomers, lipases and enzymatic polymerization of
polyesters and polyamides is introduced briefly. Then, the current research progress
in the field of the enzymatic polymerization of biobased polyesters and polyamides
is discussed in details. At last, this chapter ends with an overview of the research
work presented in this thesis.

1.2 Polyesters and Polyamides

Polyesters are polymers in which the monomer units are linked together by ester
groups, while in polyamides the repeating units are connected by amide bonds.
According to the composition of the main chain, polyesters and polyamides can be
classified to three types: aliphatic, semi-aromatic and aromatic (Scheme 1.2). In this
research, we focus on the enzymatic synthesis of aliphatic polyesters, semi-aromatic
polyesters and semi-aromatic polyamides.

![Scheme 1.2. General chemical structures of aliphatic, semi-aromatic and aromatic
polyesters and polyamides.](image)

Most known aliphatic polyesters could be produced as biobased polymers, as the
majority of their building blocks can be derived from biomass feedstocks. Aliphatic
polyesters are also (bio)degradable materials which can be recycled and have a low
environmental impact upon disposal, compost and incineration. Aliphatic
polyesters are widely used as thermoplastics and thermoset resins, with many
commodity and specialty applications. Among them, poly(lactic acid) (PLA) is the
most well-known aliphatic polyester, which can be used as fibers, food packaging
materials and durable goods, with a global demand of around 360 kilo tons in 2013. Poly(butylene succinate) (PBS) is another important commodity polyester which can be applied as packaging films and disposable cutlery, with a global market of around 10 - 15 kilo tons per year. In addition, aliphatic polyesters have found potential applications in biomedical and pharmaceutical fields such as in sutures, bone screws, tissue engineering scaffolds, and drug delivery systems, due to their biodegradability, biocompatibility and probable bioresorbability.

Compared to aliphatic polyesters, semi-aromatic polyesters generally possess better thermal and mechanical properties, which can be used as commodity plastics and thermal engineering plastics. Poly(ethylene terephthalate) (PET) is the most commonly used semi-aromatic polyester. It is the fourth-most-produced plastic, with a global supply of more than 19.8 million tons in 2012. PET has been widely used as beverage bottles, food containers, fibers and fabrics, packing films, photographic and recording tapes, engineering resins, and so on.

Similar to semi-aromatic polyesters, semi-aromatic polyamides consist of both aliphatic and aromatic fragments in the polymer main chain. Especially polyphthalamides (PPAs) are a type of semi-aromatic polyamides that are defined as “polyamides in which at least 55 mol % of the carboxylic acid portion of the repeating unit in the polymer chain is comprised by a combination of terephthalic acid (TPA) and isophthalic acid (IPA)”.

Semi-aromatic polyamides possess many merits such as high chemical, thermal, abrasion and corrosion resistance, good dimensional stability, excellent mechanical strength and superior processing characteristics. They can be used as thermal engineering materials and high performance materials, which have found various applications in many areas such as in marine, automotive industry, oil industry, electronics, machinery, domestic appliances, and personal care. Examples of semi-aromatic polyamides are poly(hexamethylene terephthalamide) (PA 6,T), poly(nonamethylene terephthalamide) (PA 9,T), and poly(decamethylene terephthalamide) (PA 10,T).

Generally speaking, polyesters and polyamides can be produced via two methods: (1) step-growth polycondensation; and (2) ring-opening polymerization. Both of these two methods have some merits and also suffer from some drawbacks. On the one hand, the building blocks for step-growth polycondensation are generally easily obtained at a relatively cheap price. However, elevated reaction temperatures (≥ 150 °C), long reaction times, high vacuum condition, heavy metal catalysts and a precise stoichiometric balance between monomers are normally required for polycondensation. In addition, side-reactions and volatilization of monomers may occur at elevated temperatures or under high vacuum. On the other hand,
removal of by-products is not required by ring-opening polymerization and, therefore, high molecular weight products can be obtained under relatively mild conditions in a matter of minutes. Besides, side reactions can be greatly suppressed during ring-opening polymerization. However, extra synthesis steps and heavy metal catalysts are often required for the preparation of the starting materials, cyclic monomers and cyclic oligomers.

**Table 1.1.** A selected list of commercially available biobased aliphatic polyesters, semi-aromatic polyesters and semi-aromatic polyamides

<table>
<thead>
<tr>
<th>Entry *</th>
<th>Type</th>
<th>Biosourcing (%) b</th>
<th>Manufacturer</th>
<th>Trademark</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>Aliphatic polyester</td>
<td>up to 100</td>
<td>NatureWorks (USA)</td>
<td>Ingeo™, NatureWorks®</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Synbra (NL)</td>
<td>BioFoam®</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Zhejiang Hisun (CN)</td>
<td>REVODE 100 and 200</td>
</tr>
<tr>
<td>PHAs</td>
<td>Aliphatic polyester</td>
<td>100</td>
<td>Metabolix and ADM (USA)</td>
<td>Mirel™</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Meredian (USA)</td>
<td>Nodax™</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tianjin Green Biosciences (CN)</td>
<td>GreenBio</td>
</tr>
<tr>
<td>PBS</td>
<td>Aliphatic polyester</td>
<td>50</td>
<td>PTT MCC BIOCHEM (TH)</td>
<td>BioPBST™</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Showa Denko K.K. (JP)</td>
<td>Bionolle™</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mitsubishi Chemical (JP)</td>
<td>GS Pla®</td>
</tr>
<tr>
<td>PEF</td>
<td>Semi-aromatic polyester</td>
<td>100</td>
<td>Avantium (NL)</td>
<td>-</td>
</tr>
<tr>
<td>PET</td>
<td>Semi-aromatic polyester</td>
<td>up to 30</td>
<td>Coca Cola (USA)</td>
<td>PlantBottle™</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Toyota Tsusho Corporation (JP)</td>
<td>GLOBIO®</td>
</tr>
<tr>
<td>PTT</td>
<td>Semi-aromatic polyester</td>
<td>37</td>
<td>DuPont (USA)</td>
<td>Sorona®</td>
</tr>
<tr>
<td>PA 10,T</td>
<td>Semi-aromatic polyamide</td>
<td>50</td>
<td>EMS-GRIVORY (DE)</td>
<td>Grilamid® HT3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Evonik (DE)</td>
<td>VESTAMID® HTplus M3000</td>
</tr>
<tr>
<td>PPA</td>
<td>Semi-aromatic polyamide</td>
<td>&gt; 70</td>
<td>Arkema (DE)</td>
<td>Rilsan® HT</td>
</tr>
</tbody>
</table>

* PLA = poly(lactic acid); PHAs = polyhydroxyalkanoates; PBS = poly(butylene succinate); PEF = poly(ethylene furanoate), PET = poly(ethylene terephthalate); PTT = poly(trimethylene terephthalate); PA 10,T = poly(decamethylene terephthalamide); PPA = polyphthalamide; b Biosourcing (%): the percentage of carbon originating from biomass sources among the total organic carbon.

Regarding to the equipment and the reaction conditions followed, the polymerization steps in polyester and polyamide synthesis are similar.55 However, with respect to the formation of high molecular weight products, the polymerization of polyamides differs from that of polyesters to some extent. Firstly, the chemical equilibrium is favored for the amide formation but is less favored for the ester formation. Secondly, when dicarboxylic acids are used as starting materials, salts are formed in polyamide synthesis, but there is no salt formation in polyester synthesis. In this case, stoichiometric equivalence can be much more easily achieved in polyamide
synthesis. Thirdly, the amide interchange reactions (transamidations) are much slower than the ester interchange reactions (transesterifications).

At present, several biobased aliphatic polyesters, semi-aromatic polyesters and semi-aromatic polyamides are already commercially available. A selected list is shown in Table 1.1.\textsuperscript{56-61} Among them, polyhydroxyalkanoates (PHAs) are produced by microorganisms; PLA can be synthesized via ring-opening polymerization and step-growth polycondensation, using chemical catalysts; and the other biobased polyesters and polyamides are normally prepared form step-growth polycondensation in the presence of organometallic catalysts.

However, polymers including polyesters, polyamides and other types, are mainly produced industrially from petrol-based monomers. The production capacity of biobased polymers represented only a 2% share of the total polymer production in 2013 and will increase to 4% by 2020.\textsuperscript{3}

1.3 Biobased Monomers Used in this Research

1.3.1 Biobased Aliphatic Diacids

Succinic acid is a naturally occurring dicarboxylic acid, which is predominantly produced commercially through petrochemical routes by catalytic hydrogenation of maleic acid or anhydride.\textsuperscript{62, 63} Succinic acid can also be produced by fermentation of carbohydrates or glycerol using engineered bacteria or yeast. The current bio-route for succinic acid is based on proprietary \textit{E. coli} or yeast strains.\textsuperscript{63} To lower the cost, other microorganisms and yeast have been developed, like \textit{Coryne}-type bacteria, which shows a significantly higher productivity compared to \textit{E. coli}.\textsuperscript{32} Currently, four companies have built up commercial facilities for the production of biobased succinic acid: Reverdia, Succinity, Bioamber and Myriant.\textsuperscript{28}

Itaconic acid is an attractive unsaturated monomer that has already been produced industrially by sugar fermentation using \textit{Aspergillus terreus} early in the 1960s.\textsuperscript{64, 65} To reduce the cost and increase the sustainability, current studies mainly focus on strain improvement of microorganisms by mutagenesis, development of more cost-effective process methodologies, and the use of alternative cheap substrates such as cellulosolytic biomass.\textsuperscript{66}

Adipic acid is one the most important commodity chemicals, which is mainly used for the production of nylon 6,6.\textsuperscript{32, 67} At present, over 90% of adipic acid is manufactured industrially by oxidation of cyclohexanol or KA-oil (a mixture of cyclohexanol and cyclohexanone) using concentrated nitric acid.\textsuperscript{67-70} In recent years,
two prospective biosynthetic pathways to biobased adipic acid have been developed and are under commercialization evaluation at the moment.\textsuperscript{30,32} (1) chemo-catalytic conversion of biologically derived precursors such as \textit{cis,cis}-muconic acid or D-glucaric acid; and (2) direct biological conversion of vegetable oils and sugars using yeast.

In addition, suberic acid, sebacic acid and dodecanedioic acid are also (potentially) biobased monomers which can be converted from plant oils.\textsuperscript{30,71-73}

### 1.3.2 Biobased Aliphatic Diols and Polyols

1,3-Propanediol (1,3-PDO) is a commodity chemical used for the production of various polymers. At present, there are two chemical processes for the industrial production of 1,3-PDO, starting from petrol-based acrylaldehyde or ethylene oxide.\textsuperscript{74,75} Nowadays, biobased 1,3-PDO is commercially synthesized via fermentation of D-glucose based on corn using a genetically engineered \textit{E.coli}.\textsuperscript{74} In addition, it is promising to produce 1,3-PDO from biomass-derived glycerol using a bacterial fermentation process.\textsuperscript{74-78}

1,4-Butanediol (1,4-BDO) is widely used as a building block for polymer synthesis.\textsuperscript{79} The industrial production of 1,4-BDO dominantly depends on petrol-based chemicals such as maleic anhydride, acetylene, butane, propylene and butadiene. Since late 2007, Genomatica (USA) started to develop a biological process for the synthesis of biobased 1,4-BDO from sugars using a genetically-modified strain of \textit{E.coli} bacteria.\textsuperscript{78-81} This process has already been commercialized.\textsuperscript{30} Alternatively, biobased 1,4-BDO can be produced by reduction of sugar-derived succinic acid and this process is under commercialization preparation stage.\textsuperscript{30}

![Scheme 1.3. Chemical structures of 1,4:3,6-dianhydrohexitols (DAHs).](image)

1,4:3,6-Dianhydrohexitols (DAHs) are sugar-derived aliphatic diols with rigid and chiral structures.\textsuperscript{82} It is of great interest to synthesize DAH-based polymers with high glass transition temperatures (\(T_g\)) and/or with special optical properties.\textsuperscript{83} According to the chirality, DAHs have three possible stereoisomers: isosorbide, isomanide and isoidide (Scheme 1.3). Due to their different positions of the hydroxyl groups, the reactivity of these isomers are different, showing the following sequence:
isomannide < isosorbide < isoidide.82,83 Nowadays only isosorbide is produced at an industrial scale using sugars as the starting materials;26,82 and Roquette (France) is a leading producer. However, the purity and high price of the commercial isosorbide are two major concerns when used for polyester synthesis.

Other aliphatic diols used in this research, including 2,3-butanediol, 1,6-hexanediol, 1,8-octanediol and 1,10-decanediol, are (potentially) biobased monomers.5,84,85

Moreover, glycerol and D-sorbitol are abundant and inexpensive biobased aliphatic polyols. Glycerol is obtained as a byproduct in the production of biodiesel from vegetable oils and fats.5,86 D-sorbitol is produced industrially on large scale by reduction of glucose derived from biomass feedstocks.32

1.3.3 Biobased Aliphatic Diamine

1,8-Octanediamine (1,8-ODA) can be potentially derived from biomass. It can be produced by amination of suberic acid which can be converted from plant oils.87

1.3.4 Biobased Furan Monomers

2,5-Furandicarboxylic acid (FDCA) is an interesting biobased rigid monomer, which is considered as the most promising substitute to petrol-based terephthalic acid (TPA) and isophthalic acid (IPA).5,14,88 Currently, FDCA is readily produced from biomass feedstocks, whereas biobased TPA and IPA are still under development with big challenges. As illustrated in Scheme 1.4, biobased FDCA can be synthesized by oxidation of 5-hydroxymethylfurfural (HMF) derived from carbohydrates;18,24 or be converted from HMF via a biocatalytic approach.89 At present, FDCA is industrially produced by Avantium (NL) using an enabling chemical synthesis technology;14,30 and the price is expected to be cheaper than the petrol-based TPA.30

![Scheme 1.4. Promising biobased furan monomers for polyester or polyamide synthesis.](https://example.com/scheme14.png)
Another interesting biobased furan monomer for polyester synthesis is 2,5-bis(hydroxymethyl)furan (BHMF). BHMF can be converted by reduction of HMF or FDCA (Scheme 1.4).\textsuperscript{5, 18, 24, 90}

### 1.3.3 Biobased Aliphatic Diamine

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### 1.4 Lipases

Lipases (triacylglycerol lipases, triacylglycerol acyl hydrolases, E.C. 3.1.1.3) are enzymes which catalyze the hydrolysis of water-insoluble triglycerides with long-chain fatty acids to di-glycerides, mono-glycerides and glycerol with release of free fatty acids in aqueous solution (Scheme 1.5). In organic synthesis, lipases can be used to catalyze other reactions in non-aqueous media, for example, esterification, transesterification, interesterification, amidation, transamidation, aminolysis, aldol condensation and Michael addition.\textsuperscript{91-95}

#### Scheme 1.5. Lipase-catalyzed hydrolysis of triglyceride.

Generally, lipases possess high catalytic reactivity in nonpolar organic solvents with log \( P \) (logarithm of partition coefficient) values of more than 1.9.\textsuperscript{96-98} Examples of suitable organic solvents for lipases are benzene (2), toluene (2.5), diphenyl ether (4.05), hydrocarbons like cyclohexane (3.2) and \( n \)-hexane (3.5), and so on.\textsuperscript{99} Lipases also function in some green solvents such as ionic liquids and supercritical \( \text{CO}_2 \).\textsuperscript{100-104}

All lipases possess a very similar \( \alpha/\beta \) hydrolase fold; and the active site of lipases consists of a highly conserved catalytic triad: a nucleophilic residue (serine), a histidine base and a catalytic acidic residue (aspartic or glutamic acid, usually aspartic acid) (Scheme 1.6). In addition, many lipases exhibit a lid, a surface loop that is a lipophilic \( \alpha \)-helical domain in the polypeptide chain and covers the active sites.\textsuperscript{105, 106} The lid controls the access of substrate molecules to the catalytic center of lipases.
**Scheme 1.6.** Secondary structure diagram of the α/β hydrolase fold and the location of catalytic triad amino acid residues in lipases. Ser: serine residue; Asp or Glu: aspartic or glutamic acid residue; His: histidine residue; helixes indicate α-helixes; arrows indicate β-sheets.107

**Scheme 1.7.** General catalytic mechanism of lipases.108

The general catalytic mechanism of lipases is illustrated in Scheme 1.7, which involves an acylation step followed by a deacylation step.105,108 At the acylation step, the hydroxyl group of the catalytic serine is activated by transferring a proton among the aspartate, histidine, and serine residues of the catalytic triad, rendering an increase of the nucleophilicity of the hydroxyl residue of the serine. After that, the hydroxyl residue of the serine attacks the carbonyl group of the substrate (carboxylic ester or carboxylic acid), forming the first tetrahedral intermediate with a negative charge on the oxygen of the carbonyl group. The oxyanion hole, which is formed by hydrogen bonding between the amide groups of the amino acid residuals of the enzyme and the carbonyl group oxygen of the substrate, stabilizes the charge...
distribution and reduces the state energy of the tetrahedral intermediate by forming at least two hydrogen bonds. Then the alcohol component (R₁–OH) is released from the bond with the intermediate, while the “acidic component” of the substrate remains covalently bound to the serine residue in the acyl-enzyme intermediate. When the enzyme is attacked by a nucleophile (R₂–OH), the deacylation step occurs. The product (a new carboxylic ester or carboxylic acid) is then released, while the enzyme is regenerated. This nucleophile (R₂–OH) can be water (hydrolysis) or an alcohol (alcoholysis).

Due to the broad substrate specificity, high selectivity, and high thermal stability and catalytic reactivity, *Candida antarctica* lipase b (CALB), which was reclassified as *Pseudozyma antarctica* lipase b (PALB) more recently,¹⁰⁹ is the most popular biocatalyst which is extensively used in biocatalytic synthesis of small molecules and polymers. CALB is a globular protein that is composed of 317 amino acids (Scheme 1.8), having a molecular weight of 33 kDa. Similar to other lipases, CALB possesses a Ser-His-Asp catalytic triad (Ser105, Asp187 and His224) in its active site and two oxyanion holes (Thr40 and Gln106),¹¹⁰ and the catalytic mechanism of CALB is the same as other lipases.

Scheme 1.8. (a) The crystal structure of *Candida antarctica* lipase b (1TCA, from http://www.rcsb.org/); and (b) a photo of Novozym® 435 beads.

However, the presence of the lid structure and the interfacial activation of CALB are still under debate. Some literature suggested that the two α-helixes (α5 and α10) surrounding the active center of CALB, the most mobile part of the structure, could work as the lid,¹¹¹-¹¹⁴ and CALB is an interfacial activated enzyme. A recent study indicated the hydrophobicity of the interface and the overall size of the substrate determine the interfacial activation of CALB.¹¹³ Others suggested that CALB has no lid covering the entrance of the active site¹¹⁰ and displays no interfacial activation.¹¹⁵ In addition, CALB has a very limited available space in the pocket of active site compared to other lipases and this explains its high selectivity.¹¹⁶
CALB shows improved thermal stability and more stable performance in its immobilized form. At present, several immobilized CALB formulations are commercially available, including Novozym® 435 (N435, Novozymes A/S, Denmark), Chirazyme® L-2 (Roche Molecular Biochemicals, Germany), LCAHNHE and LCAME (SPRIN S.p.A, Italy), and CalB immo Plus™ (c-LEcta and Purolite, Germany). They are used in the industrial fields for the synthesis of chiral intermediates in the pharmaceutical industry and for the production of other high priced fine chemicals.

N435 is the primary immobilized CALB that is used both in the industrial area and academia research. N435 functions as a hydrophobic biocatalyst, which consists of 10 wt % of CALB physically absorbed within 90 wt % of Lewatit VP OC 1600 bead which is a macroporous DVB-crosslinked methacrylate polymer resin. The bead size of N435 ranges from 0.315 to 1.0 mm (> 80 %), the effective size is around 0.32 - 0.45 mm, and the average pore diameter is 15 nm. N435 can work at mild conditions and especially, can tolerate some extreme conditions such as elevated temperatures (up to 150 °C).

1.5 Enzymatic Polymerization of Polyesters

Enzymatic polymerization is defined as “in vitro (in the test tubes) chemical synthesis of polymers via a non-biosynthetic (non-metabolic) approach using an isolated enzyme as the catalyst”.

Due to the unique properties of enzymes, enzymatic polymerization inherits many merits such as high specificity and selectivity towards monomer substrates, clean-process, energy saving, gentle environmental footprint, nontoxic natural catalysts, and recyclable catalysts (after immobilization). With these, enzymatic polymerization provides an opportunity to achieve “green polymer chemistry”.

At present, 4 enzyme classes, oxidoreductases, transferases, hydrolases and ligases, are identified to induce or catalyze polymerizations (Table 1.2). Many polymers are successfully synthesized via enzymatic polymerization, for example, vinyl polymers, polysaccharides, polyesters and polyamides. Among them, polyesters are the most extensively studied polymers in enzymatic polymerization; and lipases are the most efficient biocatalysts for enzymatic polymerization of polyesters.

<table>
<thead>
<tr>
<th>Enzyme class</th>
<th>Reaction catalyzed</th>
<th>Typical enzymes</th>
<th>Typical polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC 1. Oxidoreductases</td>
<td>Oxidation</td>
<td>Peroxidase, Laccase</td>
<td>Polanilines, Polyphenol, Polystyrenes, Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>EC 2. Transferases</td>
<td>Group transfer</td>
<td>PHA synthase, Hyaluronan synthase, Phosphorylase</td>
<td>Polyesters, Hyaluronan, Amylose</td>
</tr>
<tr>
<td>EC 3. Hydrolases</td>
<td>Hydrolysis</td>
<td>Lipase, Cellulase, Hyaluronidase, Papain</td>
<td>Polyesters, Polyamides, Cellulose, (Oligo)peptides, Glycosaminoglycan</td>
</tr>
<tr>
<td>EC 6. Ligases</td>
<td>Bond formation requiring triphosphate</td>
<td>Cyanophycin synthetase</td>
<td>Cyanophycin</td>
</tr>
</tbody>
</table>

Generally speaking, three polymerization modes can be proceeded for the lipase-catalyzed polyester synthesis (Scheme 1.9): (1) step-growth polycondensation; (2) ring-opening polymerization; and (3) a combination of ring-opening polymerization and polycondensation (ring-opening addition-condensation polymerization). Among them, polycondensation and ring-opening polymerization are the most common methods used for biocatalytic polyester synthesis.
Table 1.2. Enzymes and typical examples for their use in polymer synthesis, and typical polymers synthesized via enzymatic polymerization\cite{123}

<table>
<thead>
<tr>
<th>Enzyme class</th>
<th>Reaction catalyzed</th>
<th>Typical enzymes</th>
<th>Typical polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC 1. Oxidoreductases</td>
<td>Oxidation/Reduction ( AH_2 + B \rightarrow A + BH_2 )</td>
<td>Peroxidase, Laccase</td>
<td>Polyanilines, Polyphenol, Polystyrenes, Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>EC 2. Transferases</td>
<td>Group transfer ( A-X + B \rightarrow A + B-X )</td>
<td>PHA synthase, Hyaluronan synthase, Phosphorylase</td>
<td>Polymers, Hyaluronan, Amylose</td>
</tr>
<tr>
<td>EC 3. Hydrolases</td>
<td>Hydrolysis by ( H_2O ) ( A-B + H_2O \rightarrow AH + BOH )</td>
<td>Lipase, Cellulase, Hyaluronidase, Papain</td>
<td>Polyesters, Polymides, Cellulose, (Oligo)peptides, Glycosaminoglycan</td>
</tr>
<tr>
<td>EC 6. Ligases</td>
<td>Bond formation requiring triphosphate ( A + B \rightarrow A-B )</td>
<td>Cyanophycin synthetase</td>
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</tr>
</tbody>
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(1) **Step-growth polycondensation**

(a) *Carboxylic acids or their ester derivatives and alcohols*

\[
X-O-C-R-C-O-X + HO-R'-OH \xrightarrow{\text{Lipase}} \left( \begin{array}{c} \text{O} \\ \text{O} \\ \text{O} \end{array} \right) \text{C-O-R'-O-C-R-O} \right)_n
\]

\( X = \text{H, alkyl, halogenated alkyl, vinyl, etc.} \)

(b) *Oxyacids (hydroxyacids) or their ester derivatives*

\[
\begin{array}{c} \text{O} \\ \text{O} \end{array} \text{H-O-R-C-O-X} \xrightarrow{\text{Lipase}} \left( \begin{array}{c} \text{O} \\ \text{O} \end{array} \right) \text{C-O} \right)_n
\]

\( X = \text{H, alkyl, halogenated alkyl, vinyl, etc.} \)

(2) **Ring-opening polymerization of cyclic esters (lactones)**

\[
\text{Lipase} \xrightarrow{\text{O-R-C}} \left( \begin{array}{c} \text{O} \\ \text{O} \end{array} \right) \text{C-O} \right)_n
\]

(3) **Ring-opening addition-condensation polymerization of carboxylic anhydride and inorganic acids**

\[
\begin{array}{c} \text{O} \\ \text{O} \end{array} \text{C-R-O-C} + HO-R'-OH \xrightarrow{\text{Lipase}} \left( \begin{array}{c} \text{O} \\ \text{O} \end{array} \right) \text{C-O-R'-O-C-R-O} \right)_n
\]

\( \xrightarrow{\text{H}_2\text{O}} \)

**Scheme 1.9.** Main reaction modes of lipase-catalyzed synthesis of polyesters.

Four modes of elemental reactions may occur during the lipase-catalyzed polyester synthesis, inducing hydrolysis, esterification, transesterification (alcoholysis and acidolysis), and interesterification (Scheme 1.10). These reactions are all reversible.
Therefore, to facilitate the ester formation, it is crucial to remove the remaining water and byproducts like alcohols from the reaction mixture, for example, by adding absorbing and drying agents like molecular sieves, applying reduced pressure, using azeotropic distillation conditions, and so on.

\[
\begin{align*}
(1) \text{Hydrolysis} & \quad \text{Lipase} \quad \text{H}_2\text{O} \quad \text{R}_1\text{C}O\text{R}_2 + \text{H}_2\text{O} \quad \text{R}_1\text{C}O\text{H} + \text{R}_2\text{OH} \\
(2) \text{Esterification} & \quad \text{Lipase} \quad \text{H}_2\text{O} \quad \text{R}_1\text{C}O\text{H} + \text{R}_2\text{OH} \quad \text{R}_1\text{C}O\text{R}_2 + \text{H}_2\text{O} \\
(3) \text{Transesterification} \quad & \\
\quad (a) \text{Alcoholysis} & \quad \text{Lipase} \quad \text{R}_1\text{C}O\text{R}_2 + \text{R}_3\text{OH} \quad \text{R}_1\text{C}O\text{R}_3 + \text{R}_2\text{OH} \\
\quad (b) \text{Acidolysis} & \quad \text{Lipase} \quad \text{R}_1\text{C}O\text{R}_2 + \text{R}_3\text{COH} \quad \text{R}_3\text{C}O\text{R}_2 + \text{R}_1\text{COH} \\
(4) \text{Interestereification} & \quad \text{Lipase} \quad \text{R}_1\text{C}O\text{R}_2 + \text{R}_3\text{C}O\text{R}_4 \quad \text{R}_1\text{C}O\text{R}_4 + \text{R}_3\text{C}O\text{R}_2 \\
\end{align*}
\]

Scheme 1.10. Basic modes of elemental lipase-catalyzed reactions in biocatalytic polyester synthesis.

The first lipase-catalyzed polymerization was reported by Okumara et al. in 1984.\textsuperscript{132} They investigated the enzymatic polymerization of aliphatic diacids and diols by a lipase from \textit{Aspergillus niger} NRRL 337 (Scheme 1.11). However, only oligoesters with \(\overline{M}_n\)'s of around 1000 g/mol were obtained.

\[
\begin{align*}
\text{Diacid, } x = 4-12 \quad \text{Diol, } y = 2, 3 \quad \text{Lipase} \quad \text{H}_2\text{O}, 30^\circ\text{C}, 16 \text{h} \\
\text{Diacid} + \text{Diol} \quad \text{Oligoesters} \\
\end{align*}
\]

Scheme 1.11. Lipase-catalyzed polycondensation of aliphatic diacids and diols.

The lipase-catalyzed ring-opening polymerization was firstly reported in 1993 by two independent groups.\textsuperscript{133, 134} Gutman \textit{et al.}\textsuperscript{133} investigated lipase-catalyzed ring-opening polymerization of \(\varepsilon\)-caprolactone (\(\varepsilon\)-CL); and polycaprolactone (PCL) with a \(\overline{M}_n\) of up to 4400 g/mol was successfully produced in \(n\)-hexane (Scheme 1.12). At the same time, the enzymatic ring-opening polymerization of lactones was performed in bulk by Kobayashi \textit{et al.},\textsuperscript{134} using different lipases as catalysts. The
enzymatic polymerization gave PCL and polyvalerolactone with $M_n$’s of up to 7700, and 1900 g/mol, respectively.

In the late 1990s, the use of N435 in the enzymatic ring-opening polymerization of lactones was introduced by Gross et al. Since then, N435 became the working horse in biocatalytic polyester synthesis.

After these pioneer works, various combinations of diacids/diesters and diols, hydroxyacids/esters, and cyclic monomers such as lactones, cyclic diesters and cyclic ketene acetals, are studied for lipase-catalyzed polymerization. The recent progress in this field is comprehensively summarized in some review articles.

It should be pointed out that the large scale production of aliphatic polyesters via lipase-catalyzed polymerization is feasible. As reported by Binns et al., adipic acid and 1,6-HDO were polymerized by N435 at a multi-kilogram scale, using a two-stage method (Scheme 1.13). The enzymatic polymerization yielded poly(hexamethylene adipate) with a $M_w$ of 16400 g/mol. They also claimed that the enzymatic production can be scaled up to the pilot plant level (2.0 tons) without undue problems. Besides, poly(hexamethylene adipate) produced from the enzymatic polymerization possesses a lower acid number, higher degree of crystallinity and super crystalline growth rate compared to the conventional counterparts.

![Scheme 1.13. N435-catalyzed synthesis of poly(hexamethylene adipate) in large scale, using a two-stage method.](image)

Although a great number of aliphatic polyesters are readily synthesized with high molecular weights via lipase-catalyzed polymerization, only limited amount of aromatic (co-)polyesters are enzymatically produced. This could be mainly due to the high melting temperature ($T_m$) of aromatic polyesters and their low solubility in the reaction media, as well as, the lack of reactivity of aromatic monomers in enzymatic polyesterification. However, by using cyclic aromatic oligomers in the lipase-catalyzed polymerization, high molecular weight poly(alkylene terephthalate), poly (alkylene isophthalate)s and poly(benzenedimethanol adipate)s were obtained, with $M_w$’s of up to 107000 g/mol.
1.6 Enzymatic Polymerization of Polyamides

Lipases, proteases and other enzymes are capable of catalyzing the formation of amide bonds and therefore, they are suitable enzymes for the \textit{in vitro} polyamide synthesis.\textsuperscript{129} In this research, we focus on the lipase-catalyzed polymerization of synthetic polyamides.

Similar to biocatalytic polyester synthesis, the lipase-catalyzed polyamide synthesis can proceed via three basic modes: (1) step-growth polycondensation of diacid/diesters and diamines or \(\omega\)-amino carboxylic acids/esters; (2) ring-opening polymerization of lactams; and (3) a hybrid of step-growth polycondensation and ring-opening polymerization.

Two basic modes of elemental reactions are commonly used in the biocatalytic polyamide synthesis: directly amidation and transamidation (aminolysis) (Scheme 1.14).

\textbf{Scheme 1.14.} Basic modes of elemental lipase-catalyzed reactions in biocatalytic polyamide synthesis.

The lipase-catalyzed polymerization of polyamides has not been well studied.\textsuperscript{131} This could be attributed mainly to two reasons: (1) the high T\textsubscript{m} of polyamides, and (2) the poor solubility of polyamides in common organic solvents. On the one hand, polyamides like nylons and TPA-based polyamides are semi-crystalline polymers which normally possess a high T\textsubscript{m} above 100 °C. At such elevated temperatures, the catalytic reactivity of lipases is significantly decreased due to the occurrence of protein denaturation and deactivation. On the other hand, many polyamides can be only dissolved in some aggressive solvents such as formic acid, concentrated H\textsubscript{2}SO\textsubscript{4}, and trifluoroacetic acid, in which lipases cannot act.

Nevertheless, some oligoamides and polyamides are successfully produced via the lipase-catalyzed polymerization.\textsuperscript{129-131} Some typical examples are given below.

Cheng \textit{et al.}\textsuperscript{151, 152} investigated the lipase-catalyzed polymerization of diamines and diesters in bulk (Scheme 1.15), which resulted in aliphatic polyamides with \(M_w\)’s of
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Nevertheless, some oligoamides and polyamides are successfully produced via the lipase-catalyzed polymerization. Some typical examples are given below.

Cheng et al. investigated the lipase-catalyzed polymerization of diamines and diesters in bulk (Scheme 1.15), which resulted in aliphatic polyamides with *M*ₙ of around 3000 - 15000 g/mol. This is the first report showing that high molecular weight polyamides can be produced from lipase-catalyzed polymerization.

Scheme 1.15. Lipase-catalyzed synthesis of aliphatic polyamides.

The CALB-catalyzed ring-opening polymerization of ε-caprolactam was reported by Kong *et al.* They claimed that the enzymatic ring-opening polymerization gave nylon 6 with a high *M*ₘₚ of 212000 g/mol.

Aliphatic polyamides such as nylon 6,13, nylon 8,13 and nylon 12,13 were synthesized via the N435-catalyzed ring-opening addition-condensation (Scheme 1.16). The *M*ₙ’s of the resulting nylons were around 5600 – 8300 g/mol.

Scheme 1.16. N435-catalyzed ring-opening addition-condensation of ethylene tridecanedioate with various diamines.

In our group, enzymatic polymerization of polyamides is one of the focused research area. For example, the enzymatic polymerization of 2-azetidinone was firstly studied in our laboratory (Scheme 1.17). A different mechanism for the enzymatic ring-opening polymerization of β-propiolactam was revealed and a catalytic cycle for the oligomerization of β-lactam that rationalizes the activation of the monomers was proposed. Moreover, aliphatic oligoamides, semi-aromatic oligoamides, and poly(ester amide)s are successfully prepared via lipase-catalyzed polymerization in our laboratory.

Scheme 1.17. Enzymatic ring-opening polymerization of 2-azetidinone.
1.7 Lipase-Catalyzed Synthesis of Sustainable Polyesters and Polyamides from Biobased Monomers

At present, most research on enzymatic polymerization is still focused on the use of “traditional” monomers derived from fossil resources. Due to the growing awareness of energy safety and environmental pollution, and increased interest for the development of novel polymeric materials, utilization of biobased monomers in enzymatic polymerization becomes an appealing topic both in the academic and industrial fields. Currently, some biobased polyesters are readily synthesized via lipase-catalyzed polymerization, for example, aliphatic polyesters, vegetable oil-based polyesters, and sugar and sugar alcohol-based polyesters. In the following section, the recent progress in the lipase-catalyzed synthesis of biobased saturated aliphatic poly(alkylene dicarboxylate)s, unsaturated aliphatic poly(alkylene dicarboxylate)s, furan polyesters, and furan polyamides are discussed.

1.7.1 Biobased Saturated Aliphatic Poly(alkylene dicarboxylate)s

Aliphatic poly(alkylene dicarboxylate)s belong to a special family of aliphatic polyesters. Most of them can be synthesized from polycondensation of biobased monomers. Among them, PBS is probably the most significant biobased and biodegradable poly(alkylene dicarboxylate)s with great commercial interest, which is normally synthesized via polycondensation of succinic acid or succinic anhydride with 1,4-BDO at elevated temperatures, using a chemical catalyst. The lipase-catalyzed synthesis of PBS was studied by Gross et al., using a two-stage method which is similar to those used for industrial production but at much lower temperatures (Scheme 1.18). They found that the solvent-free enzymatic polycondensation with succinic acid gave oligomers. However, by replacing succinic acid with diethyl succinate, the two-stage method in diphenyl ether resulted in PBS with a $M_w$ of 38000 g/mol and a dispersity of 1.39.

Scheme 1.18. Enzymatic polycondensation of succinic acid or dimethyl succinate and 1,4-butandiol, using a two-stage method.

To synthesize PBS with higher molecular weights, another two enzymatic strategies were developed: (1) using cyclic oligomers, and (2) co-polymerization of succinic acid and 1,4-BDO with succinate anhydride. By using cyclic butylene succinate oligomers in the N435-catalyzed polymerization, PBS with a $M_w$ of up to 130000 g/mol and a dispersity of 1.6 was obtained. However, under similar reaction conditions, the direct enzymatic polycondensation gave PBS with a lower $M_w$ (45000 g/mol) and a broader dispersity (3.7) (Scheme 1.19). On the other hand, the enzymatic co-polymerization of succinic acid and 1,4-BDO with succinate anhydride resulted in PBS with a $M_w$ of 73000 g/mol and a dispersity of 1.7 (Scheme 1.20). However, although high molecular weight PBS can be enzymatically produced via these two approaches, an extra synthesis step is required.

Many other (potential) biobased poly(alkylene dicarboxylate)s are also synthesized via lipase-catalyzed polycondensation. For instance, the lipase-catalyzed solvent-free polycondensation of aliphatic diacids (C2 ~ C12) and aliphatic diols (C2 ~ C12) was performed by Kobayashi et al.. The enzymatic polymerization yielded various aliphatic polyesters with $M_w$'s and dispersities of around 1300 - 14000 g/mol, and 1.1 - 2.3, respectively; and the lipase-catalyzed polymerization of aliphatic diesters and diols in β-cyclodextrin gave saturated aliphatic polyesters with $M_w$'s of around 5300 to 44600 g/mol.
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Scheme 1.19. N435-catalyzed synthesis of poly(butylene succinate) by cyclization with subsequent ring-opening polymerization of the cyclic oligomers.\textsuperscript{173}

Scheme 1.20. Synthesis of poly(butylene succinate) via N435-catalyzed co-polymerization of succinic acid and 1,4-butanediol with succinate anhydride.\textsuperscript{174}

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1.7.2 Biobased Unsaturated Aliphatic Polyesters

Currently, the synthesis of biobased unsaturated polyesters, especially itaconate-based unsaturated polyesters, has not been well studied. This is because the sensitive C=C bond can be deteriorated easily under conventional polymerization conditions such as elevated temperatures and metal catalysts. However, this problem can be easily overcome by using enzyme catalysts in the polymerization, due to the mild synthetic conditions and the high catalytic specificity of the enzyme catalysts.

However, the lipase-catalyzed direct polycondensation of itaconate and aliphatic diols with short chain length generally results in oligomers. As reported by Gardossi et al., the solvent-free polyesterification of dimethyl itaconate and 1,4-BDO catalyzed by CALB gave a mixture of oligomers from dimer to pentamer. Similarly, the N435-catalyzed polymerization of itaconic anhydride with aliphatic diols (C4 ~ C10) gave oligomers with $M_n$’s of around 150 - 390 g/mol, although itaconic anhydride was completely consumed. This is because the enzymatic polycondensation is hampered by the low reactivity of itaconate due to the lower electrophilicity of the acyl carbon (C_s, Scheme 1.21) adjacent to the vinyl group. However, the low reactivity of itaconate in enzymatic polymerization could be overcome by optimizing the reaction conditions: (1) improving the mass transfer and the enzyme distribution in the reaction mixture, (2) increasing the enzyme loading, (3) lowering the diol concentration, and (4) choosing more appropriate diols.178

Scheme 1.21. Enzymatic polymerization of itaconate with aliphatic diols.

Indeed, by using glycols with longer chain lengths or with a rigid structure, itaconate-based homo-polyesters with relatively higher molecular weights were obtained from the lipase-catalyzed polycondensation. As reported by Yousaf et al., the N435-catalyzed polymerization of itaconic acid and 1,4-cyclohexanedicarboxylic acid or poly(ethylene glycol) gave homo-polymers with a $M_w$ of 2600 and 8600 g/mol, respectively. On the contrary, the tin(II) 2-ethylhexanoate-catalyzed polycondensation with itaconic acid gelled within hours.

In addition, itaconate-based polyesters with high molecular weights can be prepared via lipase-catalyzed co-polymerization. The N435-catalyzed co-polymerization of...
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In addition, itaconate-based polyesters with high molecular weights can be prepared via lipase-catalyzed co-polymerization. The N435-catalyzed co-polymerization of itaconic acid, adipic acid and 3-methyl-1,5-pentanediol resulted in a co-polymer with a $M_w$ of 19000 g/mol and poly(12-hydroxystearate-co-butylene itaconate) with a $M_w$ of 30000 g/mol was obtained from the lipase-catalyzed co-polymerization of methyl 12-hydroxystearate, dimethyl itaconate and 1,4-BDO.

1.7.3 Biobased Polyamides

At present, studies related to the enzymatic synthesis of biobased synthetic polyamides are scarcer. A few potentially biobased aliphatic polyamides, such as nylon 4,10, nylon 6,10, and nylon 8,10, can be synthesized via lipase-catalyzed polymerization. However, the molecular weights of the obtained polyamides were quite low.

Landfester et al. studied the N435-catalyzed polycondensation of diethyl sebacate and 1,8-octanediamine (Scheme 1.22). The enzymatic polymerization gave nylon 8,10 with $M_n$'s of around 2000 - 5000 g/mol.

Scheme 1.22. N435-catalyzed synthesis of nylon 8,10 from polycondensation of diethyl sebacate and 1,8-octanediamine.

In our group, oligomers including nylon 4,10, nylon 6,10, and nylon 8,10 were produced via the lipase-catalyzed polymerizations of diethyl sebacate with different diamines (Scheme 1.23).

Scheme 1.23. Lipase-catalyzed polycondensation of diethyl sebacate and diamines.
1.7.4 Furan Polyesters and Furan Polyamides

Furan polymers are not new polymers. In the late 1970s, poly(hexamethylene furanoate), a furan polyester, was synthesized by Moore and Kelly;\textsuperscript{182, 183} and various furan polyesters were successfully prepared by Ballauff \textit{et al.},\textsuperscript{184} Gandini \textit{et al.},\textsuperscript{185} and Okada \textit{et al.}\textsuperscript{186} since 1990s.

In recent years, the research on FDCA-based polyesters and polyamides is booming, due to the fast development of biobased FDCA and the broad potential applications of FDCA-based polymers.\textsuperscript{16} The FDCA-based polymers are promising sustainable aromatic polymer alternatives, and FDCA-based polymers possess similar or even better properties than their petrol-base counterparts. For example, recent studies suggested that poly(ethylene furanoate) (PEF) possesses better barrier properties compared to PET. PEF shows surprisingly large reductions in CO\textsubscript{2} permeability (19×), O\textsubscript{2} permeability (11×) and diffusivity (31×).\textsuperscript{187, 188}

At present, FDCA-based polyesters and polyamides are predominately synthesized via melt polycondensation at elevated temperatures of around 200 °C. However, decarboxylation of FDCA takes place at around 195 °C and other side-reactions may occur at such elevated temperatures,\textsuperscript{16, 189-191} which may lead to the discoloration of the resulting polymers and the formation of low molecular weight products.

![Scheme 1.24. N435-catalyzed polymerizations with furan monomers: BHMF, HMFA and FDCA.\textsuperscript{192, 193}}](image)

These drawbacks could be circumvented by using enzyme catalysts. However, the enzyme-catalyzed synthesis of furan polyesters has not been well studied up until now. Only two reports referred to the enzymatic polymerization of furan polyesters. As reported by Habeych N.\textsuperscript{192} and Boeriu \textit{et al.},\textsuperscript{193} the lipase-catalyzed polymerization with FDCA, BHMF or 5-hydroxymethyl-2-furancarboxylic acid (HMFA) gave only a mixture of linear and cyclic furan oligomers (scheme 1.24).
Therefore, it is of great interest to establish a robust enzymatic approach towards high molecular weight furan polyesters.

Moreover, the lipase-catalyzed synthesis of furan polyamides has not been studied up to now.

1.8 Scope of this Thesis

The aim of this research is to develop an eco-friendly approach for the synthesis of sustainable polyesters and polyamides. In this approach, various sustainable polyesters and polyamides are produced via lipase-catalyzed polycondensation of biobased monomers. The biobased monomers used are aliphatic diacid ethyl esters \((n = 2, 3, 4, 6, 8 \text{ and } 10)\) - number of methylene units in the diacid fragment), itaconic acid and its ester derivatives, aliphatic diols \((n = 3, 4, 6, 8 \text{ and } 10)\), polyols, and furan monomers including dimethyl FDCA and BHMF. The enzyme catalyst applied is N435.

At first, we focus on the enzymatic polymerization of biobased saturated aliphatic poly(butylene dicarboxylate)s and itaconate-based unsaturated aliphatic polyesters, poly(butylene dicarboxylate-co-itaconate)s.

Although many saturated aliphatic poly(alkylene dicarboxylate)s are enzymatically synthesized, the systematic study on the lipase-catalyzed synthesis of aliphatic poly(butylene dicarboxylate)s has not been studied up to now. In addition, the physical properties of these polyesters have not been well documented yet.

Moreover, the synthesis of high molecular weight itaconate-based unsaturated polyesters is quite challenging by conventional or biocatalytic approaches. Although a few itaconate-based (co-)polyesters with high molecular weights were enzymatically produced, the co-monomers used were petrol-based, or possess long chain lengths. Furthermore, the enzyme-catalyzed synthesis of biobased poly(butylene dicarboxylate-co-itaconate)s has not been reported yet.

In Chapter 2 we focus on the enzymatic polycondensation of succinate, itaconate and 1,4-butanediol, to produce fully biobased poly(butylene succinate-co-itaconate)s (PBSIs). The effect of polymerization conditions, including solvents, solvent dosage, oligomerization time, vacuum and itaconate structures, are carefully studied; and the optimized conditions are obtained. With the optimal conditions, PBSIs with high molecular weights and different molar compositions are obtained. Moreover, the chemical structures and thermal properties of the resulting PBSIs are fully characterized.
Chapter 3 describes the enzymatic synthesis of fully biobased PBS and PBSIs by using different monomer substrates and by applying different polymerization methods. In addition, the enzymatic polymerization mechanism is revealed by the microstructure study using $^{13}$C-NMR. Moreover, the crystalline properties of PBS and PBSIs, as well as, the thermal and mechanical properties of the cross-linked PBSIs are investigated.

Chapter 4 addresses the preparation of series of (potentially) biobased saturated aliphatic polyesters and itaconate-based unsaturated polyesters via the enzymatic polymerization of dimethyl itaconate and 1,4-butanediol with diacid ethyl esters differing in chain length. The specificity of CALB for the tested diacid ethyl esters is studied, and the structure-crystalline/thermal properties of the obtained aliphatic polyesters are investigated. Furthermore, the synthetic unsaturated aliphatic polyesters are cross-linked by UV light; and the thermal and mechanical properties of the cross-linked materials are studied.

From Chapters 2 - 4 we can draw the conclusion that enzymatic polymerization offers a great feasibility and flexibility for the synthesis of high molecular weight aliphatic polyesters, as well as, functional polymers containing sensitive groups.

Then we expand our research to study the enzymatic polymerization of furan polyesters, which are biobased alternatives to semi-aromatic polyesters. Generally speaking, it is difficult to produce semi-aromatic polyesters with high molecular weights by enzymatic polymerization. The literature study also shows that only furan oligoesters were obtained from the enzymatic polymerization with furan monomers (see section 1.7.4). However, for the first time, we establish a biocatalytic approach for the production of high molecular weight furan polyesters with diverse structures.

Chapter 5 explores the enzymatic synthesis of a series of biobased furan polyesters from polycondensation of BHMF and various diacid ethyl esters. The enzymatic polymerization kinetics are investigated by $^1$H-NMR. Moreover, the chemical structures, microstructures and end groups, and crystalline and thermal properties of the obtained BHMF polyesters are characterized; and the effects of the number of the methylene units in the dicarboxylic units on the physical properties of the BHMF polyesters are discussed.

Chapter 6 outlines the enzymatic synthesis of various high molecular weight FDCA-based furanic-aliphatic polyesters. They are promising sustainable alternatives to semi-aromatic polyesters, which can be used as thermal engineering plastics. The enzymatic polymerization conditions are optimized by studying the effect of diol structures, the reaction temperature and reaction time. With the
established method, FDCA-based furanic-aliphatic polyesters are obtained with high molecular weights. Moreover, the structure-properties relationships are established by investigating the chemical structures, microstructures and end groups, and crystalline and thermal properties of the obtained FDCA-based furanic-aliphatic polyesters.

At last, we apply the well-established methodology from the enzymatic polymerization of furan polyesters to synthesize FDCA-based furanic-aliphatic polyamides (Chapter 7). These FDCA-based furanic-aliphatic polyamides are sustainable analogues to polyphthalamides (semi-aromatic polyamides) and can be applied as thermal engineering plastics and high performance materials. A one-stage method and a temperature-varied two-stage method are applied for the enzymatic polymerization; and FDCA-based furanic-aliphatic polyamides with high molecular weights are obtained. Moreover, the chemical structures, microstructures and end-groups, and crystalline and thermal properties of the obtained furan polyanides are investigated. To the best of our knowledge, this is the first time that the enzymatic synthesis of furan polyanides has been addressed.

In Chapters 5 - 7 we demonstrate that enzymatic polymerization is a robust approach towards furan polymers; and FDCA-based polyesters and polyamides possess similar or comparable crystalline and thermal properties compared to their petrol-based counterparts.

1.9 References

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Introduction

Chapter 2

Enzyme-Catalyzed Synthesis of Biobased Unsaturated Aliphatic Polyesters: Effects of Polymerization Conditions and their Thermal Properties

Abstract: Succinate, itaconate and 1,4-butanediol are commercially available biobased monomers. They are enzymatically co-polymerized in solution via a two-stage method, using *Candida antarctica* lipase b (CALB, in immobilized form as Novozym® 435) as the biocatalyst. The effects of reaction conditions on the enzymatic polymerization are extensively investigated, and the optimal polymerization conditions are obtained. With the established method, poly(butylene succinate) (PBS) and a series of poly(butylene succinate-co-itaconate)s (PBSIs) are produced, with tunable compositions and satisfying reaction yields. 1H-NMR results confirm that all carbon-carbon double bonds are well preserved in the obtained PBIS. In addition, Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA) results indicate that the amount of itaconate has no obvious effect on the glass-transition temperature (Tg) and the thermal stability of the obtained polyesters, but has significant influences on the melting temperature (Tm).

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