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Chemo-enzymatic Routes Towards the Synthesis of Bio-based Monomers and Polymers.

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1. Introduction

Industrial biotechnology (IB) has had a significant impact on the pharmaceutical and chemical industry facilitated by the advancements in the field of directed evolution and genome sequencing. This has led to the development of cleaner, and efficient manufacturing processes, producing less waste and avoiding the use of harmful solvents and reagents. Consumer demands for green and sustainable products with minimal environmental impact has, perhaps, been the biggest driver in adopting IB and it continues to be the driving force behind sustainability. The introduction of the United Nations sustainable development goals has accelerated the development and implementation of sustainable practices including the use non-food waste biomass as feedstock, employing circular business principles and efficient production processes. As a result, IB has seen a renaissance in recent years with the commercialization of several processes in an effort to reduce waste and contribute towards resource and environmental sustainability. Biodegradable polymers have gained enormous attention in recent years attributable to the relative ease by which they can be degraded under mild aerobic and anaerobic conditions. With governmental restrictions on single-use plastics, the biopolymer market has seen a resurgence with a predicted compound annual growth rate (CAGR) of 18.6% between 2016-2021. A similar trend has been seen in academia with a 150% increase in publications on bio-based polymers between 2012-2017. While biopolymers are derived from bio-based feedstock, not all are biodegradable. For example, polylactic acid produced from waste biomass can be broken down by bacteria, [1,2] however, biopolyethylene from bacterial fermentation [3] (a bio-based drop in chemical) has the same property as oil derived polyethylene and therefore is non-biodegradable. The production of Active Pharmaceutical Ingredients (APIs) using chemo-enzymatic and biocatalytic routes is well-known and has been employed in a plethora of synthetic processes. However, the production of high-value monomers from cheap and renewable feedstocks are rarely reported. The aim of this review is to present an overview of (chemo)-enzymatic approaches to structurally diverse monomers found in polyesters, polyurethanes and in novel terpene-based polymers.

2. Polyester and polyhydroxyalkanoates (PHA)

This is a class of polymers containing ester linkages typically produced from the polycondensation of dicarboxylic acid and diols. The most common is the condensation of terephthalic acid and ethylene glycol in the production of polyethylene terephthalate (PET). Historically, polyesters were the first family of synthetic polymers investigated in the 1930's by Carothers and later developed by Whinfield *et al* which led to the discovery of PET.[4] This was subsequently commercialized by Du Pont in 1951 under the tradename Dacron in

the US and Terylene in UK. Due to it being lighter than glass, it was cheaper to transport, and as a result many companies in the FMCG industry adopted PET packaging. The mass adoption of non-biodegradable polymers has had a substantial impact on the environment, which has led to several initiatives to minimize the production of plastics derived from crude oil. A number of strategies have been explored, one of which is the use of biocatalysis to produce renewable and environmentally friendly monomers and polymers.

2.1 Synthesis of diacid monomers

Microbial production of terephthalic acid (TPA) from *p*-xylene and *m*-xylene was one of the earliest attempts to sustainably produce TPA from fermentation.[5] Using *p*-xylene, *p*-Toluic acid (*p*-TA) and TPA as the sole carbon source, a panel of bacterial colonies were screened for growth and survival. Bacteria from the genus *Burkholderia* was selected harboring the requisite enzymes for the transformation of xylene isomers to TPA. Subsequent work explored integrating key enzymes in *E. coli* to convert *p*-xylene to 4-carboxybenzyl alcohol (4-CBAL) followed by chemical oxidation to TPA.[6] This approach was adopted and extended further by integrating the whole biosynthetic pathway in *E. coli* to produce TPA from *p*-xylene. Gene expression was optimized by employing two different origins of replication and promoters producing 13.3 g of TPA from 8.8 g of *p*-xylene.[7]

Scheme 1. Biocatalytic and synthetic biology routes to TPA monomer.

Morgan and colleagues reported the use of chloroperoxidase (CPO) from *Caldariomyces fumago* which catalyzes the conversion of 1,4-benzenedimethanol (1,4-BDM) to terephthalaldehyde (TPDA) followed by oxidation of TPDA by xanthine oxidase (XO) to give TPA in 65% overall yield.[8] Frost and co-workers proposed a novel approach by combining renewable isoprene units with propiolic acid in a 4+2 cycloaddition to afford *p*-TA.[9] Oxidation of the methyl group by $\text{Co}(\text{OAc})_2/\text{Mn}(\text{OAc})_2$ in the presence of oxygen afforded TPA in 85% yield.

The challenges associated with producing TPA through biocatalytic processes has encouraged industry to seek alternative monomers with similar properties to TPA. Furan dicarboxylic acid (FDCA), a compound with similar functional properties to TPA can be synthesized from renewably sourced 5-hydroxymethylfurfural (HMF). An efficient

biotransformation process was developed to obtain FDCA using HMF/furfural oxidoreductase from *Cupriavidus basilensis* (*hmfH*). The gene was introduced into *Pseudomonas putida* S12 and the resulting biocatalyst was used to produce FDCA using glycerol as the carbon source. In fed-batch experiments 30.1 g L⁻¹ of FDCA was produced with an isolated yield of 97%. [10] A recently discovered FAD-containing enzyme, HMF oxidase (HMFO), was used to conduct three sequential oxidative processes to produce FDCA from HMF with excellent conversion (Scheme 2). [11] The low substrate concentration and the requirement for compound **2** to be hydrated in the final step led to the development of an enzymatic cascade utilizing galactose oxidase M₃₋₅ (GOase M₃₋₅) and aldehyde oxidase (PaoABC). [12] PaoABC carried out the direct oxidation of **2** to FDCA with an isolated yield of 74% at a substrate concentration of 100 mM. Immobilized magnetic laccase with TEMPO as the mediator showed remarkable activity towards the direct conversion of HMF to FDCA (scheme 2). [13] The cascade afforded quantitative conversion after 96 hours with minimal side-products or intermediates.

Scheme 2. Synthesis of high-value FDCA monomer via enzymatic cascade and its uses in packaging and electronics.

Production of FDCA directly from lignocellulosic biomass provides an opportunity to develop efficient biomanufacturing processes using renewable feedstock. Thermal hydrolysis of microalgae biomass followed by inoculating the mixture with *Bukholderia cepacia* H-2 produced 1.2 g L⁻¹ of FDCA from 2 g L⁻¹ of HMF (obtained from thermal hydrolysis). [14] FDCA is an important building block for renewable biodegradable polymers and has been used in the packaging industry [15] to replace PET and also in flexible polymer films for optoelectronics. [16]

Aliphatic C-4 diacids such as succinic and malic acid are important platform chemicals and a gateway to several high value intermediates used in the pharmaceutical and chemical industry. Due to the renewable nature and non-toxicity of these compounds they have found use as high-value monomers providing unique functional properties for next generation materials. Polymers derived from malic acid exhibit high water solubility and biodegradability, therefore increasing bioavailability, ideal for drug delivery systems. [17,18] Chemical production of malic acid goes via the hydration of fossil-derived maleic anhydride. Developments in renewable production of diacids has spawned several biocatalytic and fermentation routes to malic acid. A carbon fixation approach was used to produce malic acid by carboxylating pyruvate with hydrogencarbonate (HCO₃²⁻). [19] The photoredox chemo-enzymatic cascade was designed using Zn-Chl-e₆, ferredoxin-NADP-reductase (FNR), NADPH and malic enzyme (ME). Irradiation of the mixture with visible light gave 0.65 mmol dm³ of malic acid after 3 hrs (scheme 3a). Alternatively, baker's yeast can be used to reduce sodium diethyl oxaloacetate **4** to malate ester **6** or by using an esterase (PLE) in a resolution to produce enantiopure malate monoester (*S*)-**7** (scheme 3b). [20] While biocatalysis is a promising route to malic acid, there are challenges in producing malic acid on an industrial scale. Production through fermentation is a viable alternative to produce the

target acid in high titers and yield. Van Marris and co-workers optimized a fermentative route to malic acid from glucose. By overexpressing the native pyruvate carboxylase and the relocation of malate dehydrogenase to the cytosol produced 59 g L⁻¹ of malic acid.[21]

Scheme 3. a) Chemo-enzymatic route to malic acid from pyruvic acid using malic enzyme (ME) facilitated by visible light irradiation b) Enzymatic reduction of prochiral **4** and esterase catalysed resolution of racemic **6**.

Succinic acid is a versatile building block and is one of a few renewable chemicals which has been commercialized and its production is competitive to petroleum based routes. The acid is primarily produced through fermentation [22] and in 1996 Donnelly and co-workers conducted the first experiment to overproduce succinic acid in *E. coli*. [23] Overexpression of phosphoenolpyruvate (PEP) carboxylase improved conversion by 70% producing 10.7 g L⁻¹ of succinic acid. Other bacterial and fungal species are also capable of producing succinic acid in high titers. Using a modified strain of *Mannheimia succiniciproducens* LPK7, Lee and co-workers obtained 1.77 g L⁻¹h⁻¹ of succinic acid. [24] Substituted analogues of succinic acid decorated with aromatic and aliphatic side-chains can be obtained using a chemo-enzymatic approach. [25] Sequential alkylation of dimethyl malonate with 2,3-dibromopropene and *t*-butyl bromoacetate gives the tri-ester **8**. Decarboxylation followed by resolution of the intermediate ester by subtilisin affords a panel of substituted succinic acids **9a-e** with isolated yields of 40-50%. Removal of the *tert*-butyl group gives access to highly functionalized diacid monomers with properties suited for healthcare applications. For example, polymers containing thiazole functional groups (**9d**) have shown to exhibit excellent antimicrobial properties against *E. coli*, MRSA, *P. aeruginosa* and *S. aureus*. [26]

Scheme 4. Panel of succinic acid analogues produced via sequential alkylations of dimethyl malonate followed by enzymatic resolution using subtilisin.

Succinic acid is a key component in polybutylene succinate (PBS); a next generation thermoplastic elastomer (TPE) displaying high tensile strength and flexibility [27]. The properties of PBS can be significantly enhanced or tailored to specific application by

introducing different blends. For example, the stability of PBS can be improved without compromising elasticity by blending the polymer with cellulose triacetate. [28] Hemsri *et al.* used a similar approach by combining acrylonitrile butadiene rubber (NBR) with PBS to afford a mixture with enhanced thermal properties and 1270% improvement in elongation compared to PBS alone. [29]

2.2 Synthesis of chiral hydroxy acids

Hydroxy acids are a versatile class of compounds found in natural products, pharmaceuticals [30] and increasingly used in the production of biodegradable polyesters. Due to their importance as high-value chemical intermediates, several biocatalytic routes have been explored towards the synthesis of chiral and aliphatic hydroxy acids (scheme 5)

Scheme 5. Different biocatalytic routes towards the synthesis of aromatic and aliphatic chiral α -hydroxy acids.

One of the earliest works on enzymatic resolution of α -hydroxy esters was reported in 1903 by H. D. Dakin. [31] A racemic mixture of methyl mandelate in the presence of a lipase afforded an optically active mixture, therefore indicating the first example of enriching an enantiomer using enzymes. Later, Sivakumar and co-workers employed Novozyme-435 to obtain (*R*)-mandelic acid in 78% ee after 24 hrs. [32] Lipase from *Pseudomonas cepacia* (PSL) was used in a biphasic mixture of heptane and phosphate buffer to produce (*R*)-ethyl 2-hydroxy-4-phenylbutyrate (**10**) and the free acid (*S*)-**11** from a racemic mixture of **10**. The inhibiting product (*S*)-**11**, was removed in a diafiltration process using a ceramic membrane leading to the production of (*R*)-**10** with a space-time-yield of 275 g L⁻¹d⁻¹ with an ee of >99% (scheme 6).[33]

Scheme 6. Production of long chain aromatic α -hydroxy acid (*R*)-**10** via enantioselective hydrolysis of *rac*-**10**.

Lee *et al.* developed a whole-cell biocatalyst displaying lipase from *Pseudomonas fluorescens* on the surface of *E. coli* capable of catalyzing the kinetic resolution of hydroxy acids. When racemic mixtures of 3-hydroxybutyric acid and methyl mandelate were treated with the biocatalyst, (*R*)-3-hydroxybutyric acid and (*S*)-mandelic acid were obtained with ee values >99%. [34] Yet and co-workers reported the kinetic resolution of a panel of substituted aromatic mandelic acid derivatives (*rac*-**12**) using lipase from *Pseudomonas sp.* (scheme 7).[35] In the presence of vinyl acetate, the enzyme selectively catalyzed the transesterification of *rac*-**12** to (*R*)-**12** with 27-46% isolated yield and >96% ee.

Scheme 7. Kinetic resolution of aromatic mandelic acid derivatives.

Enantioselective hydrolysis of α -hydroxynitriles using nitrilases offer a facile approach to chiral hydroxy acids with theoretical yields of 100% in a dynamic kinetic resolution (DKR) process. Robertson *et al.* identified 137 nitrilases after screening 651 environmental samples of which 44 were found to be (*R*) and 4 were (*S*)-selective on mandelonitrile. [36] Further investigation revealed nitrilase I and II to be proficient in the DKR of aryl nitriles (*rac*-**14** and **15**) towards the synthesis of a panel of mandelic acid (*R*)-**14** and phenyllactic acid derivatives (*S*)-**15**. [37]

Scheme 8. Chemo-enzymatic DKR of α -hydroxynitriles towards the production of aromatic hydroxy acids.

Dehydrogenases provide an alternative route to hydroxy acids via the asymmetric reduction of the pro-chiral ketone in α -keto esters. Fusion proteins consisting of glutathione S-transferase *N*-terminally linked to putative dehydrogenases from *S. cerevisiae* (Ypr1p and Gre1p) were used to reduce ethyl 2-oxo-4-phenylbutanoate to the corresponding alcohol with high enantiopurity. [38] Further systematic investigation by the same group on 18 reductases fused with GST showed activity towards α - and β -keto esters. [39] Five enzymes encoded from the yeast gene YDLI24w, YAL060w, YGL157w, YDR541c and YGL151w displayed excellent enantioselectivity across all three α -keto esters, affording the corresponding alcohol in >99% ee. Xu and co-workers engineered a highly stereoselective D-lactate dehydrogenase (D-LDH) towards a panel of aliphatic and aromatic α -ketoesters. [40] The Y52L mutant of D-LDH showed enhanced activity compared to the wild-type across the whole substrate panel (scheme 9).

Scheme 9. Panel of α -keto acids using engineered D-lactate dehydrogenase (D-LDH). Values in brackets show % change in specific activity between wt and Y52L mutant.

Busto *et al.* developed an artificial linear cascade employing two enzymes to convert L-amino acids to either the (*R*) or (*S*)-hydroxy acids. [41] Oxidation of **18** by L-amino acid deaminase gives the pro-chiral intermediate which is subsequently reduced by D or L-hydroxyisocaproate (HicDH) to afford (*R*) or (*S*)-**19** with excellent conversions and >99% ee (scheme 10).

Scheme 10. Linear cascade towards the synthesis of functionalized hydroxy acids starting from L-amino acid analogues.

Synthetic strategies to obtain chiral α -hydroxy acids has largely relied on starting from highly functionalized starting substrates (ketones, nitriles and racemic alcohols) followed by either reduction, oxidation or kinetic resolution to obtain the target compound. Li and co-workers developed a modular biocatalytic cascade using four enzymes to obtain enantiopure chiral hydroxy acids starting from the bulk chemical styrene. [42] Module 1 catalyzes the formation of the diol intermediate (S)-**22** via epoxidation of the alkene functionality by styrene monooxygenase (SMO) followed by hydrolysis of (S)-**21** using epoxide hydrolase from *Sphingomonas* sp. HXN-200 (SpEH). The second module is comprised of the oxidation enzymes AlkJ from *P. putida* (a membrane bound ADH) and phenylacetaldehyde dehydrogenase (EcALDH) from *E. coli* to produce mandelic acid analogues (S)-**24** from (S)-**23**.

Scheme 11. A four enzyme modular biocatalytic cascade in the synthesis of (S)- α -hydroxy acids.

Polymers of longer chain hydroxy acids have unique properties suited for medical applications. For example, poly 3-hydroxypropionic acid (3-HP) is 120 times more flexible than polylactic acid (PLA) with a lower glass transition temperature (T_g). [43] A similar trend is seen on going from poly 3-HP to poly 4-hydroxybutyric acid (4-HP) with substantial improvement in flexibility and with a significantly lower T_g making it suitable to be used as sutures [44] and in tissue engineering.[45,46] Since 3-HP is listed as one of the top value-added products of the future by the US Department of Energy in 2004, several manufacturing processes have emerged in the last decade from fermentation to biocatalysis. It has been demonstrated that recombinant strains of *Pseudomonas denitrificans* heterologously expressing glycerol dehydratase (DhaB) and glycerol dehydratase reactivase (GdrAB) from *Klebsiella pneumoniae* produce 54.7 mmol L⁻¹ of 3-HP.[47] Recently, *E. coli* has shown to be a robust host for the production of 3-HP using a dual synthetic pathway. The authors inserted an α -ketoglutaric semialdehyde dehydrogenase (ALDH) from *Azospirillum brasilense* and the Pdu pathway from *Klebsiella pneumoniae* to obtain a yield of 54% mol mol⁻¹ of 3-HP from glycerol (scheme 12).[48]

Scheme 12. Overexpression of dual synthetic pathway in *E. coli* to improve production of 3-HP.

Despite the success in fermentation, there are several issues preventing successful commercialization of 3-HP such as productivity, high substrate tolerance and redox imbalance. Biocatalysis offers a promising route to 3-HP and longer chain hydroxy acids, mitigating issues with toxicity and the process can be scaled to reach industrial production level. For example, nitrile hydratase (NHase) is used on an industrial scale to produce acrylamide from acrylonitrile at a capacity of 30,000 tons per year.[49] The closely related enzyme nitrilase, plays a similar function by hydrolyzing nitrile functional groups to carboxylic acids.[50] Zhu and co-workers reported the use of immobilized whole cells harbouring nitrilase to successfully hydrolyze 3-HPN to 3-HP with a titer of 184.7 g L^{-1} and a productivity of $36.9 \text{ g L}^{-1} \text{ h}^{-1}$ (scheme 13).[51]

Scheme 13. Production of 3-HP using immobilized whole cells harbouring nitrilase.

A similar approach using a two-enzyme system produced a panel of aromatic β -hydroxy acids in excellent yield and enantiopurity (scheme 14).[52] Carbonyl reductase from *Candida magnoliae* (CmCR) was employed to afford the intermediate β -hydroxy nitrile followed by hydrolysis using nitrilase from cyanobacterium *Synechocystis sp.* to give aromatic 3-HP (**27a-i**) with excellent isolated yield.

Scheme 14. Synthesis of aromatic PHA analogues from the corresponding β -ketonitrile (R)-**26** using a two enzyme system.

Introducing monomers with aromatic side chains in PHA polymers can have a profound effect on the thermal property of the final compound. Feeding a recombinant strain of *Ralstonia eutropha* with 3-hydroxyphenyl propionic acid and 3-HP produced co-polymers harbouring both aromatic and aliphatic regions. DSC analysis revealed polymers with higher aromatic content had higher T_g making it suitable for use in packaging.[53] Conversely, reducing the steric bulk of the polymer and using longer chain hydroxy acids increases the flexibility of the polymer which is more suited for medical applications. Park and co-workers

developed a multi-enzymatic cascade to obtain long chain bi-functional monomers from renewable fatty acids.[54] Hydration of oleic acid by hydratase followed by oxidation using ADH from *Micrococcus luteus* gives the ketone intermediate **29**. BVMO catalyzed lactone formation and hydrolysis of the ester affords ω -hydroxynonanoic acid (**31**) in 60% yield. Long chain aliphatic di-functional monomers (**31**) play a crucial role in developing novel biocompatible polymers with increased flexibility and tensile strength. Polyesters obtained from plant oil fatty acids similar to poly-**31** have shown to be compatible with injection molding, electrospinning and film extrusion [55] for use as packaging materials, fibres and plastic films respectively.

Scheme 15. Biocatalytic production of long chain hydroxy acid from renewable Oleic acid

2.3 Chiral lactones as bio-based monomers

Alternative routes to functionalized chiral polyesters can be achieved through the ring opening polymerization (ROP) [56] of substituted lactones affording a simple one-step process to biodegradable polyesters. Earliest attempts date back to 1966 where ROP of lactide in the presence of SnCl_4 afforded polyesters used in surgical implants.[57] Since then, aliphatic polyesters (PHAs) have been employed in the design of artificial skins, [58] dental implants [59] and pins for internal bone fractures.[60] ROP of larger cyclic ketones such as of δ -valerolactone and allyl- δ -valerolactone provide additional properties such as flexibility and elasticity making it suitable for implantable drug delivery systems.[61] Poly- ϵ -caprolactone (PCL) from the ROP of ϵ -caprolactone (ϵ -CL) is used as a biocompatible material in tissue engineering and surgical sutures.[62] Smaller rings, for instance, γ -butyrolactone (γ -BL; **33a**) has proved difficult due to its thermodynamic stability, however co-polymerization with ϵ -CL affords PBL-co-PCL copolyester with improved biodegradability (scheme 14b).[63] Cyclic lactone monomers such as ϵ -CL and **33a** are accessible through biocatalysis, for example, Turrini *et. al.* reported the synthesis of a panel of γ -BL analogues (**33a-e**; scheme 14a) from the corresponding unsaturated lactone (**32a-e**) using ene-reductase (EREDs).

Scheme 16. a) Asymmetric reduction of unsaturated lactone to the corresponding 5-membered substituted lactone using EREDs b) Co-polymerization of **33a** and ϵ -caprolactone.

The six membered δ -valerolactone can be obtained from the open-chain racemic hydroxy ester (**34**) via lipase catalyzed kinetic resolution followed by lactonization to afford enantiopure α -methyl- δ -valerolactone (*S*)-**36**.^[64] A ruthenium catalyst was used to catalyze the racemization of alcohol **34** followed by acetylation by lipase from *P. fluorescens* to produce (*S*)-**35** with high conversion and enantioselectivity. Kobayashi and co-workers used **36** as a substrate for lipase catalyzed ROP to obtain poly-**36** with an M_n of 8.4 kg mol^{-1} .^[65]

Scheme 17. Chemo-enzymatic synthesis of substituted lactone (*S*)-**36** and subsequent lipase catalysed ROP to the polyester.

Introducing functional groups by chemical modification ^[66,67] is a common strategy to diversify applications of PHA.^[68] For example, branched PHA with aliphatic side chains increases melt strength, consequently making the polymer suitable for thermoforming and foaming processes to manufacture utensils and packaging materials. PHAs with aliphatic side chains can be synthesized from the corresponding lactone monomer. Delgove *et. al.* explored the substrate scope of a library of BVMOs towards sterically demanding cyclohexanone analogues.^[69] Three cyclohexanone monooxygenases from *Acinetobacter calcoaceticus* (AcCHMO), *Rhodococcus sp.* (RhCHMO), *Thermocrispum municipale* DSM 44069 (TmCHMO) as well as cyclopentadecanone monooxygenase from *Pseudomonas sp.* (PsCPDMO) was screened for activity. All four enzymes showed conversion to compounds (**37-42**) with varying levels of regioselectivity (abnormal/normal lactone) and conversion (scheme 18). The discrepancy on forming either the normal or abnormal lactone or mixtures highlights the tunability of these biocatalysts. Engineering BVMOs to selectively produce a single regioisomer can significantly alter the polyester backbone leading to new properties and in some cases make it easier for transesterification in polymer synthesis.

Scheme 18. BVMO catalysed synthesis of functionalised lactone monomers.

3. Terpene based bio-polymers

Lactone **37** resembles natural products belonging to a class of diverse compounds known as monoterpenoids. More than 80,000 compounds have been identified so far and they have gained considerable interest from industry as pharmaceuticals, fragrances, antiseptics, biofuels, and more recently as building blocks for the synthesis of sustainable polymers and other advanced materials.[70–72] The terpenoid scaffolds are produced from the universal linear C5 isoprenoid precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are combined by prenyl transferases to generate pyrophosphate substrates of varying lengths (C10, C15, C20, etc). These prenyl pyrophosphates are then used by terpene cyclases/synthases to produce a large variety of terpene scaffolds.

Scheme 19. Proposed mechanism for the formation of β -myrcene, limonene, and α - and β -pinene catalysed by monoterpene cyclases/synthases (mTC/S).

The monoterpene cyclases/synthases (mTC/S) are responsible for the conversion of a single linear substrate, geranyl diphosphate (GPP) into a variety of linear and (bi)cyclic products. All mTC/S enzymes catalyze high-energy cyclisation reactions involving unstable carbocation intermediates. The reaction is initiated by the divalent metal ion-dependent ionization of GPP; the resulting geranyl cation can then react along several channels to form linear, monocyclic or bicyclic monoterpene hydrocarbon scaffolds. The formation of all cyclic products requires the isomerization of the geranyl cation to the linalyl cation (via linalyl diphosphate), which can cyclize via a 1,6-ring closure to yield the α -terpinyl cation (scheme 19). Linear products, such as β -myrcene are formed following the deprotonation of either the

geranyl or linalyl cation prior to cyclisation. Limonene is formed following the deprotonation of the α -terpinyl cation at C8. The formation of pinene requires a second cyclisation of the α -terpinyl cation (2,7-ring closure) leading to the pinyl cation, which upon deprotonation results in the bicyclic products α - or β -pinene.

3.1 Production of monoterpenes limonene, β -myrcene and α - and β -pinene.

Most industrially relevant monoterpenes can be extracted from plant-based materials, such as essential oils and turpentine; the latter is a by-product from the wood pulp industry and is a major source for α - and β -pinene. However, inefficient extraction processes and low yields limit the use of these natural sources.[72] Metabolically engineered microbes provide an attractive alternative method for the production of monoterpenoids. In the last decade or so technologies have been developed for the production of monoterpenoids and other isoprenoids using engineered microbes, such as *E. coli* [73] and *Saccharomyces cerevisiae* [74], or cell-free systems.[75] All of these systems rely on engineered enzymatic pathways delivering the C5 isoprenoid precursors IPP and DMAPP from simple carbohydrates, glycerol, or more complex renewable carbon sources such as lignocellulosic biomass.[76,77] The target compounds β -myrcene[78], (-)-limonene [77,79] and α - and β -pinene[80–82] are important precursors for next generation polymers and have all been produced in engineered microbes at titres ranging from 58 mg L⁻¹ for β -myrcene [78] to 435 mg L⁻¹ for (-)-limonene[79]. Recently a flexible ‘Plug and Play’ platform was developed for the production of over 30 different monoterpene hydrocarbon scaffolds in *E. coli*, including, β -myrcene, (+)- and (-)-limonene, and α - and β -pinene (scheme 20) [83]

Scheme 20. Synthesis of a panel of monoterpenoids using the plug and play platform in *E. coli*.

Most batch microbial monoterpene production systems rely on a two-phase culture system where an organic overlay is used to trap the volatile monoterpene products produced. The trapped hydrophobic compounds can be easily recovered from the organic phase using two-phase partitioning, silica gel column chromatography, and reversed-phase preparative high-

performance liquid chromatography.[84] Higher monoterpene yields can be achieved using fermentation conditions. For example fed-batch fermentation experiments with engineered *E. coli* strains resulted in α -pinene and (-)-limonene titres of 0.97 and 1.35 g L⁻¹ respectively.[77,82] The volatility and toxicity of monoterpenoids requires the continuous removal of the product during fermentation. Jongedijk and colleagues [85] showed that the continuous capturing of limonene from a yeast culture from the headspace during growth, using an adsorbent material, resulted in an 8-fold increase in limonene yield compared to extraction by an organic overlay, yielding pure limonene essential oil.

3.2 Monoterpenes as highly functionalized monomers

The natural diversity of terpenes makes it a valuable source for renewable building blocks for biomaterials. Monoterpenes such as menthone, dihydrocarvone and carvomenthone have been exploited as valuable precursors for pressure sensitive adhesives [86], shape memory polyesters [87] and as thermoset elastomers.[88] Terpenes with a lactone functional group can be obtained through the BVMO oxidation of natural products found in the peppermint biosynthetic pathway.[89] Wild-type BVMO generally exhibits poor substrate selectivity and conversion towards monoterpene scaffolds.[69] To overcome this, Bornscheuer and co-workers identified three important residues in the active site that are thought to play a vital role in substrate binding and catalysis.[90] The triple mutant F299A/F330A/F485A switched the selectivity of CHMO from *Arthrobacter* sp. (CHMO_{Arthro}) from abnormal to normal lactone production. Introduction of similar point mutations by Hanan *et. al.* in the active site of CHMO from *Rhodococcus* sp. Phi1(CHMO_{Phi1}) predominantly produced the normal lactone of (-)-menththide and (+)-dihydrocarvide (scheme 21).[91] Subsequent ROP of the lactones using Mg(BHT)₂(THF)₂ [92] as the catalysts produced polymers with molecular weights of 6.55 kg mol⁻¹ for polymenththide (PM) and 6.11 kg mol⁻¹ for polydihydrocarvide (PDC). A Mark-Houwink plot revealed differences in chain flexibility influenced by the different substitution patterns of PM and PDC highlighting the importance of aliphatic side chains in tuning the property of polymers.

Scheme 21. Chemo-enzymatic synthesis of PM and PDC from naturally occurring monoterpenes (-)-menthone and (+)-dihydrocarvone.

The presence of dual alkene functionality in limonene makes it a versatile monomer that has been used in the synthesis of thermoplastic polymers and in coating applications.[93] Epoxidation of the endocyclic alkene to limonene oxide (LO) followed by ring opening copolymerisation with CO₂ affords polylimonene carbonate (PLimC) with a T_g similar to that of polycarbonates but with higher thermal and scratch resistance property (scheme 22).[94]

Scheme 22. Synthesis of limonene based polycarbonates with antimicrobial properties (PLimC-NQ) or those similar to rubber (PLimC-B3MP).

Additional modifications utilizing the exocyclic alkene provides access to unique functionalities and properties. Insertion of a thiol-functionalized ester lowers the T_g of PLimC from 130°C to 5°C with an eight-fold improvement in elongation resulting in the final polymer having properties closer to silicone rubber (scheme 21, PLimC-B3MP).[95] Insertion of a quaternary ammonium group using thiol-ene click chemistry [96] afforded polymer PLimC-NQ displaying excellent antimicrobial properties.[95]

Both α - and β -pinene are important precursors to a diverse range of renewable polymers. Out of the two isomers, β -pinene is the more reactive owing to the exocyclic methylene group which readily undergoes polymerization. A direct comparison between the two isomers found β -pinene to give the best polymer yield of 95% compared to 35% for α -pinene.[97] In the presence of TiCl_4 and 2-chloroethyl vinyl ether (CEVE) β -pinene undergoes cationic polymerization via the β -scission of the four-membered ring to give poly- β -pinene.[98] Quilter and colleagues converted the reactive exocyclic methylene group to the ketone via ozonolysis to afford (+)-nopinone (scheme 23). This was followed by a three-step chemical modification to 4-isopropylcaprolactone **37**. [99] ROP of **37** using a zirconium initiator gave poly-**37** with an M_n of 110 kg mol⁻¹.

Scheme 23. a) Conversion of β -pinene to (+)-nopinone followed By ring opening and ROP to give poly-**37** b) Beckmann rearrangement of oxime **38** gives lactam **39**. ROP of **39** gives polyamide similar to Nylon-6.

Recently Winnacker et. al. illustrated the versatility of terpene compounds by exploiting the ketone group in (+)-nopinone by forming an oxime intermediate (**38**). Beckmann rearrangement of **38** gave the bicyclic lactam **39** and subsequent ROP afforded poly-**39** with properties similar to Nylon-6. Decomposition temperature of >400°C and a melting temperature of 308°C, suggest Poly-**39** could be used as a high-performance polymer.

Due to the poor reactivity of α -pinene, it is usually converted to the more reactive (+)-pinocarvone **40** in quantitative yield through a photooxidation process (scheme 24). Compound **40**, an essential oil found in plants features an exocyclic methylene group similar to β -pinene. Opening of the cyclobutane ring in a β -scission process followed by radical polymerization affords poly-**40** with excellent thermal, optical and physical properties. [100]

Scheme 24. Photooxidation of α -pinene to (+)-pinocarvone **40**. Radical polymerization affords polymers with excellent physical properties.

Aliphatic terpenoids such as β -myrcene can be used to produce branched polymers with similar properties to rubber (scheme 25a).[101] Hillmyer and co-workers reported the conversion of β -myrcene to 3-methylenecyclopentene **41** by ring closing metathesis (RCM) using a second generation Grubbs catalyst. Cationic polymerization of monomer **41** in the presence of $SnCl_4$ furnished poly-**41** in quantitative yield with excellent thermal properties (scheme 25b).[102]

Scheme 25. a) Emulsion polymerization of β -myrcene in the synthesis of branched aliphatic polymers with properties similar to rubber b) Synthesis of poly-**41** via RCM of β -myrcene and cationic polymerization of the diene **41**.

The bicyclic terpene borneol can be used as a replacement for TPA in the production of bio-based packaging material. Insertion of a second hydroxyl group catalyzed by CYP101 from *P. putida* affords 5-exo-hydroxyborneol **42**. Polycondensation of diol **42** in the presence of diester **43** gave the terpene based biopolymer **44** with a T_g similar to commercial PET (scheme 26).[103]

Scheme 26. Chemo-enzymatic synthesis of polyester **44** with properties similar to commercial PET.

4. Polyurethanes

Polyurethanes (PU) are a class of material with multifunctional properties with the carbamate group as the core repeating unit. Formed between the polyaddition of polyols and

diisocyanates, synthesis of the polymer was first reported in the 1930's and later developed by Otto Bayer in 1947.[104] Originally used as replacement of rubber during world war 2, it has become one of the most versatile materials in construction, healthcare and coatings (figure 27).

Figure 27. Commercial applications of common polyurethane formulations. Polyurethaneimides (PUI).

Among the different applications, rigid PU foam is the most widely used material, consuming 50% of all PU production.[105] To manage industry demands, several chemical routes have been developed since the discovery of PU, many of which have diverted away from the use of phosgene in diisocyanate synthesis.[106,107] A comprehensive review was reported by Cramail and co-workers exploring chemical routes to polyurethane (scheme 28). [108] This section will focus on the use of bio-derived diamines in the production of PU.

Scheme 28. Chemical and bio-based routes to the diisocyanate monomer used in PU production.

4.1 Diamines in polyurethanes

The amine motif is ubiquitous in high performance polymers and is an essential component in polyamides such as Nylon and recently in isocyanate free PU.[108] Industry has shifted towards developing new routes to PU by avoiding the use of phosgene in the synthesis of isocyanate (section 4; scheme 28) and instead employing cyclic carbonates and diamines as monomer for non-isocyanate polyurethane (NIPU) synthesis. One of the earliest example of NIPU was reported by Piotrowska and Rokicki by combining bio-derived putrescine (1,4-

diaminobutane) with cyclic carbonate (**45**) and 1,6-hexanediol (**47**) to obtain PU (**48**) with an overall yield of 69%.^[109]

Scheme 29. Synthesis of [3,6]-polyurethane from bio-derived putrescine monomer.

Longer chain aliphatic diamines have also found use in NIPU synthesis. Endo and co-workers combined monomers **49** and **50** in the presence of LiCl to produce PU decorated with free primary and secondary hydroxyl groups in the polymer backbone. Further functionalization by either esterification or silylation afforded polymers (**51a-b**) with hydrophobic properties and a lower T_g compared to poly(hydroxyurethane).^[110]

Scheme 30. Synthesis of highly functionalized polymers **51a-c** from long chain aliphatic diamine **50** and cyclic bi-carbonate **49**.

Limonene di-carbonate (**53**) derived from waste feedstock is a versatile synthetic intermediate and an important monomer in the synthesis of bio-based PU. Both mono and di-epoxide (**52**) of limonene can be accessed through lipase-catalysed epoxidation via *in-situ* formation of peroxy acids^[111] followed by carbonation by CO_2 .^[112] The corresponding bi-cyclic carbonate has been used in the synthesis of NIPU thermoplastics with cadaverine as the amine partner.^[113]

Scheme 31. Carbonation of intermediate **52** gives the high-value bi-carbonate monomer **53** used in the synthesis of PU **54**.

4.2 Synthesis of diamine monomers

Synthesis of linear and aromatic diamines is a challenging endeavor requiring multi-step synthesis with several protection and deprotection steps. The advent of synthetic biology and biocatalysis has made it easier to access such compounds starting from commonly

occurring natural amino acids. Putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane) can be produced from L-arginine (ADC pathway), L-ornithine (ODC pathway) or L-Lysine respectively. The ADC route is the longer of the two pathways requiring three enzymatic transformations to obtain putrescine while the ODC approach requires a single decarboxylation step. Chromosomal deletion of the ArgF and ArgR gene upstream to L-ornithine and the heterologous overexpression of SpeC responsible for decarboxylation resulted in putrescine production of 6 g L⁻¹ (scheme 32).[114] The removal of the L-arginine production pathway meant the system requires external supplementation of this amino acid. To avoid this, a plasmid addiction system was developed for low-level ArgF expression.[115] By modifying the promoter and ribosomal binding site, production improved 3 fold to 19 g L⁻¹ of putrescine. Cadaverine can be produced from a single step decarboxylation from L-lysine. The first reported production of cadaverine was in a patent published in 2007 using resting *E. coli* cells overexpressing CadA. Decarboxylation of L-lysine produced 69 g L⁻¹ of cadaverine.[116] Lee et. al. reported the production of 9.6 g L⁻¹ of cadaverine using growing cells by overexpressing CadA, DapA (involved in L-lysine synthesis) and replacing the native promoter with the stronger *trc* promoter in the genome (scheme 32).[117]

Scheme 32. a) Production of putrescine by heterologous expression of SpeC and deletion of ArgF downstream to L-ornithine b) Cadaverine production by improving metabolic flux towards L-lysine synthesis and removal of genes downstream to CadA.

Biocatalytic routes to diamines are notoriously difficult as they generally cyclize and dimerize to form stable adducts. This is a common strategy to synthesize pyrroles, piperidines [118] and azepines [119] via spontaneous cyclization to the imine followed by enzymatic reduction using IREDs.[120–122] Kroutil and co-workers developed a self-sufficient redox-neutral cascade to aliphatic diamines starting from the corresponding diol.[123] By employing a thermostable ADH from *Bacillus stearothermophilus* (ADH-hT) and a transaminase from *Chromobacterium violaceum* (CV- ω TA) 99% conversion of the diamine was achieved by sequential oxidation/amination process. The diamine **56b** was synthesized on a preparative scale with an isolated yield of 70% starting from 174 mg of **52b**.

Scheme 33. Redox neutral enzymatic cascade towards the synthesis of long-chain aliphatic diamines.

5. Comparison of different routes and assessing feasibility of industrial production

The review has so far explored diverse biocatalytic routes towards highly functional monomers and its application in the production of bio-polymers. This section aims to provide a qualitative and quantitative comparison between the different biocatalytic and biosynthetic routes cited in this paper and assessment of suitability for commercial production. Each route is measured against three criteria 1) accessibility of substrates 2) formation of by-products and 3) yield/productivity.

Substrate and feedstock availability is crucial for embarking and implementing new manufacturing processes. Cost and ease of feedstock accessibility can influence both process conditions, costs and in the long-term impact supply chain sustainability. Fermentation usually requires glucose/glycerol as a cheap energy source, however, biocatalytic reactions may often start from advanced chemicals which can be expensive and difficult to access. Waste generation is a vital metric when developing synthetic routes, consequently affecting the viability of a potential process in terms of sustainability and can in many cases complicate downstream processing (DSP). Perhaps the best metric is yield/productivity, providing a direct comparison between different routes and highlighting issues surrounding efficiency.

Current biocatalytic TPA production lacks the scalability of chemical routes, with the best biosynthetic production starting from petroleum-based xylene.[7] While synthesis of bio-based PET has gained considerable support, it still suffers from similar issues of biodegradability and recyclability. Recently, FDCA has emerged as a replacement for TPA, producing polymers with similar properties to PET with an added advantage of being fully bio-based and biodegradable. Production has also been demonstrated to be competitive, generating 30.1g L⁻¹ of FDCA in a sequential oxidative process from biorefined HMF. With a CAGR of 7.6%, the market for bio-plastics is expected to grow in 2019 with Asia accounting for 80% of total production due to better access to feedstock and a favourable political framework.[124]

PHAs have seen a resurgence in the bio-based polymer market since their discovery in the early nineteenth century. As bifunctional, environmentally benign polyesters with remarkable flexibility and strength these are the second fastest growing bio-plastics according to a recent market report.[124] Unlike plant-based polymers, PHAs are produced by bacteria with monomers containing hydroxy acid functional groups. These are the key building blocks for next-generation biodegradable polymers and can be synthesized from several starting points (discussed in section 2.2).

A comparison using the criteria introduced earlier show synthesis from styrene to be one of the best routes for commercial production closely followed by DKR of hydroxynitriles. The cheap and readily available substrate and the modularity of the pathway is attractive for industrial application giving access to other high-value chemical intermediates such as diols and amino acids. In addition, the method is scalable with conversions >90% and ee >99% with minimal waste and accumulation of intermediates. Synthesis via the oxidation of hydroxy nitriles offers an alternative route with quantitative production of either (*R*)- or (*S*)- α -hydroxy acids through a DKR approach using nitrilase. These enzymes have proven to be robust catalysts used in the production of speciality chemicals, [125] exhibiting high thermal stability and tolerance to organic solvents.[126]

Longer chain hydroxy acids with properties suited for a range of applications and processes are perhaps more challenging to synthesize enzymatically due to the size and flexibility of the starting compound. 3-HP and its derivatives can be synthesized from β -ketonitriles in a two-step enzymatic process. The starting nitrile is easily accessible from either substituted α,β -unsaturated ketone via cyanation or from the coupling between acetonitrile and an

ester.[127] The production entails the use of purified CmCR, as the presence of ethanol (in whole cell biotransformation) can lead to alkylated side-products.[52] Monomers with > 4 carbon chain length can be produced from renewable fatty acids in a three-step biocatalytic process. The method affords the target product in good yield but also producing heptanoic acid as a by-product complicating DSP.[54] A two-step chemoenzymatic process may be better suited for industrial synthesis as demonstrated by Messiha *et al* (section 3.2) in the synthesis of PM from menthone.[91] These terpene-based polymers with a diversity of >80,000 compounds have generated immense interest in the speciality polymer market. Due to the complexity of these compounds, most are isolated from plants and only recently through fermentation. Chemical synthesis of monoterpenoids is difficult, laborious and wasteful as they frequently produce several by-products and often start from advanced chemicals. Microbial production is perhaps the most sustainable method to produce these compounds avoiding the use of plants and agricultural land for chemical production.

With diamines playing an important role in the development of bio-polymers, sustainable production of these monomers has stifled progress in recent years. This paper introduces two approaches to producing diamines; fermentation via the catabolism of naturally occurring amino acids and through cascade biocatalysis. The synthetic biology approach has been successful in producing putrescine[114,115] and cadaverine[116,117] with titers competitive for industrial production. The lack of flexibility in producing diamines of varying length is a disadvantage of this approach and would require designing and engineering enzymes that make use of longer chain non-natural amino acids. In contrast, the biocatalytic route[123] is a lot more flexible with the potential of producing diamines of varying chain length. Comparison of the different routes and their commercial viability can be found in **table 1**, highlighting areas which require further developments and improvement for industrial production.

Table 1. Comparison of different biotechnological route to high-value monomers. Highlighting formation of by-products, yield and commercial viability.

Product [Ref.]	Substrate/Feedstock chemicals	By-Products	Yield	Challenges and Commercial viability
TPA [7]	Xylene	-	97 mol% from <i>p</i> -xylene	Process produces good yield of TPA with minimal by-product. Has the potential for large scale production.
TPA [8]	1,4-BDM	CPO and XO oxidation give mixtures of intermediates	65%	Demonstrated only on small scale. Long reaction times and CPO enzyme is deactivated under high [H ₂ O ₂]
TPA [9]	Glucose	Mixtures of <i>para</i> - (72%) and <i>meta</i> -cycloadducts (24%)	85%	Synthetic route demonstrated on preparative scale. Mixtures of by-products and unreacted material may pose DSP issues.
FDCA [10]	HMF/Glycerol	Minimal accumulation of intermediates	75.9%	Feedstock chemicals are renewably resourced with a simple isolation protocol.
Malic acid [20]	4	Mixture of product and starting compound.	38-94%	Satisfactory yield and poor ee with some substrates make it unlikely for commercial production.
Malic acid [21]	Glucose	Small amounts of pyruvate and succinate	59 g L ⁻¹	High productivity with minimal by-products make it viable for large-scale synthesis.
Succinic acid [23]	Glucose	Negligible concentrations of Lactic and pyruvic acid.	10.7 g L ⁻¹	Good productivity and an important platform chemical make it attractive for commercial production.
α -hydroxy acid [33]	<i>rac</i> - 10	Unreacted substrate and the removal of inhibitory product (<i>S</i>)- 11	275 g L ⁻¹ d ⁻¹	Excellent yield using a robust catalyst. However, <i>in situ</i> removal of (<i>S</i>)- 11 can be costly.
α -hydroxy acid [37]	<i>rac</i> - 14 and 15	-	85%	DKR demonstrated on preparative scale producing a panel of hydroxy acid derivative.
α -hydroxy acid [42]	styrene	Minimal build-up of intermediates.	71-97%	Modularity of the process including high conversion and ee make it feasible for pilot-scale

				production.
3-HP [51]	3-HPN	-	184.7 g L ⁻¹	Method can be applied to large-scale production due to enzyme stability, reusability and excellent productivity.
3-HP [52]	25	Alkylated products when using whole-cells.	80-90%	Two step enzymatic process demonstrated on a 10 mM scale. Promising route to aromatic PHA, requires scale-up optimization.
ω -hydroxynonanoic acid [54]	Oleic acid	Heptanoic acid	60%	Elegant multi-enzyme cascade to produce flexible monomers from renewable feedstock. Process lacks scalability with current method at mM scale.
Chiral lactones [69]	Cyclic ketones	Can produce a mixture of normal and abnormal lactone	50->99%	Method requires enzyme engineering to obtain single regioisomer and process optimization for scale-up.
Monoterpenes [77, 78, 82]	Glucose	-	0.058 – 1.37 g L ⁻¹	Recent developments in process conditions and enzyme discovery/engineering has facilitated transition to preparative scale production. Several optimized routes to novel functional polymers exist for commercial applications.
Terpene-based lactone [91]	(-)-Menthone	-	93.5%	Demonstrated large-scale biocatalytic synthesis of Menthide followed by chemical ROP.
Long chain diamines [123]	52a and b	-	94%	Environmentally benign and sustainable process producing PU precursors.
Putrescine [114]	Glucose	-	6 g L ⁻¹	Optimized production strain with minimal accumulation of by-products.
Cadaverine [116]	Glucose	-	69 g L ⁻¹	Patented method with optimized pathway.

6. Conclusion and perspective

In view of the current environmental crisis, there is an urgent need to develop biodegradable plastics and new manufacturing processes utilizing renewable feedstock for consumer grade polymers and in medical applications. Biocatalysis and synthetic biology, which has predominantly been used in the synthesis of API precursors, have the potential to play a significant role in developing new bio-based materials with unique properties and applications. The discovery and evolution of enzymes has expanded the biocatalytic toolbox, giving access to new chemical transformations and development of novel molecular architectures which would otherwise be difficult to achieve through traditional methods.

By exploiting the selectivity and complementarity of biological catalysts, enzymatic cascades have been developed to produce biodegradable monomers used in high performance materials or as flexible biocompatible polymers in tissue engineering. Perhaps the greatest potential comes from the rapid developments in synthetic biology; incorporating novel enzymes to create artificial biosynthetic pathways towards industrial production of high-value monomers. While this review has illustrated the potential of IB and synthetic biology in the production of bio-degradable polymers, there are key challenges in manufacturing that still need to be addressed. The polymer industry consumes large amounts of monomers per year to keep up with rising market demands. A typical TPA plant produces >500000 tons per annum (tpa) and a specialty monomer production facility can produce between 10 to 100 tpa. Current bio-manufacturing processes are lacking the scalability and productivity of petroleum-based routes; however, governmental regulations and consumer pressure has led the chemical industry to adopt and implement sustainable production processes. For example, DuPont in partnership with Tate & Lyle have commercialized the production of 1,3-propanediol (bio-PDO) from waste biomass at a capacity of 45,000 tpa – a key component in the synthesis of polytrimethylene terephthalate (PTT). The sustainable production of bio-PDO highlights the potential of biotechnology to reduce waste and improve resource efficiency, therefore playing a vital role in addressing the current challenges in sustainability.

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