Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Harnessing biocatalysis to achieve selective functional group interconversion of monomers

Madan R Gopal[1,2] and Aditya M Kunjapur[1,2]

Polymeric materials are ubiquitous to modern life. However, reliance of petroleum for polymeric building blocks is not sustainable. The synthesis of macromolecules from recalcitrant polymer waste feedstocks, such as plastic waste and lignocellulosic biomass, presents an opportunity to bypass the use of petroleum-based feedstocks. However, the deconstruction and transformation of these alternative feedstocks remained limited until recently. Herein, we highlight examples of monomers liberated from the deconstruction of recalcitrant polymers, and more extensively, we showcase the state-of-the-art in biocatalytic technologies that are enabling synthesis of diverse upcycled monomeric starting materials for a wide variety of macromolecules. Overall, this review emphasizes the importance of functional group interconversion as a promising strategy by which biocatalysis can aid the diversification and upcycling of monomers.

Addresses
[1] Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE, USA
[2] Center for Plastics Innovation, University of Delaware, Newark, DE, USA

Corresponding author: Kunjapur, Aditya M (kunjapur@udel.edu)

Current Opinion in Biotechnology 2024, **86**:103093

This review comes from a themed issue on **Tissue, Cell & Pathway Engineering**

Edited by **Wilfred Chen** and **Millicent Sullivan**

Available online xxxx

https://doi.org/10.1016/j.copbio.2024.103093

Introduction

Over the last decade, biocatalysis has increasingly permeated a wide range of industrial processes for small-molecule transformations, particularly in the pharmaceutical industry [1,2]. As biocatalytic technology for industrial processes is maturing, and as new enzymes are continually discovered or engineered, opportunities to harness biocatalysis in other industries are emerging. The materials industry is one notable example, with a sharp rise in interest enabled by the discovery and engineering of enzymes for plastic degradation [3] and chemical methods for breakdown and functionalization of lignocellulosic biomass (LCB) [4]. The ability to efficiently break down new classes of recalcitrant polymers creates myriad opportunities for innovation in the materials sector. Given that society will still rely on a wide range of materials, one attractive option is to harness biocatalysis for chemical recycling of waste polymers. When interpreted strictly, chemical recycling means the remaking of the identical virgin polymer from the same monomers [5]. Another option is to catabolize the deconstruction products of polymers toward central carbon metabolism. While this could vastly broaden the range of possible products to include any molecule that appears in metabolism as well as nonnatural targets of engineered biosynthetic pathways, this option often results in poorer atom economy and an imbalance between the market sizes of the plastic feedstock and the alternative product. A third option is to chemically upcycle recalcitrant polymers, where we interpret 'upcycling' as achieving goals of either increasing product value, creating a greener path to form other existing polymer classes, or introducing recyclability by design [6–8]. The growing number of biocatalytic and chemical processes capable of deconstructing macromolecules foreshadow an increasing number of processes ripe for intensification. It is reasonable to anticipate that a larger number of macromolecular linkages will be broken, resulting in more distinct terminal chemical functional groups for biochemical pathway designers to imagine manipulating. Finally, the recent growth in biocatalysts that perform selective functional group interconversion (FGI) with broad substrate specificity means that more processes could be developed in which the starting molecules could be interchanged, a broad range of starting materials could be funneled toward common products, or selective transformations could be occurring within mixed waste streams. In this review, we provide an overview of recent works in support of these trends, and with a perspective on where the field may be headed.

An overview of deconstruction methods of recalcitrant materials to access monomer pools

Biocatalytic deconstruction of plastics that contain ester linkages has recently garnered much attention. The most commonly produced polyester plastic is polyethylene terephthalate (PET). Established PET deconstruction products include terephthalic acid (TPA), mono-2-hydroxyethyl terephthalic acid (MHET),

bis-2-hydroxyethyl terephthalate (BHET), and ethylene glycol (EG). Numerous chemical and pyrolytic routes to break down PET have been reported. However, biocatalysis offers a uniquely green route to break down PET in aqueous conditions, neutral pH, and at mild temperatures. Biocatalytic efforts to break down PET focus on cutinases [9,10] and PETases [11,12]. Both cutinase and PETase have served as launching points for engineered variants with high activity and thermostability. The first commercialized enzymatic deconstruction process for PET was developed by Carbios using an engineered leaf branch compost cutinase (LCC) [13]. The most active LCC variants, named "ICCG" (LCC F243I/D238C/S283C/Y127G) and "WCCG" (LCC F243W/D238C/S283C/Y127G), both contained engineered disulfide bonds and two binding pocket mutations that resulted in nearly 90% depolymerization of amorphous PET at a low enzyme loading of 0.3 wt%. Meanwhile, other groups have continued to advance the engineering of PETases. Lu et al. used machine learning and rational design to engineer the most active PETase variant to date, dubbed FAST-PETase ("Functional, Active, Stable, and Tolerant PETase"). FAST-PETase showed high activity on pretreated PET substrates at 50 °C [14]. Although engineered PET-hydrolyzing enzymes provide a sustainable avenue for deconstruction, a critical drawback is the need for thermal or mechanical pretreatment of real PET waste streams as low enzyme activity can be attributed to high substrate crystallinity (Figure 1a). To address this, Bell et al. utilized site saturation mutagenesis and DNA shuffling to develop HotPETase, a thermostable PETase capable of hydrolyzing semicrystalline PET. Under optimal conditions, HotPETase liberated 6.07 mM of MHET + TPA in 5 h from semicrystalline PET (29.8% crystallinity) at an elevated temperature of 60 °C [15].

Deconstruction technologies of plastics that contain C–C bonds in their monomer linkages, such as polyethylene (PE) and polystyrene (PS), and C–N bonds such as polyurethanes (PUs) and polyamides (PAs), remain underdeveloped. Unlike the hydrolyzable C–O bonds present in PET, the macromolecular backbone of these plastics makes them especially recalcitrant to biological depolymerization processes [16]. The digestive tracts of several insect species that are capable of degrading PE and PS applying this technology to valorization efforts would require separation of deconstructed product from frass and a deeper characterization of the numerous chemical modifications [17,18]. Enzymatic deconstruction of PE by a PEase enzyme from wax worm saliva helps to introduce heteroatom content into the PE backbone via oxidation, which is generally the first step to biological deconstruction. The main deconstruction products from this enzymatic reaction are C10-C22 methyl ketone, sebacic acid, and 2,3-butanediol [19]. Currently, amidases and urethanases for breakdown of PAs and PUs remain underdeveloped, with only a few reports of enzymes that degrade nylons [20,21] or urethanes [22]. Accelerating enzyme discovery and improving catalytic screening of enzymes for plastics deconstruction remains an area of great interest and need.

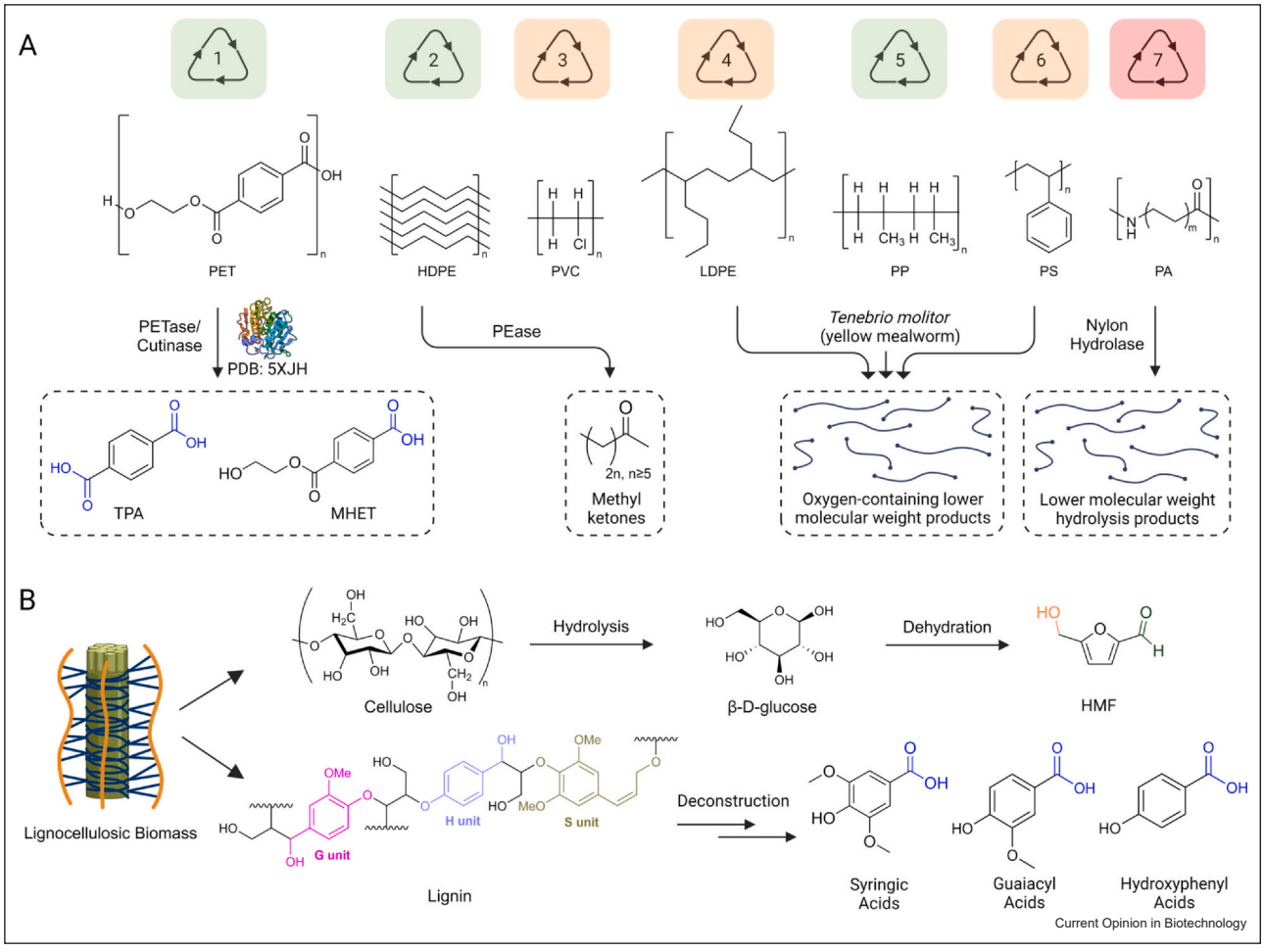
Last, LCB is the most abundant polymer on earth and represents a largely untapped source feedstock material, especially for bio-based plastic alternatives that could contain aryl or heterocyclic repeating units (Figure 1b). LCB comprises lignin, an aromatic-rich cross-linked polymer network, and cellulose and hemicellulose, both of which are polysaccharides [23]. The monomer components of LCB have garnered considerable attention as a drop-in replacement for petroleum-derived monomers. Deconstruction of cellulose to glucose and fructose followed by acid-catalyzed dehydration has previously been used to produce the oxygenated heterocycle hydroxymethylfurfural (HMF) directly from corn stover [24]. Lignin on the other hand is the most difficult of the three types of LCB components to deconstruct. The recalcitrance of lignin is largely due to its degree of functionalization in the three primary aromatic monolignol monomers, which form a highly crosslinked network based on recalcitrant C-C and ether bonds. Lignin can be deconstructed by alkaline, acidic, thermal, reductive, or oxidative routes to liberate a variety of substituted phenol, guaiacol, and syringol monomers that can be harnessed for downstream valorization [25,26].

The breadth of monomers that can be accessed from plastic waste and LCB is vast. While scalable biocatalytic deconstruction has proven economically feasible for PET, further development of technologies for C-C bond containing polymers is required. Application of the correct deconstruction strategy will allow monomers to be selectively liberated and applied toward the synthesis of value-added feedstocks for materials applications.

Biocatalytic valorization of monomers for value- and performance-enhanced materials

Deconstructed plastic wastes and LCB contain a mix of functional groups that, with the right green chemistry tools, can be functionalized into monomers that are difficult-to-access by conventional synthetic routes or into novel materials. In this section, we highlight examples of biocatalytic FGI to valorize monomers and create feedstock compounds, with the eventual goal of creating performance-enhanced materials [27]. Biocatalytic chemical transformations are growing in popularity since enzymes perform difficult chemistries in green solvents, mild reaction temperatures, and often exhibit high substrate specificity. Biocatalytic heteroatom transformations have been used extensively in the pharmaceutical and specialty chemical industries, but have yet to debut at large scale for materials applications.

Figure 1



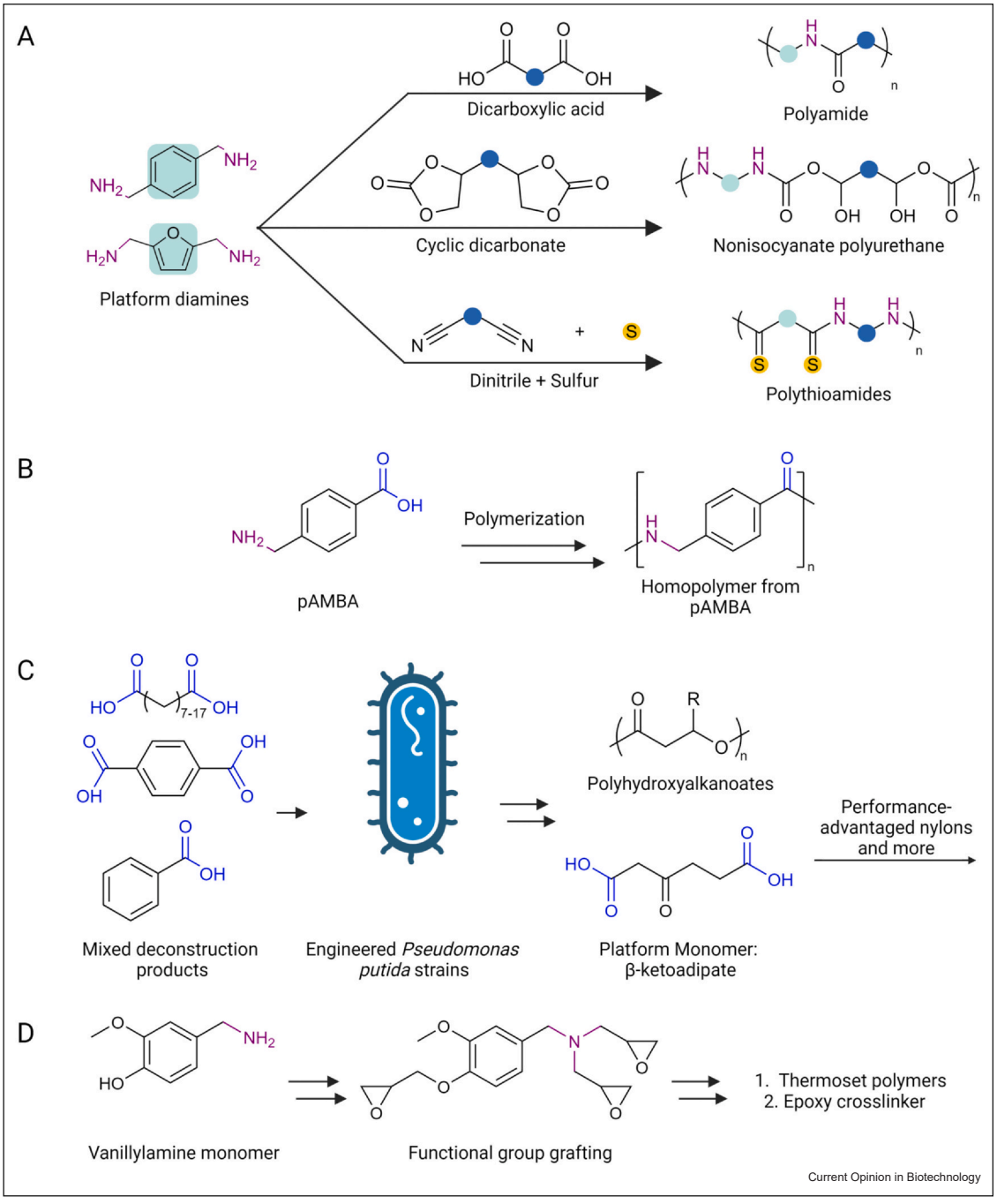
Deconstruction technologies driving the shift toward utilizing recalcitrant polymers as a source of monomers for materials synthesis. **(a)** Biological deconstruction of plastic waste into monomers or lower products. Resin Identification Codes (RIC): green indicates established recycling technologies and high-technology adoption, yellow indicates difficulty in sorting or processing, and red indicates potential opportunities for advancement. **(b)** LCB contains cellulose, a homopolymer of β-D-glucose, that can be processed into aromatic furans, while lignin, a heterogeneous aromatic-rich polymer, can be deconstructed into different phenolic monomers that are further functionalized for use in polymeric material. HPDE, high-density polyethylene; PVC, polyvinylchloride; LDPE, low-density polyethylene; PP, polypropylene. Figure created with Biorender.

The ability to engineer biocatalysts for high activity and substrate recognition, as well as the development of host organisms that can stabilize reaction intermediates, has positioned biocatalysis as an option not to be overlooked for monomer valorization.

Biocatalytic functional group interconversions of polyethylene terephthalate and mixed plastic waste monomers

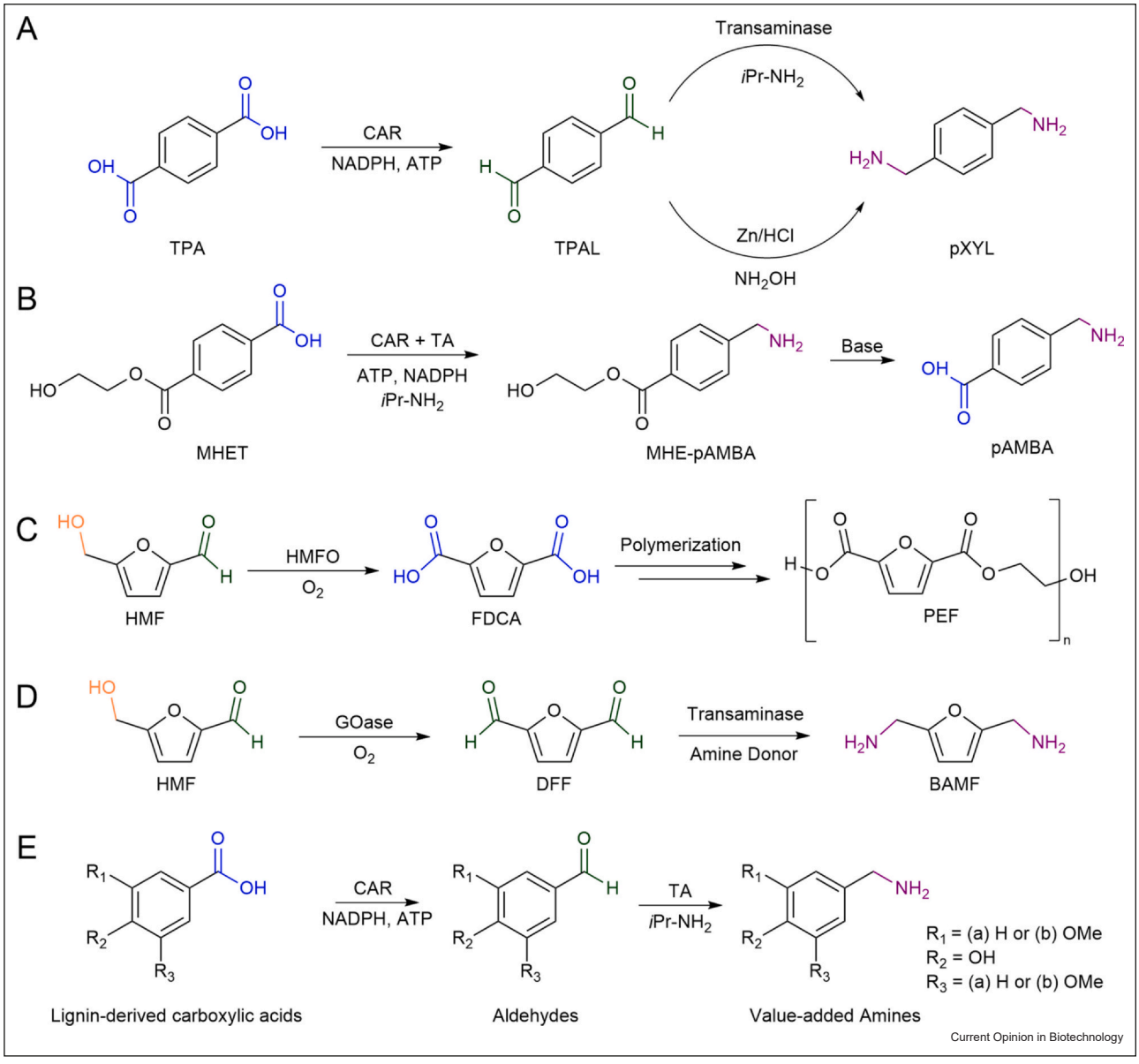
FGI using biocatalysis is a growing area of interest to a broad range of disciplines for many purposes beyond macromolecule synthesis as well [28]. There is growing interest in functionalizing carboxylic acid precursors into value-added monomers due to their stability and abundance as starting materials. However, in their protonated or anionic states, carboxylic acids are relatively unreactive, and require activation for downstream functionalization. TPA and MHET, two products from PET deconstruction, are sterically unhindered carboxylic acids which makes them a prime target for FGI to higher value molecules. In the case of TPA, which is a dicarboxylic acid, a route to functionalize both carboxylate groups can provide an avenue for repolymerization of the aromatic backbone into a different polymer class, such as one with C–N bonds, instead of the original C–O bond polyester (Figure 2a). Bayer et al. reported the bifunctional reduction of TPA to its corresponding dialdehyde terephthalaldehyde (TPAL) using a single carboxylic acid reductase (CAR) from *Mycobacterium marinum* harbored in resting *E. coli* cells [29]. The activated aldehyde allows for multiple chemical and enzymatic routes to molecules such as amine, alcohols, hydroxynitriles, and

Figure 2



Potential polymeric materials derived from value-added monomers. **(a)** Aryl and furanic diamines are highly desired for their use in rigid polymer networks, PAs, nonisocyanate polyurethanes, and polythioamides, a class of polymers with desirable optical and semiconducting properties. Polar furanic diamines can also induce hydrogen bonding within polymer networks, leading to materials with higher T_g. **(b)** Molecules containing multiple functional groups have great potential to be self-polymerized. pAMBA, an amine-containing carboxylic acid, can potentially be polymerized into a rigid aramid fiber that is similar in structure to Kevlar. **(c)** Funneling multiple deconstruction products can alleviate costly plastic separation. By engineering the central metabolism of *P. putida*, aromatic and aliphatic carboxylic acids can be funneled toward PHA or β-ketoadipate. β-ketoadipate is a performance-advantaged nylon precursor. **(d)** Grafting glycidyl functional groups onto biologically synthesized vanillylamine prepares it for subsequent use as a trifunctional epoxide monomer for use in thermoset polymers and as an epoxy cross-linker or in bio-based vitrimers.

Scheme 1



Heteroatom transformations relevant for plastic and LCB valorization. **(a)** Fully enzymatic and chemoenzymatic functionalization of TPA, a diacid, into pXYL, a diamine, *via* the aldehyde intermediate TPAL. **(b)** Synthesis of an amine-containing carboxylic acid from MHET using an enzymatic reduction and transamination cascade. **(c)** Full oxidation of HMF to FDCA by HMFO for use as a drop-in replacement to TPA. **(d)** Partial reduction of HMF to DFF followed by enzymatic transamination to BAMF. **(e)** Synthesis of aryl amines from multifunctional lignin-derived phenols.

aldols [30]. To this end, the authors used a chemical route to perform aqueous-phase reductive amination of TPAL to a diamine, *para*-xylylenediamine (pXYL), *via* a hydroxylamine intermediate, resulting in 15% yield of the desired product (Scheme 1a).

Interconversion of dicarboxylic acids to diamines is of great interest for production of PAs and PUs [31,32]. Our lab published an *in vitro* enzyme cascade with purified enzymes in which the CAR from *Segniliparus rotundus* (srCAR) reduced TPA to TPAL [33]. Furthermore, bifunctional transamination of TPAL by a single ω-transaminase (TA) from *Chromobacterium violaceum* (cvTA) resulted in stoichiometric pXYL yield from 10 mM TPAL loading. By optimizing the srCAR to cvTA ratio, and coupling srCAR to enzymatic nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine 5′-triphosphate (ATP) regeneration, a one-pot reaction converted 10 mM TPA to pXYL at 69% yield using only low micromolar enzyme concentrations. This cascade is modular

and accepts alternate PET deconstruction products such as MHET with no change to reaction conditions (Scheme 1b). MHET undergoes reduction by srCAR and transamination by cvTA to form monohydroxyethyl-*para*-aminomethylbenzoic acid (MHE-pAMBA). With the addition of base, MHE-pAMBA is hydrolyzed to pAMBA at 70% yield, which can be used as a monomer for homopolymer synthesis, in PU resins, and as a pharmaceutical product and synthon (Figure 2b) [34].

For biocatalytic systems to be economically viable, fermentation and processing costs must be kept low. This generally requires that enzymes are not used in purified form but rather are used in whole cells, and that cells are engineered to prevent competing reactions that often are catalyzed by promiscuous endogenous enzymes. In pursuit of this goal, Dickey et al. developed the *E. coli* RARE.Δ16 strain [35], based on the original *E. coli* RARE strain [36], as an enabling technology that takes advantage of translational knockouts to stabilize aldehydes in whole cells. This strain can stabilize TPAL for 24 h, and, when RARE.Δ16 cells expressing cvTA are supplied with TPAL, the desired diamine product pXYL is formed with high selectivity.

Many plastic packaging materials contain a diverse array of mixed polymers that impart desirable characteristics such as low-oxygen permeability or high strength-to-weight ratio. However, separating layered plastics and mixed plastics is often impractical and technologies that address mixed plastics waste are few. Engineered metabolic pathways in organisms such as *Pseudomonas putida* have been sought for their biotechnological potential in processing multiple plastics deconstruction products [37,38]. Several *Pseudomonas* strains process both TPA and EG into polyhydroxyalkanoates (PHA) [39,40]. The work of Bao et al. features a synthetic microbial consortia of engineered *P. putida* strains (Pp-T and Pp-E) to produce both muconic acid and PHAs when PET hydrolysates are supplied as the sole carbon source [41]. Tandem chemical deconstruction and biological upcycling of a mixed waste stream of PET, HDPE, and PS was reported in 2022 using engineered *P. putida* strains for production of two distinct products, PHA or β-ketoadipate (Figure 2c) [42]. β-ketoadipate represents a class of performance-advantaged dicarboxylate monomers that can be used in nylons and other PA applications [43], whereas PHA can be modified and further functionalized for desired polymer properties [44].

Advances in PET and plastics deconstruction have ushered in the development of valorization technologies to utilize substrates to synthesize aldehydes, alcohols, and amines using *in vitro* enzyme cascades and whole-cell biocatalysts. However, oxidative PEases also show potential in forming long-chain carboxylic acids that could be a potential feedstock for biocatalytic synthesis of fatty diamines or diols [45] for application in polymers [46]. Historically, biocatalytic amine, alcohol, and heteroatom synthesis have been used in the fine chemistry and pharmaceutical industries. Harnessing biocatalytic heteroatom transformation for monomer synthesis opens multiple avenues to diverse classes of valorized polymers to meet growing demand for sustainable materials.

Valorization of hydroxymethylfurfural to valorized monomers through functional group interconversion

Complete biocatalytic oxidation of HMF to 2,5-furandicarboxylic acid (FDCA) has garnered significant interest (Scheme 1c). FDCA can serve as a drop-in replacement for TPA in PET to form poly(ethylene furanoate), a polyester with properties akin to PET [47]. Development of a single-enzyme system that can catalyze sequential oxidations of the alcohol and aldehyde groups of HMF to carboxylic acids is desirable for decreasing process complexity and cost. Dijkman et al. reported a single, flavin adenine dinucleotide (FAD)-dependent HMF oxidase (HMFO) from *Methylovorus sp.* strain MP688 that yielded 95% yield of FDCA [48,49].

Partial oxidation of HMF to diformylfuran (DFF) also offers a compelling route for valorization (Scheme 1d). Dialdehydes are often synthetically difficult to synthesize due to the presence of a heterogeneous mix of chemical species of acids, aldehydes, and alcohol groups in the product stream [50]. Engineered galactose oxidases and whole-cell biocatalysts can provide robust conversion of HMF to DFF with yields approaching 95% in a variety of reaction conditions [51–53]. Of interest for materials synthesis is biocatalytic funneling of dialdehyde products to diamines, which allows for a sustainable route to valorized monomers. Diamines are critical for synthesis of PAs, PUs, and polythioamide materials [54]. Dunbabin et al. showed that TAs readily accept DFF as a substrate to produce *bis*-aminomethylfuran (BAMF) at 70% yield [55]. The biotransformation of DFF to BAMF has also been studied in both batch and continuous flow with immobilized TAs, with stoichiometric yields of BAMF in the batch process [56].

Bifunctional biomass-derived monomers will play a crucial role in the next generation of sustainable polymers. Chemical transformation of HMF to FDCA, DFF, and BAMF monomers highlights the versatility of biocatalytic heteroatom transformations to repurpose a single starting material into a diverse showcase of value-added starting materials driven by green synthesis techniques.

Functional group interconversions of lignin-derived monomers

The diversity in heteroatom content in lignin allows for synthesis of cross-linked thermosets with performance-enhanced properties (Figure 2d) [57,58]. One of the most-

studied biocatalytic FGIs of lignin-derived monomers is the transformation of vanillin and other methoxyphenol lignin derivatives into amines [59,60]. In 2021, Fu et al. developed a recombinant *E. coli* strain harboring a two-enzyme cascade to convert the lignin-derived monomers ferulic acid and vanillic acid to vanillylamine [61]. Ferulic acid was first converted to vanillin by heterologous expression of a feruloyl-CoA synthetase and an enoyl-CoA hydratase/aldolase from *P. putida*. Subsequent transamination of vanillin to vanillylamine was catalyzed by an ω-TA from *C. violaceum*. In the case of vanillic acid as the substrate, a CAR from *S. rotundus* was instead chosen to synthesize vanillin *in vivo* followed by transamination to vanillylamine by the ω-TA. Vanillylamine synthesis in both whole-cell cascades resulted in high yields of the desired product at approximately 95% yield.

While this cascade creates a value-added thermoset monomer, the generalizability of a biocatalysis platform for synthesis of diverse amines from lignin was not fully explored. Nain et al. explored whole-cell and cell-free extract formats to produce vanillylamine, syringylamine, cinnamylamine, 4-hydroxy-3-methoxycinnamylamine, and 3-(4-hydroxy-3-methoxyphenyl)propylamine from biomass precursors (Scheme 1e) [62]. In both formats, the CAR and ω-TA cascade shows robust activity after multiple cycles in whole cells. Primary amine synthesis by enzymatic transamination often involves the generation or supplementation of reactive aldehyde species, which are readily detoxified to alcohols or carboxylic acids by endogenous enzymes in whole-cell contexts [63]. Similar to the work of Dickey et al., engineered *E. coli* containing knockouts of highly active endogenous oxidoreductases can also be a useful tool for ensuring substrate funneling to the desired products.

Conclusions and future perspectives

The works summarized here showcase the emerging opportunity for biocatalysis and biochemical pathway design strategies to expand further into the arena of producing monomers for macromolecule synthesis. Unlike classic metabolic engineering efforts that focused on production of monomers such as 1,4-butanediol, the latest wave of research has embraced waste polymer feedstocks and the development of precision transformation opportunities. Going forward, the field will benefit greatly from advances in four areas. First, for recently demonstrated transformation routes, it will be valuable to apply them to an increasingly wide range of substrates using engineered variants or homologs. This is especially important to achieve funneling of substrates that contain common functional group chemistries in mixed waste streams toward similar upcycled monomers. Second, for the deconstruction products that can currently be generated, it will be valuable to design routes toward more complex but useful functional group chemistries. Here, leveraging the respective strengths of biocatalysis and chemocatalysis to create hybrid and compatible routes could be especially promising. Third, the identification or engineering of enzymes that break down the plastic types that cannot yet be efficiently broken down, particularly those that install or reveal heteroatom chemistry in which enzymes can readily further manipulate, will be immensely valuable. Finally, it is of utmost importance in this particular application area of biocatalysis that practitioners collaborate with polymer scientists to actually create macromolecules from the targeted monomers. In doing so, both communities can discover whether biocatalytic transformations show promise for meeting the requisite criteria for synthesis on an industrial scale and whether the resulting materials indeed exhibit performance-advantaged properties.

CRediT authorship contribution statement

M.R.G: Conceptualization, Writing – original draft, Writing – review & editing. **A.M.K:** Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

A.M.K. is a co-founder of Nitro Biosciences and a Scientific Advisory Board member of Wild Microbes. In addition, A.M.K. and M.R.G. are co-inventors on a filed patent application related to the use of enzymes for selective functional group interconversion of PET deconstruction products.

Acknowledgements

The authors acknowledge Professor Wilfred Chen (University of Delaware) for many valuable discussions about the contents of this article. M.R.G. and A.M.K. acknowledge funding support from the Center for Plastics Innovation, an Energy Frontier Research Center funded by the U.S. Department of Energy (DOE), Office of Science, Basic Energy Sciences, under Award No. # DE-SC0021166. Figures 1 and 2 are created with BioRender.com.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest.

1. Bell EL, Finnigan W, France SP, Green AP, Hayes MA, Hepworth LJ, Lovelock SL, Niikura H, Osuna S, Romero E, *et al.*: **Biocatalysis**. *Nat Rev Methods Prim* 2021, **1**:1-21, https://doi.org/10.1038/s43586-021-00044-z

2. France SP, Lewis RD, Martinez CA: **The evolving nature of biocatalysis in pharmaceutical research and development**. *JACS Au* 2023, **3**:715-735.

3. Tournier V, Duquesne S, Guillamot F, Cramail H, Taton D, Marty A, André I: **Enzymes' power for plastics degradation**. *Chem Rev* 2023, **123**:5612-5701.

4. O'dea RM, Willie JA, Epps TH: **100th Anniversary of macromolecular science viewpoint: polymers from lignocellulosic biomass. Current challenges and future opportunities**. *ACS Macro Lett* 2020, **9**:476-493.

5. Ragaert K, Delva L, Van Geem K: **Mechanical and chemical recycling of solid plastic waste**. *Waste Manag* 2017, **69**:24-58.

6. Korley LT, Epps III TH, Helms BA, Ryan AJ: **Toward polymer upcycling—adding value and tackling circularity**. *Science* 2021, **373**:66-69.

7. Hinton ZR, Talley MR, Kots PA, Le AV, Zhang T, Mackay ME, Kunjapur AM, Bai P, Vlachos DG, Watson MP, *et al*.: **Innovations toward the valorization of plastics waste**. *Annu Rev Mater Res* 2022, **52**:249-280.

8. Blank LM, Narancic T, Mampel J, Tiso T, O'Connor K: **Biotechnological upcycling of plastic waste and other non-conventional feedstocks in a circular economy**. *Curr Opin Biotechnol* 2020, **62**:212-219.

9. Müller RJ, Schrader H, Profe J, Dresler K, Deckwer WD: **Enzymatic degradation of poly(ethylene terephthalate): rapid hydrolyse using a hydrolase from T. fusca**. *Macromol Rapid Commun* 2005, **26**:1400-1405.

10. Baker PJ, Poultney C, Liu Z, Gross R, Montclare JK: **Identification and comparison of cutinases for synthetic polyester degradation**. *Appl Microbiol Biotechnol* 2012, **93**:229-240.

11. Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K: **A bacterium that degrades and assimilates poly(ethylene terephthalate)**. *Science (1979)* 2016, **351**:1196-1199.

12. Austin HP, Allen MD, Donohoe BS, Rorrer NA, Kearns FL, Silveira RL, Pollard BC, Dominick G, Duman R, Omari KEl, *et al*.: **Characterization and engineering of a plastic-degrading aromatic polyesterase**. *Proc Natl Acad Sci USA* 2018, **115**:E4350-E4357.

13. Tournier V, Topham CM, Gilles A, David B, Folgoas C, Moya-Leclair E, Kamionka E, Desrousseaux ML, Texier H, Gavalda S, *et al*.: **An engineered PET depolymerase to break down and recycle plastic bottles**. *Nature* 2020, **580**:216-219.

14. •• Lu H, Diaz DJ, Czarnecki NJ, Zhu C, Kim W, Shroff R, Acosta DJ, Alexander BR, Cole HO, Zhang Y, *et al*.: **Machine learning-aided engineering of hydrolases for PET depolymerization**. *Nature* 2022, **604**:662-667.

An engineered variant of IsPETase with high activity at mild reaction temperatures. The work represents a major advancement in protein engineering using machine learning for PET hydrolase engineering.

15. • Bell EL, Smithson R, Kilbride S, Foster J, Hardy FJ, Ramachandran S, Tedstone AA, Haigh SJ, Garforth AA, Day PJR, *et al*.: **Directed evolution of an efficient and thermostable PET depolymerase**. *Nat Catal* 2022, **5**:673-681.

A thermostable variant of *Is*PETase that shows activity on difficult to degrade PET waste like semicrystalline PET and PET/PE composite films. Of note is the high MHET to TPA ratio liberated by this PETase variant under the tested conditions.

16. Zhang Y, Pedersen JN, Eser BE, Guo Z: **Biodegradation of polyethylene and polystyrene: from microbial deterioration to enzyme discovery**. *Biotechnol Adv* 2022, **60**:107991.

17. Yang Y, Yang J, Wu WM, Zhao J, Song Y, Gao L, Yang R, Jiang L: **Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 1. Chemical and physical characterization and isotopic tests**. *Environ Sci Technol* 2015, **49**:12080-12086.

18. Brandon AM, Gao SH, Tian R, Ning D, Yang SS, Zhou J, Wu WM, Criddle CS: **Biodegradation of polyethylene and plastic mixtures in mealworms (Larvae of Tenebrio molitor) and effects on the gut microbiome**. *Environ Sci Technol* 2018, **52**:6526-6533.

19. • Sanluis-Verdes A, Colomer-Vidal P, Rodriguez-Ventura F, Bello-Villarino M, Spinola-Amilibia M, Ruiz-Lopez E, Illanes-Vicioso R, Castroviejo P, Aiese Cigliano R, Montoya M, *et al*.: **Wax worm saliva and the enzymes therein are the key to polyethylene degradation by Galleria mellonella**. *Nat Commun* 2022, **13**:5568.

Discovery of a pair of enzymes in wax worm saliva responsible for oxidative deconstruction of PE into methyl ketones as the primary product.

20. Negoro S, Shibata N, Tanaka Y, Yasuhira K, Shibata H, Hashimoto H, Lee YH, Oshima S, Santa R, Mochiji K, *et al*.: **Three-dimensional structure of nylon hydrolase and mechanism of nylon-6 hydrolysis**. *J Biol Chem* 2012, **287**:5079-5090.

21. Cahill JF, Kertesz V, Saint-Vincent P, Valentino H, Drufva E, Thiele N, Michener JK: **High-throughput characterization and optimization of polyamide hydrolase activity using open port sampling interface mass spectrometry**. *J Am Soc Mass Spectrom* 2023, **34**:1383-1391, https://doi.org/10.1021/jasms.3c00097

22. Branson Y, Söltl S, Buchmann C, Wei R, Schaffert L, Badenhorst CPS, Reisky L, Jäger G, Bornscheuer UT: **Urethanases for the enzymatic hydrolysis of low molecular weight carbamates and the recycling of polyurethanes**. *Angew Chem Int Ed* 2023, **62**:e202216220.

23. Boerjan W, Ralph J, Baucher M: **Lignin biosynthesis**. *Annu Rev Plant Biol* 2003, **54**:519-546.

24. Binder JB, Raines RT: **Simple chemical transformation of lignocellulosic biomass into furans for fuels and chemicals**. *J Am Chem Soc* 2009, **131**:1979-1985.

25. Schutyser W, Renders T, Van Den Bosch S, Koelewijn SF, Beckham GT, Sels BF: **Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading**. *Chem Soc Rev* 2018, **47**:852-908.

26. • O'Dea RM, Pranda PA, Luo Y, Amitrano A, Ebikade EO, Gottlieb ER, Ajao O, Benali M, Vlachos DG, Ierapetritou M, *et al*.: **Ambient-pressure lignin valorization to high-performance polymers by intensified reductive catalytic deconstruction**. *Sci Adv* 2022, **8**:7523.

Chemical deconstruction of lignin into various phenolic compounds enables efforts to upcycle phenolic monomers polymers. Authors demonstrate synthesis of acrylated vanillyl alcohol monomers and subsequent polymerization.

27. Dickey RM, Forti AM, Kunjapur AM: **Advances in engineering microbial biosynthesis of aromatic compounds and related compounds**. *Bioresour Bioprocess* 2021, **8**:91.

28. Hanefeld U, Hollmann F, Paul CE: **Biocatalysis making waves in organic chemistry**. *Chem Soc Rev* 2022, **51**:594-627.

29. Bayer T, Pfaff L, Branson Y, Becker A, Wu S, Bornscheuer UT, Wei R: **Biosensor and chemo-enzymatic one-pot cascade applications to detect and transform PET-derived terephthalic acid in living cells**. *iScience* 2022, **25**:104326.

30. Cutlan R, De Rose S, Isupov MN, Littlechild JA, Harmer NJ: **Using enzyme cascades in biocatalysis: highlight on transaminases and carboxylic acid reductases**. *Biochim Biophys Acta Proteins Prote* 2020, **1868**:140322.

31. • Fedorchuk TP, Khusnutdinova AN, Evdokimova E, Flick R, Di Leo R, Stogios P, Savchenko A, Yakunin AF: **One-pot biocatalytic transformation of adipic acid to 6-aminocaproic acid and 1,6-hexamethylenediamine using carboxylic acid reductases and transaminases**. *J Am Chem Soc* 2020, **142**:1038-1048.

First-of-its-kind study using wild-type and engineered CARs and TAs for functionalization of adipic acid to hexamethylenediamine, an aliphatic diamine used in nylons and other PAs.

32. Khusnutdinova AN, Flick R, Popovic A, Brown G, Tchigvintsev A, Nocek B, Correia K, Joo JC, Mahadevan R, Yakunin AF: **Exploring bacterial carboxylate reductases for the reduction of bifunctional carboxylic acids**. *Biotechnol J* 2017, **12**:1-12.

33. •• Gopal MR, Dickey RM, Butler ND, Talley MR, Nakamura DT, Mohapatra A, Watson MP, Chen W, Kunjapur AM: **Reductive enzyme cascades for valorization of polyethylene terephthalate deconstruction products**. *ACS Catal* 2023, **13**:4778-4789.

First example of one-pot multienzyme synthesis of pXYL from TPA. This functional group interconversion yielded 69% diamine from 10 mM of starting material and featured minimal accumulation of byproducts. The same system is modular and accepts MHET as a substrate for synthesis of a value-added amine-containing carboxylic acid, pAMBA.

34. Hirata S., Mitsutsuji Y., Bang S., Tsuchiya A.: Fiber reinforced thermoplastic resin molding material, and fiber reinforced thermoplastic resin molded article**.** 2019.

35. • Dickey RM, Jones MA, Butler ND, Govil I, Kunjapur AM: **Genome engineering allows selective conversions of terephthalaldehyde to multiple valorized products in bacterial cells**. *AIChE J* 2023, **69**:e18230, https://doi.org/10.1002/aic.18230.
A genome engineered *E. coli* strain stabilizes aldehydes and produces valorized aldehydes, amines or diamines depending on the subset of gene knockouts.

36. Kunjapur AM, Tarasova Y, Prather KLJ: **Synthesis and accumulation of aromatic aldehydes in an engineered strain of Escherichia coli**. *J Am Chem Soc* 2014, **136**:11644-11654.

37. Dissanayake L, Jayakody LN: **Engineering microbes to bio-upcycle polyethylene terephthalate**. *Front Bioeng Biotechnol* 2021, **9**:656465.

38. Kenny ST, Runic JN, Kaminsky W, Woods T, Babu RP, Keely CM, Blau W, O'Connor KE: **Up-cycling of PET (Polyethylene Terephthalate) to the biodegradable plastic PHA (Polyhydroxyalkanoate)**. *Environ Sci Technol* 2008, **42**:7696-7701.

39. Werner AZ, Clare R, Mand TD, Pardo I, Ramirez KJ, Haugen SJ, Bratti F, Dexter GN, Elmore JR, Huenemann JD, *et al.*: **Tandem chemical deconstruction and biological upcycling of poly (ethylene terephthalate) to β-ketoadipic acid by Pseudomonas putida KT2440**. *Metab Eng* 2021, **67**:250-261.

40. Tiso T, Narancic T, Wei R, Pollet E, Beagan N, Schröder K, Honak A, Jiang M, Kenny ST, Wierckx N, *et al.*: **Towards bio-upcycling of polyethylene terephthalate**. *Metab Eng* 2021, **66**:167-178.

41. Bao T, Qian Y, Xin Y, Collins JJ, Lu T: **Engineering microbial division of labor for plastic upcycling**. *Nat Commun* 2023, **14**:5712.

42. • Sullivan KP, Werner AZ, Ramirez KJ, Ellis LD, Bussard JR, Black BA, Brandner DG, Bratti F, Buss BL, Dong X, *et al.*: **Mixed plastics waste valorization through tandem chemical oxidation and biological funneling**. *Science (1979)* 2022, **378**:207-211.
Biological approaches to tackling mixed plastic streams are vital. In this work, two strains of *P. putida* harness engineered metabolic pathways to either produce PHAs or β-ketoadipate from a heterogenous mix of carboxylic acids from PE, PS and PET deconstruction.

43. Rorrer NA, Nicholson S, Carpenter A, Biddy MJ, Grundl NJ, Beckham GT: **Combining reclaimed PET with bio-based monomers enables plastics upcycling**. *Joule* 2019, **3**:1006-1027.

44. Raza ZA, Riaz S, Banat IM: **Polyhydroxyalkanoates: properties and chemical modification approaches for their functionalization**. *Biotechnol Prog* 2018, **34**:29-41.

45. Citoler J, Derrington SR, Galman JL, Bevinakatti H, Turner NJ: **A biocatalytic cascade for the conversion of fatty acids to fatty amines**. *Green Chem* 2019, **21**:4932-4935.

46. Susa A, Bijleveld J, Hernandez Santana M, Garcia SJ: **Understanding the effect of the dianhydride structure on the properties of semiaromatic polyimides containing a biobased fatty diamine**. *ACS Sustain Chem Eng* 2018, **6**:668-678.

47. Hu L, He A, Liu X, Xia J, Xu J, Zhou S, Xu J: **Biocatalytic transformation of 5-hydroxymethylfurfural into high-value derivatives: recent advances and future aspects**. *ACS Sustain Chem Eng* 2018, **6**:15915-15935.

48. Dijkman WP, Groothuis DE, Fraaije MW: **Enzyme-catalyzed oxidation of 5-hydroxymethylfurfural to furan-2,5-dicarboxylic acid**. *Angew Chem Int Ed* 2014, **53**:6515-6518.

49. Dijkman WP, Fraaije MW: **Discovery and characterization of a 5-hydroxymethylfurfural oxidase from Methylovorus sp. strain MP688**. *Appl Environ Microbiol* 2014, **80**:1082-1090.

50. Dörwald: **FZaragoza: monofunctionalization of symmetric difunctional substrates**. Side Reactions in Organic Synthesis: A Guide to Successful Synthesis Design. Wiley-VCH; 2005:333-353.

51. Qin YZ, Li YM, Zong MH, Wu H, Li N: **Enzyme-catalyzed selective oxidation of 5-hydroxymethylfurfural (HMF) and separation of HMF and 2,5-diformylfuran using deep eutectic solvents**. *Green Chem* 2015, **17**:3718-3722.

52. McKenna SM, Leimkühler S, Herter S, Turner NJ, Carnell AJ: **Enzyme cascade reactions: Synthesis of furandicarboxylic acid (FDCA) and carboxylic acids using oxidases in tandem**. *Green Chem* 2015, **17**:3271-3275.

53. Millán Acosta A, Cuesta Turull C, Cosovanu D, Sala Martí N, Canela-Garayoa R: **Novel and efficient biotechnological approach to produce 2,5-diformylfuran from biomass-derived 5-hydroxymethylfurfural**. *ACS Sustain Chem Eng* 2021, **9**:14550-14558.

54. Froidevaux V, Negrell C, Caillol S, Pascault JP, Boutevin B: **Biobased amines: from synthesis to polymers; present and future**. *Chem Rev* 2016, **116**:14181-14224.

55. • Dunbabin A, Subrizi F, Ward JM, Sheppard TD, Hailes HC: **Furfurylamines from biomass: transaminase catalysed upgrading of furfurals**. *Green Chem* 2017, **19**:397-404.
An in-depth study on TAs-mediated interconversion of biomass derived aldehydes into amines in both batch and flow reactors.

56. Heinks T, Merz LM, Liedtke J, Höhne M, van Langen LM, Bornscheuer UT, Fischer von Mollard G, Berglund P: **Biosynthesis of furfurylamines in batch and continuous flow by immobilized amine transaminases**. *Catalysts* 2023, **13**:875.

57. Mahajan JS, O'Dea RM, Norris JB, Korley LSTJ, Epps TH: **Aromatics from lignocellulosic biomass: a platform for high-performance thermosets**. *ACS Sustain Chem Eng* 2020, **8**:15072-15096.

58. Fache M, Montérémal C, Boutevin B, Caillol S: **Amine hardeners and epoxy cross-linker from aromatic renewable resources**. *Eur Polym J* 2015, **73**:344-362.

59. Manfrão-Netto JHC, Lund F, Muratovska N, Larsson EM, Parachin NS, Carlquist M: **Metabolic engineering of Pseudomonas putida for production of vanillylamine from lignin-derived substrates**. *Micro Biotechnol* 2021, **14**:2448-2462.

60. Du CJ, Rios-Solis L, Ward JM, Dalby PA, Lye GJ: **Evaluation of CV2025 ω-transaminase for the bioconversion of lignin breakdown products into value-added chemicals: synthesis of vanillylamine from vanillin**. *Biocatal Biotransformation* 2014, **32**:302-313.

61. Fu B, Xiao G, Zhu Y, Chen Y, Lai Y, Yuan J: **Renewable vanillylamine synthesis from lignin-derived feedstocks**. *ACS Agric Sci Technol* 2021, **1**:566-571.

62. •• Nain P, Dickey RM, Somasundaram V, Sulzbach M, Kunjapur AM: **Reductive amination cascades in cell-free and resting whole cell formats for valorization of lignin deconstruction products**. *Biotechnol Bioeng* 2023, **121**:593-604, https://doi.org/10.1002/bit.28604.
A recent study demonstrating valorization of lignin-derived aryl carboxylic acids to aryl amines. The study features comparative analysis of biocatalysts in cell-free and resting whole-cell formats.

63. Butler ND, Anderson SR, Dickey RM, Nain P, Kunjapur AM: **Combinatorial gene inactivation of aldehyde dehydrogenases mitigates aldehyde oxidation catalyzed by E. coli resting cells**. *Metab Eng* 2023, **77**:294-305.