Green chemistry and biocatalysis

Engineering a sustainable future

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Green chemistry and biocatalysis: Engineering a sustainable future

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ABSTRACT

The increasing role of biocatalysis in the green and sustainable manufacture of chemicals is discussed. In the last two decades the breadth and scope of biocatalysis has increased enormously as a result of remarkable advances in metagenomics, protein engineering and bioinformatics. Moreover, the use of enzymes has become more cost-effective through advances in immobilization technologies and the application of the immobilized enzymes in continuous flow operation in packed bed reactors. Consequently, biocatalysis is already the method of choice for the synthesis of enantiopure chiral products for the pharmaceutical and fine chemical industries. Further applications in commodity chemicals manufacture are currently being stimulated by the increasing maturity of biocatalysis and the ongoing transition to a bio-based circular economy based on the valorization of organic waste on the road to net zero manufacturing.

1. Introduction

The grand challenge facing humanity in the twenty first century is to facilitate the ongoing transition to a decarbonized, renewable source of energy and greener processes for the sustainable manufacture of chemicals and materials. This will be achieved through efficient use of non-fossil resources, elimination of waste and evading the use of toxic and hazardous substances. Waste organic material, in its many manifestations, is the root cause of numerous ecological problems [1]. Solutions to this global waste problem require a paradigm shift in how we assign economic value to replacing fossil resources with renewable energy and raw materials.

2. Waste elimination and the quest for net zero

The major sources of waste are depicted in Fig. 1. The introduction of the E-Factor in 1992 [2] drew attention to the copious amounts of waste produced in the production of pharmaceuticals and fine

chemicals. In the meantime, it has become crystal clear that waste carbon dioxide resulting from the use of fossil resources - oil, coal

and natural gas - in heating, transport fuels and chemicals production, e.g. in the manufacture of steel and cement and commodity chemicals, is a major cause of climate change. In principle, the E-Factor always included energy usage, which can be expressed as kgs of CO_2 waste formed per kg of product, but at that time the data for energy consumption in chemicals production were not widely available. Subsequently, Christensen and coworkers [3] proposed a Climate Factor (C-Factor), defined as the amount of CO_2 emitted divided by the amount of product formed, in order to quantify the carbon footprints of processes.

Another ubiquitous source of undesirable waste is derived from discarded materials, particularly single use plastics, that are polluting our countryside, rivers and oceans [4–7]. Yet another major source of organic waste, most of which ends up as land-fill, is food supply chain waste (FSCW) [8,9] arising from the production, distribution and consumption of food. However, the primary waste problem is currently the staggering amounts of CO_2 and other greenhouse gases (GHGs) that enter the atmosphere as a result of the burning of fossil resources.

The primary solution to pollution is to avoid the formation of waste in the first place. However, not all waste is avoidable

; agricultural and forestry residues [10], for example, are unavoidable but they can be utilized as a raw material for producing liquid fuels, sustainable bio-energy and chemicals in biorefineries [11,12] on an overall carbon neutral basis. In the longer term algal biomass, i.e. third generation (3 G) biomass could become a primary raw material for the production of chemicals and fuels. It has several advantages compared with 2nd generation (2 G), i.e. lignocellulosic biomass: no competition with land crops, a short growth cycle, and a reduction in carbon dioxide emissions [13].

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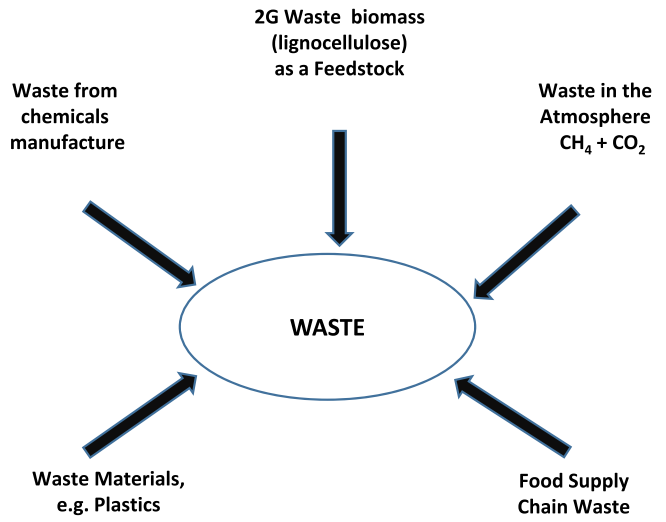

Fig. 1. Major sources of waste.

3. The metamorphosis of chemicals manufacture

The industrial revolution, starting in the mid-18th century, was driven by an unbridled consumption of fossil resources, in particular the combustion of coal to drive the steam engine. It served humanity well for more than two centuries, enabling the growth of the global population to unimaginable proportions based on ever-increasing agricultural and industrial productivities. However, we have clearly reached the limits to a growth based on the burning of fossil resources. A metamorphosis in chemicals production is needed and will be facilitated by the ongoing energy transition from a fossil resource-based to a sustainable resource–based energy sector for heating, transportation and building.

As we have noted elsewhere [14], the burning of fossil resources, as an energy source for chemicals production will be superseded by electrons and photons that support electrocatalysis and photocatalysis, respectively. In a Power-to-X industry liquid fuels and chemicals will be produced in electrolytic processes conducted in e-(bio)refineries using off-peak electricity generated with sustainable energy sources (solar, wind, hydroelectric, geothermal and nuclear). This chemistry will be facilitated by both chemo- and biocatalysis.

3.1. The role of catalysis and atom efficiency

The formation of copious amounts of waste in the manufacture of fine chemicals was largely a consequence of the widespread use of antiquated technologies employing stoichiometric amounts of inorganic and organic reagents [1]. Hence, the way forward was to reduce these enormous amounts of waste by replacing these antiquated technologies with cleaner catalytic alternatives and this has been widely implemented in the fine chemical and pharmaceutical industries in the last thirty years. Prime examples include the replacement of oxidations and reductions using stoichiometric inorganic oxidants and metal hydrides with more atom efficient catalytic oxidations and hydrogenations, respectively [15].

The displacement of “stoichiometric” technologies with catalytic alternatives was mainly restricted to the production of fine chemicals and pharma as the production of commodity chemicals already involved the use of relatively atom efficient catalytic processes, with some notable exceptions such as propylene oxide [16] and caprolactam [17].

However, although fine chemicals manufacture generates less waste per kg of product, the total amounts of commodity chemicals are generally 1–2 orders of magnitude higher and, hence, the total amounts of waste generated are higher. Moreover, although the original E-factor concept included waste (expressed as kgs CO_2) derived from energy usage, the data was not widely available at the time and, hence, was not included. In the meantime, the amount of waste CO_2 generated has become of crucial importance in connection with climate change mitigation. It is of particular importance in commodity chemicals manufacture that typically involves more energy-intensive processes. Hence, the carbon footprint of chemicals manufacture [18] has become a key issue in the current drive to net zero chemicals manufacture [19].

4. Biocatalysis is green and sustainable

Biocatalysis can now be considered as a key enabling technology for the implemention of sustainable chemicals manufacture [20], not only in fine chemicals and pharmaceuticals manufacture but also in the production of commodity chemicals and bio-based plastics. Biocatalysis is Green and Sustainable [21]. It is in accordance with 10 of the twelve principles of Green Chemistry, with the remaining two being concerned with product rather than process design.

Enzymes are biodegradable, biocompatible and are derived from renewable resources and, hence, fit seamlessly into a bio-based economy. They circumvent the use of precious metals and their costly removal, to very low levels, from products. Enzymatic reactions are conducted under mild conditions – largely in water at ambient temperature and pressure – and are, hence, less energy intensive and have no need for specialised equipment. They are highly chemo- and stereoselective and, hence, produce higher quality products. In short, they produce less waste and are environmentally friendly with reduced costs. Moreover, spectacular advances in molecular biology and biotechnology, over the last two decades, have enabled the development of eminently sustainable biocatalytic processes that combine low cost with a low environmental footprint.

In the preceding two decades they have been widely applied in the resource-efficient industrial synthesis of active pharmaceutical ingredients (APIs), flavour and fragrance compounds, vitamins, food additives and other high value-added products. The rapidly advancing science of (meta)genomics, supported by recent developments in bioinformatics, has enabled the rapid discovery of numerous enzymes that were previously unknown.

4.1. Enabling biocatalytic processes with protein engineering

Spectacular developments in protein engineering have facilitated the optimization of the key properties of enzymes, such as activity, catalytic productivity, thermal stability and substrate and stereo-specificity. It is now eminently feasible to develop enzymatic transformations that fit predefined parameters to afford processes that are truly sustainable by design.The classic example of optimizing the biocatalyst to fit the dream process is the Codexis three enzyme process for the synthesis of a key intermediate for the cholesterol-lowering agent, atorvastatin, (Fig. 2 [22].

DNA shuffling [23] was used to optimize the key parameters of all three enzymatic steps to a predetermined level. The parameters of the novel dehalogenase catalyzed step are shown in Table 1. This process is widely regarded as the gold standard in directed evolution.

Similarly, directed evolution formed the basis of a biocatalytic process (Fig. 3) for the synthesis of a key intermediate for sitagliptin, the active ingredient of the antidiabetic drug Januvia [24]. In a joint effort by Codexis and Merck scientists a combination of protein engineering techniques was used to develop an amine transaminase (ATA) for the conversion of the ketone precursor, prositagliptin, to the chiral primary amine. The starting point was an R-selective ATA that contained the necessary machinery but exhibited zero activity towards prositagliptin.

A combination of computer-aided design of the active site and saturation mutagenesis subsequently afforded a variant that gave 0.7% conversion in 24 h using an enzyme loading of 10 $g.L^{-1}$ at a substrate loading of 2 $g.L^{-1}$ in 50% aqueous DMSO. For commercial viability the enzyme was evolved, in multiple rounds of DNA shuffling. It afforded a

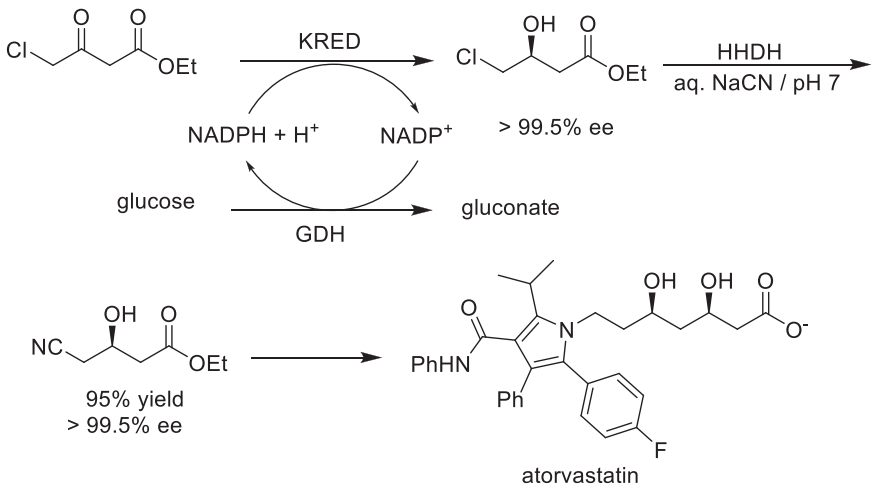

Fig. 2. Codexis process for an atorvastatin intermediate.

Table 1
HHDH process design vs wild-type enzyme and best variant.

Parameter	Process design	Wild-type	Best variant
[Substrate] (g.L-1)	120	20	140
[Enzyme] (g.L-1)	1.5	30	1.2
Catalyst productivity (g/g)	80	0.7	117
STY (g.L-1 day-1)	>360	7	672
Isolated yield (%)	>90	67	92
Chemical purity (%)	>98	>98	>98
ee (%)	>99.5	>99.5	>99.5
Reaction time (h)	8	72	5
Phase separation (min.)	<10	>60	<1

variant that at 6 $g.L^{-1}$ with 100 $g.L^{-1}$ substrate, 1 M isopropylamine in 50% DMSO in water and a temperature of 45^0 C for 24 h afforded sitagliptin in 92% yield and >99.95% ee.

Compared with the rhodium-catalyzed asymmetric hydrogenation of an enamine that it superseded, the biocatalytic process provided a 19% decrease in E-Factor, a 13% increase in overall yield, a 53% increase in space-time yield $g.L^{-1}h^{-1}$ and topped with a superb enantioselectivity of >99.95% compared with 97% in the Rh-catalyzed process.

5. The scope of biocatalysis in organic synthesis

As a direct result of the spectacular advances in (meta) genomics, protein engineering and bioinformatics, industrially viable biocatalytic methods are now available for a broad variety of synthetic organic transformations [25]. Consequently, they have been widely applied in the industrial synthesis of Active Pharmaceutical Intermediates (APIs). Indeed, based on its exquisite, often near-perfect, enantioselectivities, biocatalysis has become the method of choice in the synthesis of enantiopure pharmaceuticals.

5.1. Biocatalytic synthesis of chiral alcohols and amines

Two of the most commonly used intermediates in the synthesis of enantiopure APIs are chiral secondary alcohols and chiral secondary and primary amines. Following the spectacular results obtained in the

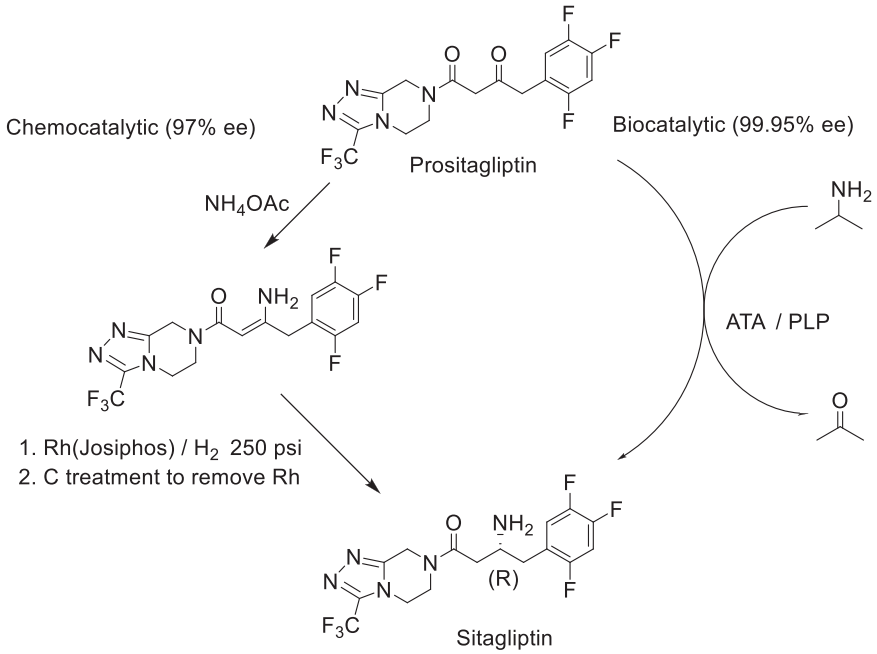

Fig. 3. Enantioselective synthesis of sitagliptin.

synthesis of the chiral alcohol intermediate for atorvastatin, enzymatic reduction catalyzed by ketoreductases (KREDs) became the method of choice for the industrial synthesis of a wide variety of enantiopure secondary alcohols [26–28]. Efficient regeneration of co-factors is achieved by adding a second alcohol, e.g. isopropanol, or a second enzyme (Fig. 4).

Notwithstanding the spectacular results obtained with highly engineered KREDs, the real success story of biocatalysis in enantioselective synthesis is in the production of enantiopure amines. For example, amine transaminases (ATAs) are a sub-class of amine transferases that use simple primary amine donors, such as isopropylamine, for the enantioselective production of chiral secondary amines by reaction with prochiral ketone acceptors, as in the above described synthesis of sitagliptin (Fig. 3). The method has been widely used in the synthesis of APIs in the last decade [29–32] including the use of a highly engineered ATA in the synthesis of the blockbuster cardiovascular drug, Sacubtril (Fig. 5) [33].

Noble metal catalyzed reductive amination of ketones, with a mixture of hydrogen and ammonia, is a widely used method for the synthesis of secondary amines in fine and bulk chemicals. Asymmetric versions, employing chiral ligands, have been widely used to produce chiral amines. An enzymatic equivalent, employing NAD(P)H and ammonia (Fig. 6), was not known until Bommarius and co-workers, in 2012, generated an amine dehydrogenase (AmDH) by subjecting a leucine dehydrogenase to several rounds of protein engineering [34]. Now, a decade later, AmDHs are widely used in the synthesis of enantiopure amines [35].

Imine reductases (IREDs) are another recent addition to the repertoire of enzymatic methods for enantioselective amine synthesis. IREDs catalyze both the reduction of imines (Fig. 6) and reductive aminations of ketones with secondary amines. Interestingly, an IRED was used in a novel synthesis of S-nicotine [36].

Alternatively, an overall redox neutral process can be obtained by combining an amine dehydrogenase with an alcohol dehydrogenase (ADH), i.e. a KRED in reverse (Fig. 7) in an overall process, that is also a widely known chemocatalytic approach [37] referred to as hydrogen borrowing [38,39].

5.2. *Expanding the Scope of Biocatalysis: New to Nature reactions*

Arnold and coworkers [40,41] used an elegant combination of mechanism-guided chemomimetic design and directed evolution to expand the scope of biocatalysis in organic synthesis. For example, they used highly engineered variants of Cyt-P450 monooxygenases from *P. megaterium* to catalyze cyclopropanations and aziridinations with ethyl diazoacetate and tosyl azide via carbene and nitrene transfer, respectively (Fig. 8).

However, these transformations generally involved the use of reagents that are costly and result in poor atom economy and stoichiometric amounts of organic waste. The nitrene insertion reactions, for example, involve the use of stoichiometric amounts of tosyl azide, which would certainly be an issue on an industrial scale. It is interesting, therefore, that Arnold and coworkers [42] reported that an engineered heme enzyme from *Pyrobaculum arsenaticum* protoglobin was able to catalyze nitrene insertion reactions using hydroxylamine hydrochloride, an inexpensive commodity chemical, as the stoichiometric reagent. (Fig. 8).

6. From bench scale to industrial process

Enzymes are water soluble and are generally used on a single use, throw-away basis which is neither cost-effective nor conducive to a circular economy. Moreover, the chemical industry prefers the use of heterogeneous catalysts that are easily separated from reaction mixtures, by simple filtration or centrifugation, and are more readily adapted to continuous processing, e.g. in packed bed reactors. Thus, from a processing viewpoint, it is more advantageous to use enzymes in the form of water-insoluble powders i.e. as immobilized enzymes. In order to compete with heterogeneous chemocatalysts in the commodity chemicals arena, the performance of biocatalytic methods as reflected in volumetric ($kg.L^{-1}$) and catalyst productivities (kg/kg) will need to be increased by 1–2 orders of magnitude at concentrations of 200–400 g.

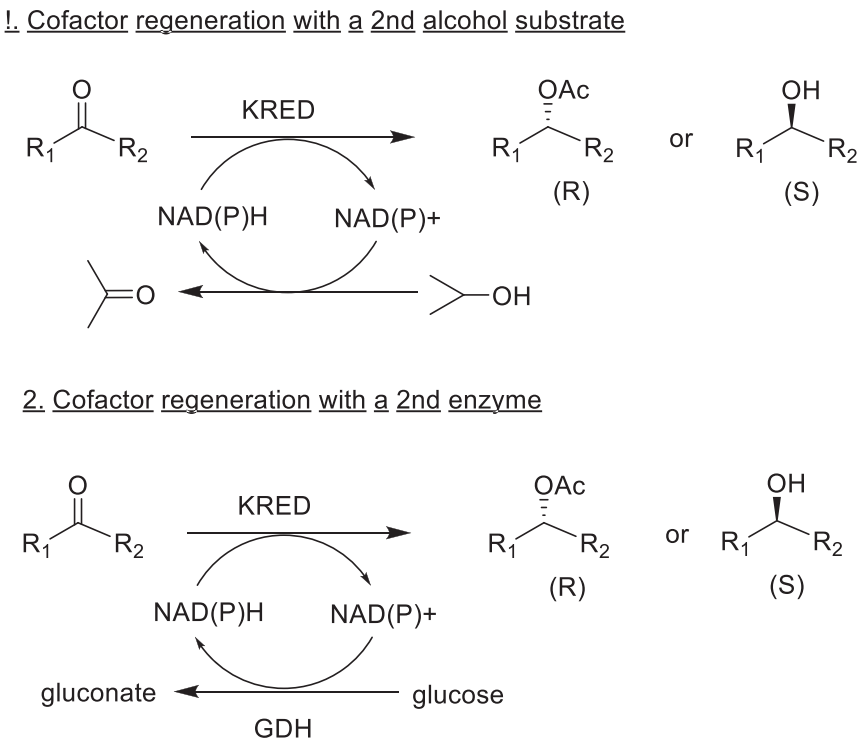

Fig. 4. Enzymatic synthesis of chiral sec-alcohols.

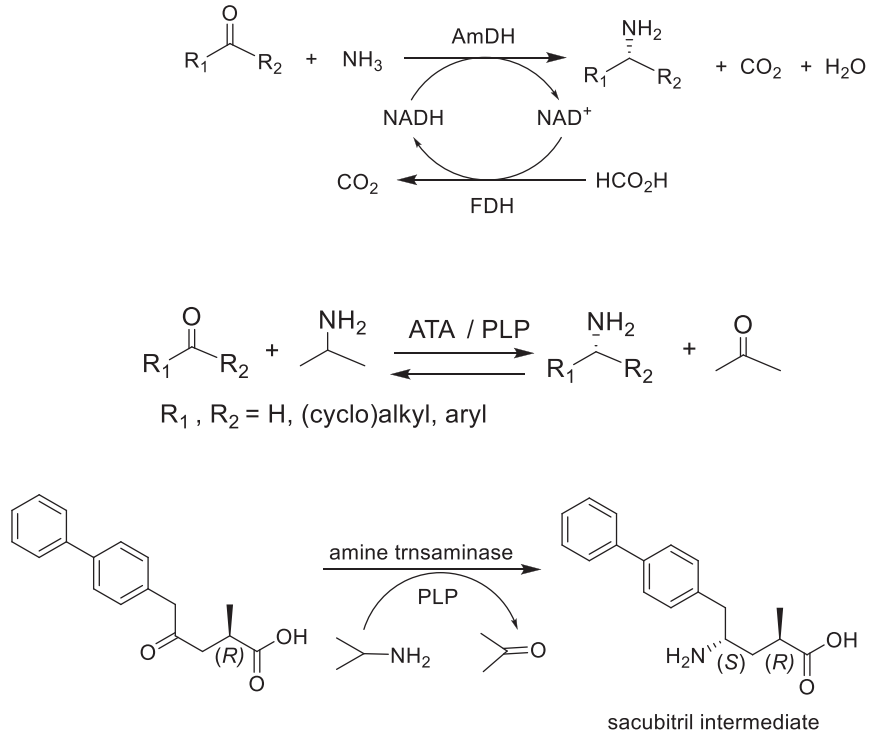

Fig. 5. Enzymatic synthesis of chiral amines with amine transaminases.

L^{-1} [43,44]. Enzymes will need to compete with the performance observed with heterogeneous catalysts in petrochemical production, i.e. space time yields (STYs) of 1−10 kg·L^{-1}·h^{-1} rather than the 0.001−0.1 kg·$L^{-1}h^{-1}$ characteristic of the production of APIs [45].

Enzymes are soluble in water and as we have noted elsewhere[46]: the best solvent is no solvent and if a solvent (diluent) is needed then water is preferred. It is nontoxic, nonflammable, abundantly available and inexpensive. After completion the product is often extracted into an organic phase, e.g. ethyl acetate. Alternatively, this can be back-integrated by performing the reaction as aqueous biphasic catalysis whereby the catalyst resides in the water phase and the product is dissolved in the organic phase for recovery by simple phase separation. This mode of operation was used in the Codexis process for the atorvastatin intermediate (see Section 4.1). On the other hand, it has been pointed out [47] that the advantages are largely nullified by the relatively low concentrations of reactants, e.g. 20 g.L^{-1}. However, the problem is low activity of the enzyme not the substrate solubility in water and the activity can be substantially increased using protein engineering. The Codexis process is a perfect illustration of this. The substrate concentration obtained with the wild-type dehalogenase (see Table 1) was 20 g.L^{-1} The target for protein engineering in process design was 120 g.$L^{-1}$and, in practice, ⁻the best variant managed 140 g.L^{-1}.

As pointed out by Dominguez di Maria and co-workers [48], the use of aqueous biphasic systems requires that the carbon footprint of the (fossil resource-derived) organic solvent be taken into account. They also note that the key to reaching a diminished environmental footprint is the type of wastewater treatment that needs to be implemented.

Another possibility is to add non-ionic surfactants that can accelerate enzymatic reactions in biphasic aqueous organic media [49]. However, this also means that the recovery, recycling and eventual treatment of both the enzyme and the surfactant have to be taken into account [49,50].

6.1. *Enzyme immobilization: an enabling technology*

Enzymes are readily immobilized on solid carriers (supports) through simple adsorption or ionic or covalent bonding [51]. Simple adsorption on, for example, hydrophobic resins such as polymethyl methacrylates, is widely used at commercial scale for reactions in water-free media. A striking example of immobilization by simple adsorption is the amine transaminase (ATA) used in the synthesis of sitagliptin (see Section 4). Protein engineering afforded spectacular increases in activity and (enantio)selectivity but the resulting process needed to be performed in 50% aqueous DMSO on a single use basis, i.e. distinctly disadvantageous from a sustainability viewpoint.

In stark contrast, immobilization of the ATA, at 4% loading on a commercially available hydrophobic octadecyl polymethacrylate resin, afforded a solid catalyst that produced sitagliptin in 91% yield and 99% ee at 200 gL^{-1} substrate concentration in isopropyl acetate saturated with water at 50 °C. No loss of activity was observed, over 10 consecutive recycles in a 200 h period, thus enabling a >90% reduction in the amount of enzyme used, despite the 45% activity loss compared with the soluble enzyme. The free lyophilised enzyme, on the other hand, was immediately denatured in the organic solvent and exhibited zero activity. Interestingly, the ATA was recently co-immobilized with the PLP cofactor, by covalent attachment to an epoxy resin carrier, and used for the synthesis of sitagliptin in continuous operation in packed bed reactors [52].

A serious disadvantage of immobilized enzymes involving simple adsorption on or ionic binding to carriers is that the enzyme is susceptible to leaching in aqueous media thus limiting its application to water free systems. This is avoided by employing covalent bonding to carriers through reaction of free amino groups in the enzyme with reactive functional groups, e.g. aldehyde or epoxide, on the surface of the carrier. In addition to simple adsorption, ionic and covalent bonding, there is a fourth method: affinity immobilization. The latter involves the coordination of the histidine groups in His-tagged enzymes to transition metal ions, e.g. Ni(II), Fe(II), Cu(II), Co(II), contained in chelate complexes on the surface of a carrier such as controlled pore glass.

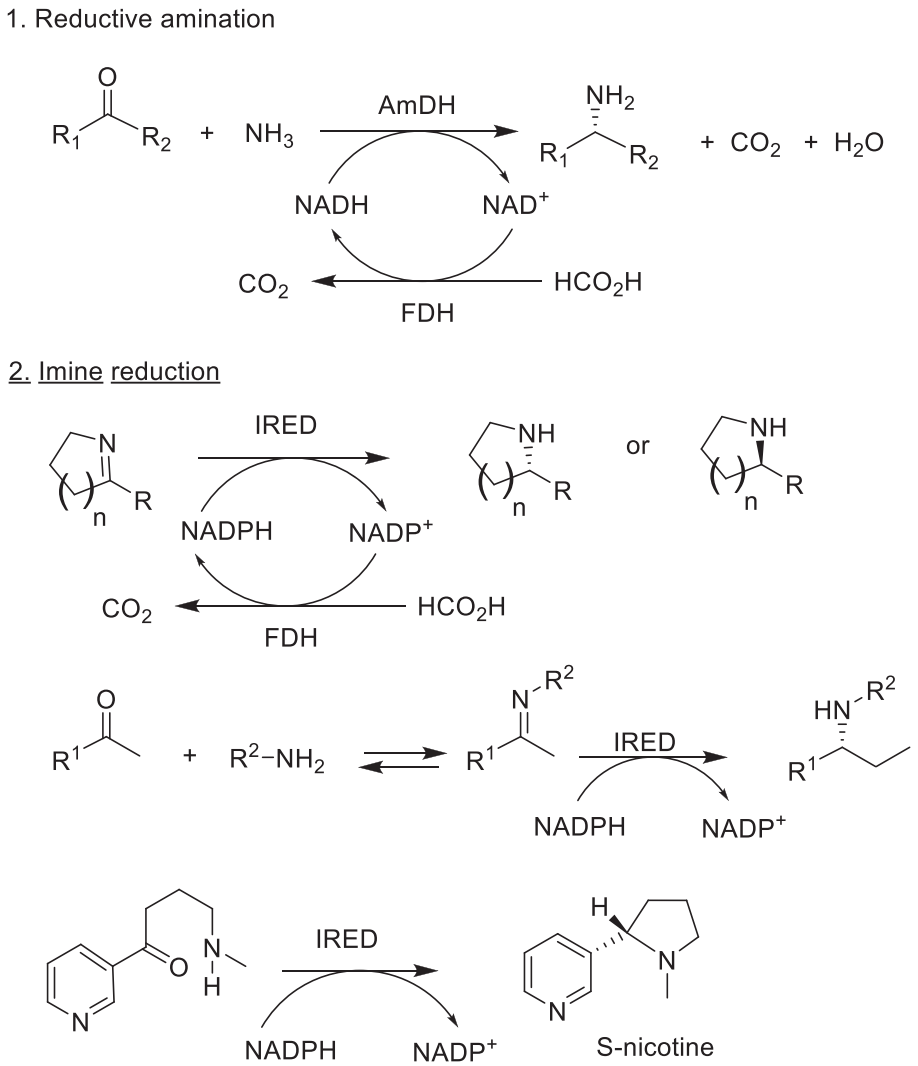

Fig. 6. Amine dehydrogenases and imine reductases.

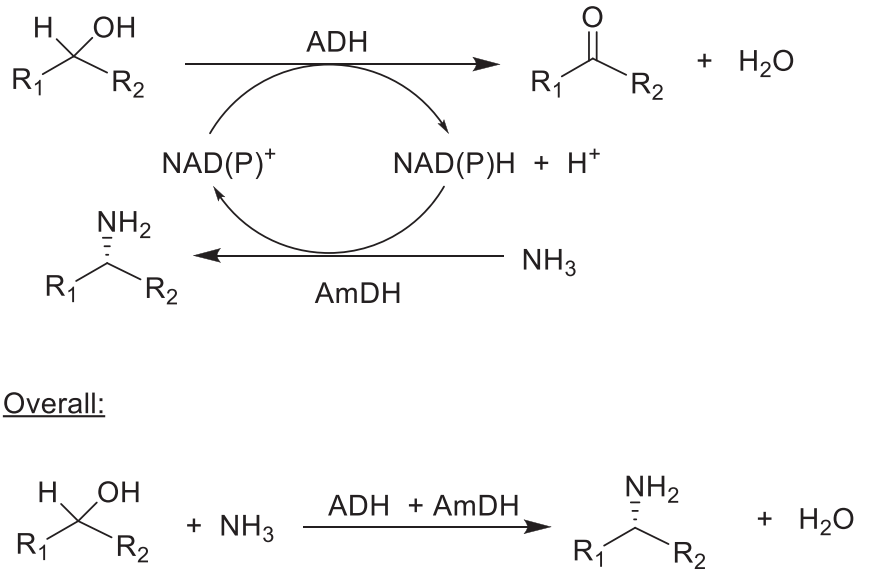

Fig. 7. Biocatalytic conversion of an alcohol to an amine.

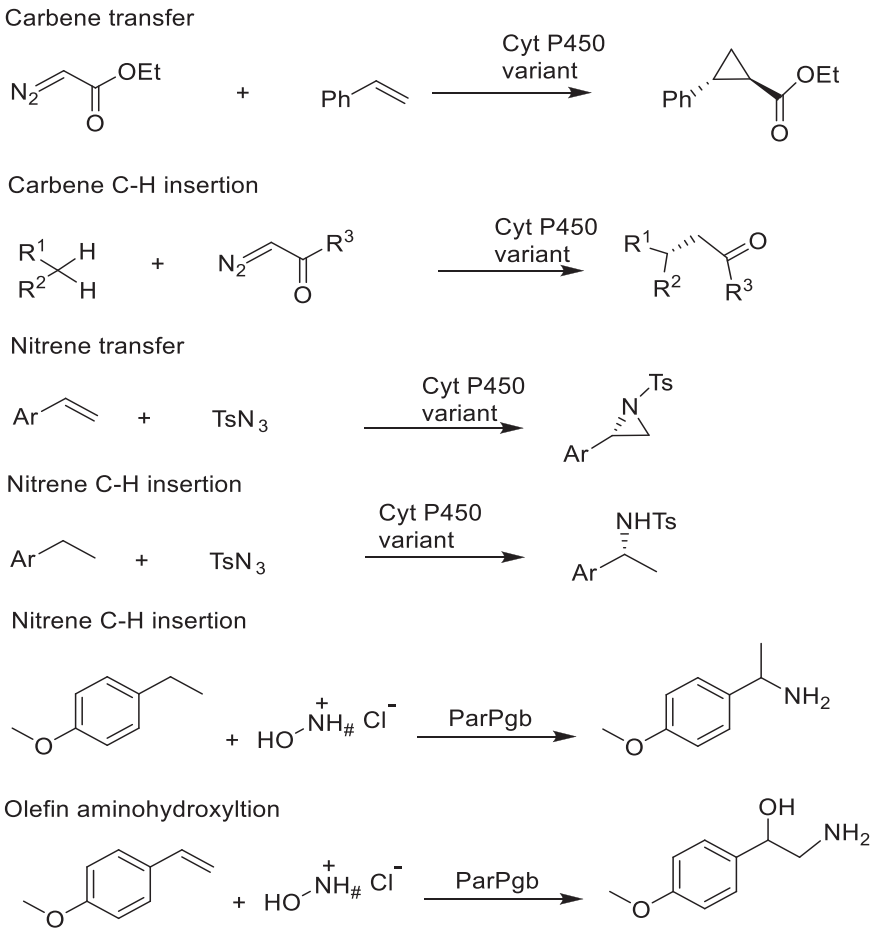

Fig. 8. New to nature reactions.

Recombinant proteins are normally produced with a so-called His-tag attached to the C- or N-terminus to facilitate their separation from crude cell lysate. Such non-invasive binding is very specific and leads to high enzyme loading and activity retention [53]. Turner and co-workers [54] demonstrated that this so-called EziG^{TM} technology (EnginZyme AB Sweden) has broad scope in organic synthesis.

In order to be broadly applicable, an enzyme immobilization technique must fulfill a number of requirements [49]:

- High enzyme loading (> 10 wt%)
- High activity recovery (> 50%)
- No leaching
- Tolerant to organic solvents
- Recyclable > 20 recycles)
- Mechanically stable in batch and flow
- Good mass transport
- Broad applicability in organic synthesis

A shortcoming of carrier-bound enzymes in general is that they involve a huge dilution of activity that translates to low space-time yields and productivities. This can be circumvented by using carrier-free self-immobilization techniques in which enzyme molecules are covalently linked together, forming microscopic particles. The most convenient and popular method for self-immobilization of enzymes is as cross-linked enzyme aggregates (CLEAs) [55].

A general problem with immobilized enzymes is that small particles exhibit high activities but are difficult to separate and large particles are easier to separate but have low activities. However, both high activities and facile separation can be combined using magnetically recoverable catalysts derived from enzyme immobilization on magnetic carriers or in magnetic CLEAS (mCLEAs) [55].

The fine tuning of overall biocatalytic process development can be further streamlined by integrating an enzyme immobilization step into the screening process of directed evolution to afford immobilized biocatalyst engineering (IBE) which combines the strengths of protein engineering and enzyme immobilization [56]. However, even in this combination the immobilization step remains a separate step in the overall production of the immobilized biocatalyst, i.e. the production of the free enzyme is *in vivo* while the immobilization is *in vitro*.

In contrast, engineering the enzyme to self-assemble *in vivo*, avoids the necessity for binding to prefabricated carriers *in vitro*. In this approach, genetic engineering is used to fuse a self-assembling partner protein to the enzyme. For example, genetic fusion of the polyester synthase, the central enzyme of polyhydroxyalkanoate (PHA) biosynthesis, results in the formation of insoluble PHA beads displaying the target enzyme covalently attached to the bead and in a functional mode.

Various microorganisms produce PHAs as a means of energy storage and they are used as bio-based plastics. Hence, this methodology constitutes an elegant example of a biocatalyst immobilized on a bio-based plastic and produced in one step from a renewable raw material [57].

Alternatively, genetic insertion of non-canonical amino acids (NCAAs) into enzymes can be used to create functional groups that enable precise formation of cross-linked enzyme aggregates or coupling to carriers via bio-orthogonal coupling reactions in crude cell lysate[58]. This elegant method effectively combines enzyme purification and immobilization into a single, cost-effective unit operation and avoids activity losses caused by undesirable side-reactions in standard procedures [59].

6.2. Continuous biocatalysis: go with the flow

From a chemical engineering viewpoint, there are two types of processes: batch and continuous. Traditionally, large volume specialty chemicals and commodity chemicals were produced in continuous processes and relatively small volume fine chemicals and pharmaceuticals were prepared in batch processes. However, in the last decade there is an increasing trend towards the production of pharmaceuticals in continuous flow processes, particularly those involving biocatalysis. Merging of biocatalysis and continuous processing is the solution to a number of problems associated with batch processes, namely feedback inhibition and intermediate degradation.

Biocatalysis in flow is rapidly becoming a key enabling technology and is has been applied to a broad range of transformations [60–65], including the use of multi-enzyme cascade biocatalysis in flow [66] The use of immobilized enzymes in packed bed reactors has a positive effect on the sustainability of processes by, inter alia, eliminating downtime thus increasing productivities, optimizing resource utilization and reducing the amount of waste compared with batch processing. In short, biocatalysis in flow is more cost effective and more sustainable. The switch from batch to continuous flow is facilitated by the many improvements that have been made in the development of cost-effective techniques for enzyme immobilization for use in packed bed reactors. For example, the hydrogen borrowing cascade for the synthesis of chiral amines from the corresponding alcohols (Fig. 9) has also been performed with the two enzymes co-immobilized on controlled porosity glass Fe^{3+} ion-affinity beads in continuous flow operation [51,67]. Similarly, the hydrogen borrowing cascade can also be performed with the co-immobiized whole cell biocatalysts [68].

6.3. Biocatalytic and chemoenzymatic cascades

Multi-step organic syntheses are generally performed step-by-step with product isolation and often purification following each step. This inevitably leads to low volumetric productivities and considerable waste generation. In contrast, telescoping into one-pot procedures reduces the number and amounts of solvents used and the amount of waste generated in fewer unit operations, smaller reactor volumes and in shorter cycle times with higher productivities. However, integration of multiple steps is often problematic owing to differences in, inter alia, operating temperatures and pressures and solvents used in the individual steps.

In stark contrast, metabolic pathways *in vivo* involve an exquisite orchestration of multiple steps, catalyzed by different enzymes in multi-enzyme cascades and all in water at ambient temperature and pressure. It is, therefore, eminently feasible to construct multi-enzyme and sometimes even chemo-enzymatic cascade processes *in vitro* [64,69–72] thus affording green and sustainable processes for the synthesis of relatively complex molecules. This can involve combinations of highly evolved enzymes into multi-enzyme or chemo-enzymatic cascades for the synthesis of molecules of ever increasing complexity [73–75].

A striking example is provided by the development, in a Merck/Codexis collaboration [76], of a sustainable and efficient synthesis of

Molnupiravir, an antiviral agent for treatment of COVID-19 (Fig. 10). It involves a novel, three-step biocatalytic cascade, incorporating an innovative phosphate recycling system and based on commodity raw materials and simple procedures involving robust reactions and only a single solid isolation step.

In contrast with the five-step chemical synthesis, the biocatalytic route involves only three steps, all proceeding in over 95% yield and is 70% shorter with a 7 times higher yield (69%). The cascade was optimized to make efficient use of the ribose starting material. Two of the three enzymes involved were evolved to afford 80- and 100-fold higher activity on the non-natural substrates compared to that of the natural enzymes. Remarkably, the entire cascade was developed within six months, thus demonstrating the enormous potential of engineered enzyme cascades to rapidly develop synthetic pathways for high-demand APIs.

7. Chemicals from valorization of waste biomass: towards a circular bio-based economy

Traditionally, the main applications of biocataysis were in food, beverages and detergents. In the last two decades developments in (meta)genome mining, protein engineering and bio-informatics, have enabled applications in the industrial scale synthesis of APIs, flavors and fragrances and a variety of other fine chemicals. Now, in the current quest for carbon neutrality, through the substitution of fossil-derived hydrocarbon feedstocks with alternatives based on waste biomass [77, 78], carbohydrates and triglycerides are set to replace hydrocarbons as the base chemicals in biorefineries, as oil refineries become largely obsolete. This radical change provides the foundations for a veritable renaissance in carbohydrate chemistry whereby both chemo- and biocatalytic methodologies [79] are utilized to upgrade bio-based resources including both the primary conversion of the feedstock and downstream processing of the base chemicals. In short, biocatalysis can contribute in various ways to enabling a circular bio-based economy. This includes not only the production of commodity chemicals and polymers but also the biocatalytic recycling of the polymers back to the constituent monomers.

7.1. Conversion of bio-based feedstocks to base chemicals

Bio-based feedstocks, as replacements for crude oil, are primarily polysacharides and triglycerides, although they could be simply mixed organic waste streams. The bio-based polysaccharides are first hydrolyzed, chemically or enzymatically, to their hexose and pentose building blocks. The latter are subsequently converted by fermentation to a variety of oxygenates, such as diols and dicarboxylic acids, many of which are industrial monomers [80–83]. In contrast, in an oil refinery complex mixtures of hydrocarbons in crude oil are converted to, *inter alia,* lower olefins, aromatic hydrocarbons and methanol as the base chemicals. In the petrochemical industry roughly 90% of the downstream products are polymers roughly 40% of which are polyolefin plastics. We can reasonably assume, therefore, that ca 90% of the commodity chemicals produced from biomass in a biorefinery will also be industrial monomers but not necessarily the same polymers.

Production of commodity chemicals from C_6 and C_5 sugars can involve either (i) fermentation to lower alcohols - ethanol, 1-butanol and isobutanol – followed by dehydration to ethylene, 1-butene and isobutene, respectively, and further processing via established petrochemical technologies or (ii) direct, redox-economic conversion to oxygenates as platform chemicals. The latter route provides the

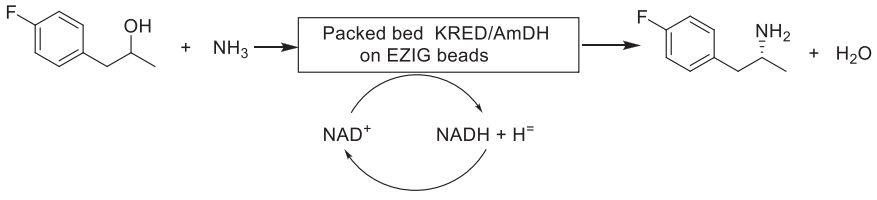

Fig. 9. Continuous flow synthesis of chiral amines.

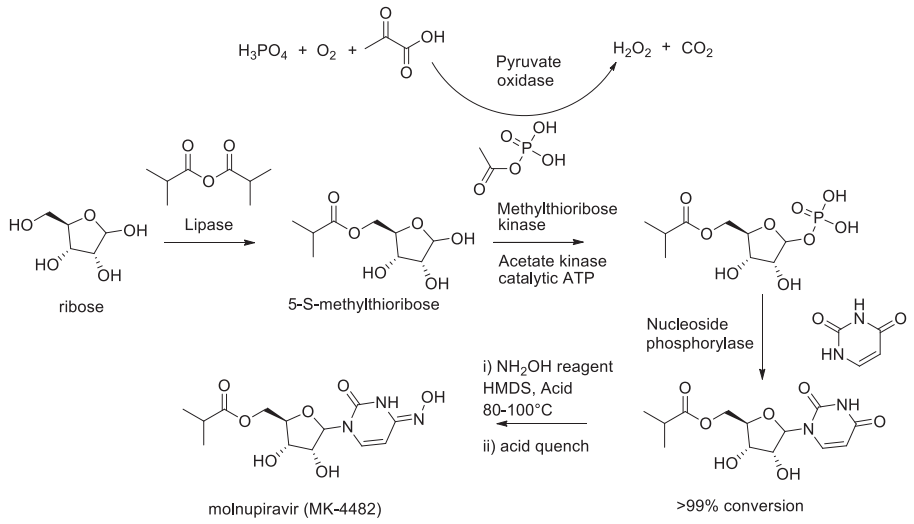

Fig. 10. Biocatalytic synthesis of molnupiravir.

possibility of creating alternative bio-based polyesters and polyamides to replace polyolefins in e.g. plastics. For example, glucose can be hydrolyzed to 5-hydroxymethyl furfural (HMF). Aerobic oxidation of the latter, using chemocatalytic or biocatalytic [84,85] methods affords 2, 5-furandicarboxylic acid (FDCA) (Fig. 11). Subsequent polymerization of FDCA with ethylene glycol produces polyethylene furanoate (PEF), the all-bio equivalent of the more well-known polyethylene terephthalate (PET). PF and PET have similar mechanical, thermal and barrier properties [86]. However, the weak link in the chain is the conversion of glucose to HMF owing to the low stability under the acidic conditions. A possible solution is to convert HMF in situ to 5-chloromethylfurfural (CMF) by reaction with aq. HCl. The lipophilic CMF is more stable and can be further oxidized to FDCA in high yield [87]. Indeed, CMF in itself is an interesting bio-based platform chemical.

Other polymer building blocks can also be produced from HMF, e.g. biocatalytic or chemocatalytic reduction and reductive amination produce the corresponding diol or diamine, as building blocks for polyesters or polyamides, respectively [88].

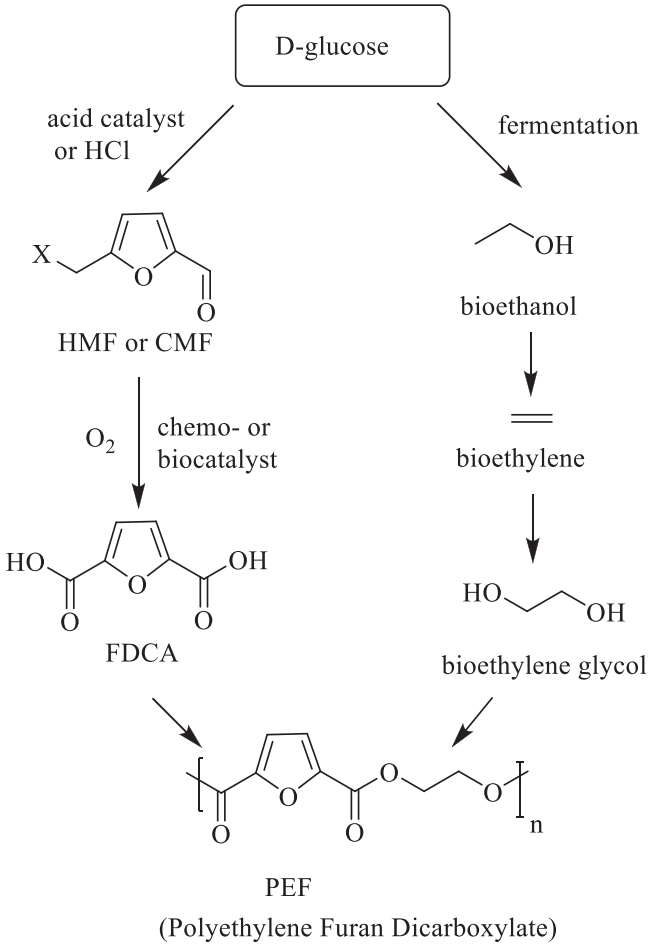

Fig. 11. Production of polyethylene furanoate.

7.2. *The plastic pollution challenge and the circular economy*

According to a 2017 estimate [89], only 30% of all the plastics that have ever been produced are currently in use. The remaining 70%, amounting to in excess of 6 billion tonnes, has primarily accumulated in landfills or in our natural environment. Moreover, estimates of the projected production of plastics in the period up to 2050 suggest that a further 6–12 billion tonnes of waste will be generated. This simly cannot be allowed to happen. The linear take-make-use-dispose economy must be superseded by a sustainable circular economy that optimizes resource utilisation through multiple rounds of recycling [90]. Landfill is no longer a viable option and chemical recycling by converting waste plastic to e.g. fuel is not an attractive proposition. The most sustainable and sensible option is closed loop recycling by conversion to the original plastic through recovery of the monomers.

However, closed loop recycling of polyethylene and polypropylene is not technically feasible. Perhaps we should see this more as a golden opportunity than a problem, by motivating the replacement of fossil resource derived polyolefins with eminently recyclable plastics derived from bio-based polymers containing readily hydrolysable bonds, e.g. polyesters and polyamides. For example, polyethylene furanoate (PEF), and polybutylene succinate (PBS), can be converted to the original monomers by acid- or base-catalysed or enzyme-catalysed hydrolysis. Most of the reported studies of enzymatic hydrolysis of polyesters to the original monomers concern the widely used polyethylene terephthalate (PET) [91]. The current status of research on PET-hydrolyzing enzymes for industrial scale application [92] and the possibilities and limitations of biotechnological recycling of plastics in general were recently reviewed [93,94]. It seems clear that technologies for (bio)catalytic depolymerisation of polyesters are on the verge of industrial viability particularly when the costs of virgin vs recycled plastic are compared with extended producer responsibility included in the pricing.

7.3. Direct conversion of organic waste to polymers

Yet another possibility is to produce bio-based polymers directly by fermentation of organic waste streams, a prime example being the polyhydroxyalkanoates (PHAs). The synthesis and applications of PHAs were recently reviewed [95]. They function as a source of both energy and carbon in acetogenic bacteria and can reach up to 90% of the dry weight of the microorganism. They are produced by fermentation of essentially any organic waste stream, e.g. municipal wastewater [96]. Their physical properties are highly dependent on the exact structure and some PHAs have properties comparable with those of PE and PP and are, in principle, suitable as single use plastics. However, they cost more than the polyolefins that they seek to replace. It is worth noting, however, that the cost-price of polyolefins has been optimized over a period of more than half a century. This scenario could drastically change in the near future as the price of fossil resource-derived base chemicals substantially increase and the concept of extended producer responsibility (EPR) is finally introduced in the plastics industry.

8. Conclusions and future prospects

In order to meet the grand challenges for chemistry and chemical engineering by solving the ubiquitous waste problem - from waste CO_2 that is the underlying cause of climate change to the notorious fouling of our own nest with plastic pollution. When it is not properly managed waste becomes pollution. The solution is more sustainable chemistry based on a more efficient use of resources in a circular bio-based economy and products that are recyclable by design. The key words are *pollution prevention*. In Ernest Callenbach's Ecotopia plastics were derived solely from living biological sources (plants) rather than fossilized ones [97].

On the basis of the spectacular advances in (meta)genomics, directed evolution and bioinformatics, biocatalysis has already become a technology to be reckoned with in the production of chemicals. Further optimization of enzyme performance has been achieved, through the development of improved immobilization techniques and the use of immobilized enzymes in e.g. packed bed reactors in continuous operation.

In addition to the substantial improvements in performance the use of biocatalysis is being further stimulated by the ongoing extensive decarbonization of the energy sector and defossilization of chemicals manufacture. These developments underpin the transition to a bio-based economy that utilizes (waste) biomass as the raw material and produces bio-based plastics that are recyclable to the original polymers. Biocatalysis has the right credentials to play an important role in this transition to a future bio-based chemical industry. Moreover, the increasing electrification of the energy sector and the increased use of photovoltaic systems will stimulate the use of electro- and photo-biocatalysis in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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