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Development of Sustainable Biocatalytic Reduction Processes for Organic Chemists
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1.1 Introduction

Among the different factors contributing to a good chemical manufacturing process, the process efficiency and specifically volume–time output in terms of reactor capacity and cycle time, respectively, have been given the largest weight – among the conversion costs, with material cost being the second [1]. Raw materials or intermediates with a higher oxidation state than the target products are often preferred to oxidations on an industrial scale due to process safety and toxicity concerns [2] and therefore have to be transformed in one or more reduction processes to the desired oxidation state; the importance to use as few redox steps as possible in a multistep synthesis has been outlined in the concept of redox economy [3–5]. Nonselective reductions often require additional protection–deprotection steps influencing process economy and leading to waste that scales stoichiometrically with increasing production. Therefore, the reduction of the number of synthetic steps by highly selective and sustainable reduction processes in organic synthesis is of key importance and has influenced the development of reduction processes, reagents, and tools (see Figure 1.1 for route selection in reductions). The variety of reducing agents, from simple molecular hydrogen with chiral or nonchiral catalysts in catalytic hydrogenations to reducing equivalents from inorganic or organic reagents with the required reducing power for the specific reduction, has enabled a large number of selective reduction reactions. The scope of reducing agents has been greatly expanded from the use of hydrogen gas in catalytic hydrogenation, the preparation of nongaseous reducing agents like lithium aluminium hydride and sodium borohydride, to the development of highly selective boranes by HC Brown, representing a milestone of organic synthesis and optically active organoboranes and providing versatile synthetic methodologies for asymmetric reductions of prochiral ketones, whereby the chiral auxiliary is recovered in an easily recyclable form [6–8]. With the growing importance of safety, health, and environment aspects, the nature of the reducing agents, the transition from stoichiometric to catalytic reductions, and the development of sustainable chemistry
have received increased attention [9]. Among the many synthetic methodologies available for reduction reactions, biocatalysis [10–20] has become an attractive choice in organic chemistry due to progress in understanding fundamental structure–function relationships and engineering of enzymes, their applications to organic synthesis, and developing novel enzymes to solve synthetic challenges in organic chemistry.

Key advances over the past 10 years have established biocatalysis as a practical, robust, and sustainable methodology in both laboratory and industrial chemical syntheses of bulk and specialty chemicals for a variety of industries [21–22]. The biocatalysts in the BRENDA database [23], which contains functional biochemical and molecular enzyme data and about 62 000 unique fully characterized reactions, can be searched according to EC subclasses for known reduction reactions in various ways. The widely used differentiation between alcohol dehydrogenases and carbonyl reductases or ketoreductases is based on the directional preference, expressed as the ratio of the reaction rate constants for the reduction and the oxidation direction, which have been for the first time reengineered by active site redesign of a parent dehydrogenase into an effectively “one-way” reductase [24]. Ready-to-use biocatalysts in the form of whole cells or isolated enzymes have become practical tools for the organic chemist to perform enzymatic reductions with high selectivity [25].

![Figure 1.1 Biocatalytic reduction processes and the optimization of the redox state changes and the number of one-pot reaction steps over the synthetic sequence, whereby additional criteria like material costs, yields, and step combinations in one-pot reaction play a role in route selection.](image-url)
1.2 Biocatalytic Reductions of C=O Double Bonds

The synthetic applications of the biocatalytic reduction of C=O double bonds are also described in detail in Chapter 4, while Chapter 6 describes the use of protein engineering to develop novel enzymes for the improved reduction of C=O double bonds.

1.2.1 Biocatalytic Reductions of Ketones to Alcohols

The biocatalytic asymmetric reduction of ketones to alcohols has been of great interest to organic chemists over many decades [26–28]. A large range of reactions with an even larger number of ketone substrates carrying a variety of substituent functional groups has been developed. Microbial reduction of phenylglyoxylic acid to mandelic acid by yeast has been found more than a century ago [29]. The investigation of the absolute stereochemical course of hydride transfer to carbonyl groups of decalin derivatives in reductions by microorganisms like *Curvularia falcata* has led Vladimir Prelog to rationalize these numerous experimental facts by a simple scheme connecting the substrate orientation in the plane of the carbonyl group with the spatial hydride transfer relative to this plane, later called Prelog’s rule (Figure 1.2), for the absolute configuration of the obtained chiral alcohols [30,31]. Prelog’s rule states that the alcohols that were formed by the microbial reductions studied had all the (S)-configuration and explains this fact by the pro-R hydride transfer from the cofactor to the Re-face of the carbonyl group, a property not only of the microbial reducing agents used but also of the oxidoreductase enzymes [32,33]. The later discoveries of microorganisms and alcohol dehydrogenases, for example, from *Mucor javanicus* [34,35], *Pseudomonas* sp., and *Lactobacillus kefir* [36,37], with the pro-R hydride transfer from the cofactor to the opposite Si-face of the carbonyl group leading to alcohols with the (R)-configuration are described to have anti-Prelog enantioselectivity. The catalytic asymmetric reduction of prochiral cyclohexanones to their corresponding axially chiral (R)- and (S)-alcohols is a reduction where chiral transition metal catalysts fail, but where excellent enantioselectivity has been achieved with alcohol dehydrogenases and the reversal of enantioselectivity by directed evolution [38].

The form of the biocatalysts used has varied widely from whole cells like baker’s yeast [39], *Geotrichum candidum* [40], or recombinant microorganisms expressing the desired alcohol dehydrogenase or ketoreductase, extracts, or isolated enzymes thereof [41,42] to the isolated animal-derived enzymes like horse liver alcohol dehydrogenase (HLADH) [43] and isolated enzymes from microorganisms like *Saccharomyces cerevisiae* [44], *Thermoanaerobium brockii* [45], and *L. kefir* [46]. The progress in the development and production of recombinant and engineered alcohol dehydrogenases or ketoreductases [47,48] has accelerated its use in laboratory and industrial-scale processes [49–55]. A recombinant short-chain alcohol dehydrogenase RasADH from *Ralstonia* sp. overexpressed in *Escherichia*
coli has been shown to reduce ketones with two bulky substituents to the corresponding optically highly enriched alcohols with very high stereoselectivity according to Prelog’s rule [56]. The search efforts for highly active and enantioselective carbonyl reductases that have a broad substrate range and tolerate high substrate and product concentrations have been shortened by the progress in bioinformatics and protein engineering, and a carbonyl reductase from *Kluyveromyces thermotolerans* able to reduce a variety of arylketones to alcohols at high concentrations and high ee values according to anti-Prelog’s rule has been found by genome mining [57]. Substituent effects in a series of ketoreductase-catalyzed reductions of aryl ketones have been evaluated on activity and enantioselectivity for different isolated recombinant ketoreductases [58,59]. Reducing enzymes like the alcohol dehydrogenases and carbonyl- or ketoreductases have become excellent tools for ketone reduction in organic synthesis (see Figure 1.3 for selected

![Figure 1.2 Prelog’s rule: stereospecific product formation by alcohol dehydrogenase-catalyzed ketone reduction, assuming the large group having higher priority in CIP rules than the small group.](image-url)
reductions), which in the case of ketone reductions outperform other ketone reduction chemistries and make them a method of first choice [60–65].

Biphasic reaction media, which are advantageous for poorly water-soluble ketones and for reactions at higher substrate concentrations, have been developed for the
asymmetric reduction of ketones with in situ cofactor regeneration, whereby both the alcohol dehydrogenase and the formate dehydrogenase remain stable [66]. Since many oxidoreductase reactions depend on the nicotinamide cofactors NAD⁺/NADH and NADP⁺/NADPH, efficient in situ cofactor regeneration systems have been engineered [67], which can be scaled up.

Dicarbonyl reductases, including diketoreductases, α-acetoxyketone reductase, and sepiapterin reductase, are of preparative interest for the direct production of chiral diols by the biocatalytic reduction of two carbonyl groups [68]. The increasing number of suitable and accessible dehydrogenases/reductases makes the enzymatic reduction processes attractive to a growing number of organic chemists due to robust enzymatic reduction technologies, which over many decades have been established and translated into industrial processes for the synthesis of chiral alcohols [69].

The biocatalytic reduction of the keto group to the alcohol group proceeds with a great tolerance of other functional groups in the substrate, for example, the enantioselective reductions of ketoesters to chiral hydroxyesters [70–73] or of 2-ketoacids to chiral α-hydroxy acids [74]. The substrate selectivity of transition metal- and lactate dehydrogenase-catalyzed enantioselective reductions of several 3-aryl-2-oxopropanoic acids has been compared [74].

1.2.2 Biocatalytic Reductions of Aldehydes to Alcohols

Biocatalytic reductions of furfural to furfuryl alcohol by yeast have been first described more than a century ago [75] and continue to attract interest even today (see Figure 1.4 for selected reductions) despite the efficient industrial chemo-catalytic reduction processes. One area where biocatalytic reductions are of particular interest is the flavor and fragrance industry and an efficient biocatalytic process for the reduction of cinnamyl aldehyde to the aroma chemical cinnamyl alcohol has been developed using recombinant whole cells overexpressing an alcohol dehydrogenase from L. kefir and a glucose dehydrogenase from Thermoplasma acidophilum [76]. The product cinnamyl alcohol was obtained in 77% yield with a high substrate concentration of 166 g/l cinnamyl aldehyde and with the reaction reaching 98% conversion [76]. Chiral metalloccenic alcohols have been obtained from racemic 1-formyl-2-methyl derivatives of tricarbonyl (cyclopentadienyl) manganese and (benzene) tricarbonyl chromium in a kinetic resolution with HLADH [77]. HLADH has also been used in the highly enantioselective biocatalytic reduction of 2-arylpropionic aldehydes to (2S)-2-aryl-propanols [78]. High yields have been achieved in a dynamic kinetic resolution process (DKR) by coupling the HLADH-catalyzed reduction to a chemical racemization step. The racemization step is represented by the keto–enol equilibrium of the aldehyde and can be controlled by modulating pH and reaction conditions. A new glycerol dehydrogenase from the acetic acid bacterium Gluconobacter oxydans shows a broad substrate spectrum in the reduction of different aliphatic, branched, and aromatic aldehydes with the highest activities observed for the conversion of D-glyceraldehyde.
in the reductive direction [79]. The kinetic resolution of racemic glyceraldehyde has been achieved and enantiopure L-glyceraldehyde was obtained on preparative scale. Coexpression of this glycerol dehydrogenase from *G. oxydans* and the enzyme for cofactor regeneration, glucose dehydrogenase from *Bacillus subtilis*, in *E. coli* BL21 (DE3) facilitated the access to L-glyceraldehyde with high enantioselectivity at 54% conversion. The whole-cell catalyst shows several advantages over the cell-free system like a higher thermal, a similar operational stability, and the ability to recycle the catalyst without any loss of activity, making the whole-cell catalyst more efficient for the production of enantiopure L-glyceraldehyde [80]. A new NADPH-dependent aldehyde reductase from *Oceanospirillum* sp. has been demonstrated to reduce a variety of substituted benzaldehydes and aliphatic aldehydes with high chemoselectivity, as shown by the chemoselective reduction of aldehydes in the

Figure 1.4 Selected biocatalytic reductions of aldehydes to alcohols.
presence of ketones, for example, the selective reduction of the aldehyde functional
group in 4-acetylbenzaldehyde or in the mixture of hexanal and 2-nonanone [81]. A
kinetic resolution of racemic 2-methyl valeraldehyde utilizing an evolved ketore-
ductase for the enantioselective reduction of the (R)-enantiomer and a scalable
method for the separation of the desired product from the (S)-enantiomer of the
starting material yielded the important chiral intermediate (R)-2-methypentanol
with high volumetric productivity [82].

1.2.3
Biocatalytic Reductions of Carboxylic Acids to Aldehydes

The reduction of carboxylic acids to aldehydes is of preparative interest and a
number of microbial carboxylic acid reductases (CARs) (aldehyde oxidoreductases)
have been found since the discovery of the tungsten-containing CAR that reduced
nonactivated carboxylic acids to the corresponding aldehydes with no further
reduction of the aldehydes to alcohols [83]. The reduction of vanillic acid to the
aldehyde vanillin has been achieved in vitro with the CAR from Nocardia sp. [84] and
as part of a de novo biosynthesis in yeast [85]. A CAR from Mycobacterium marinum
has been discovered that can convert a wide range of aliphatic fatty acids (C6–C18)
into corresponding aldehydes [86].

1.2.4
Biocatalytic Reductions of Carboxylic Acids to Alcohols

A selective biocatalytic hydrogenation of a broad range of carboxylic acids to the
corresponding primary alcohols has been achieved by Pyrococcus furiosus without
reducing isolated carbon–carbon double bonds [87]. By the combination of a CAR
from M. marinum with an aldehyde reductase, fatty alcohol concentrations of
350 mg/l have been achieved [86]. A range of short-chain carboxylic acids from
acetic acid to n-caproic acid were converted into their corresponding alcohols using
Clostridium ljungdahlii and Clostridium ragsdalei as biocatalysts and syngas as energy
and electron source [88]. A new Acinetobacter species has been found to reduce the
carboxy functional group in 5-cis,8-cis,11-cis,14-cis-arachidonic acid to 5-cis,8-cis,11-
cis,14-cis-arachidonyl alcohol [89]. Further details on biocatalytic reductions of
carboxylic acids to alcohols can be found in Chapter 2.

1.3
Biocatalytic Reductions of C=C Double Bonds

Catalytic asymmetric reductions of carbon–carbon double bonds can be done in a cis-
or trans-fashion and generate up to two new chiral centers. The biocatalytic
asymmetric reduction of carbon–carbon double bonds occurs with exclusive
trans-stereospecificity [90] except for some rare cases of cis-stereoselectivity. Bio-
catalytic reductions are therefore complementary to the high standard of transition
metal-catalyzed cis-hydrogenation and an increasing number of successful syntheses have been achieved (see Chapter 3 for further details) over the last few years using cloned enoate reductases [91,92], making this an established methodology with many benefits (see Figure 1.5 for selected C=C double bond reductions). The oxygen-stable enoate reductases 12-oxophytodienoate reductase isoenzymes OPR1 and OPR3 from tomato and the “old yellow enzyme” homolog YqjM from *B. subtilis* have been found to reduce a remarkably broad range of activated alkenes bearing an

![Figure 1.5](image)

Figure 1.5 Selected biocatalytic asymmetric reductions of C=C double bonds.
electron-withdrawing group like $\alpha,\beta$-unsaturated aldehydes, ketones, maleimides, and nitroalkenes with absolute chemoselectivity and excellent ee values up to $>99\%$ [93]. An interesting novel nicotinamide-independent asymmetric reduction of activated carbon–carbon double bonds was developed by direct hydrogen transfer from a sacrificial enone as hydrogen donor, catalyzed by enolate reductases [94]. The known substrate scopes of a large number of old yellow enzymes and old yellow enzyme-like biocatalysts include a large library of $\alpha,\beta$-unsaturated activated alkene compounds typically containing electron-withdrawing or electron-activating groups such as aldehyde, acyclic and cyclic ketones, carboxylic acid, ester, and nitro functionalities [95]. The $(R)$- and $(S)$-citronellal enantiomers have been prepared by ene reductase-catalyzed reductions from citral [96–99]. Ene reductase-catalyzed reduction of 2-hydroxy-methylacrylic acid methylester and its O-allyl, O-benzyl, and O-TBDMS derivatives yielded the corresponding $(R)$-3-hydroxy-2-methylpropionic acid methyl-esters with excellent enantioselectivities of up to $>99\%$ ee [90,100]. An interesting example for preparing two enantiomeric 2-methyl-3-aryl-propanols has been demonstrated by controlling the starting alkene regioisomer [102]. Substrate control has also been investigated in the ene reductase-catalyzed reduction of carbon–carbon double bonds of $\beta$-cyano-$\alpha,\beta$-unsaturated esters [103]. Excellent productivity improvements in the OYE3-catalyzed reduction of $(E)$-2-ethoxy-3-($p$-methoxyphenyl)prop-2-ene-1-al to $(S)$-2-ethoxy-3-($p$-methoxyphenyl)propion-1-aldehyde have been obtained by combining the use of overexpressed OYE3 with the in situ SFPR technology, making it a practical and simple process with ready-to-use isolated enzymes [104]. The use of protein engineering to develop novel enzymes for the improved reduction of $C=\!C$ double bonds is described in Chapter 5.

1.4 Biocatalytic Reductions of Imines to Amines

The enantioselective reduction of imines provides an interesting strategic route to chiral amine functions in the construction of a target molecule and is therefore of great synthetic interest. While several oxidoreductases catalyzing asymmetric reduction of water-soluble substrates have been found over the last decades, it is only recently that the synthetic applications have attracted attention [105,106]; Figure 1.6 shows selected imine reductions to amines (see also Chapter 2 for further details). Imine-reducing strains of *Streptomyces* sp. have been identified by screening to reduce 2-methyl-1-pyrroline with high $(R)$- and $(S)$-selectivity [107]. The corresponding whole-cell biocatalysts have been shown to reduce 2-methyl-1-pyrroline to $(R)$-2-methylpyrrolidine with high enantioselectivity (99.2% enantiomeric excess), while the other enantiomer $(S)$-2-methylpyrrolidine was obtained with lower enantioselectivity (92.3% enantiomeric excess) at 91–92% conversion [107]. The responsible isolated enzyme $(R)$-imine reductase of *Streptomyces* sp., a homodimer consisting of 32 kDa subunits and dependent on NADPH, also reduced 2-methyl-1-pyrroline to $(R)$-2-methylpyrrolidine with 99% enantiomeric excess in a nearly complete conversion [108]. Interestingly, under neutral conditions of pH 6.5–8.0, the 2-methyl-1-
pyrroline was reduced, while \((R)\)-2-methylpyrrolidine was oxidized under alkaline pH of 10–11.5 [108]. A NADPH-dependent \((S)\)-imine reductase from *Streptomyces* sp., a homodimer consisting of 30.5 subunits, catalyzed the enantioselective reduction of 2-methyl-1-pyrroline to the corresponding \((S)\)-amine with 92.7% ee, of 1-methyl-3,4-dihydroisoquinoline to its corresponding \((S)\)-amine with 96% ee, and of 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline to the corresponding \((S)\)-amine with >99% ee [109]. A recombinant form of the NADPH-dependent imine reductase from *Streptosporangium roseum* strain DSM 43021 has been coexpressed
with NADP-dependent glucose dehydrogenase to catalyze the synthesis of optically active (S)-amines from imines [110]. The thiazolinyl imine reductase PchG from *Pseudomonas aeruginosa* reduces the C= N double bond of a thiazoline ring to the thiazolidine ring in the synthesis of pyochelin [111] and the thiazolinyl imine reductase Irp3 from *Yersinia enterocolitica* catalyzes the NADPH-dependent reduction of a C= N double bond in the center thiazoline ring of an intermediate to make the thiazolidine ring of the product in the synthesis of yersiniabactin [112].

### 1.5 Biocatalytic Reductions of Nitriles to Amines

Contrary to the predictions based on sequence analysis, a new nitrile reductase, queF, has been discovered that catalyzes the NADPH-dependent four-electron reduction of 7-cyano-7-deazaguanine (preQ\(_0\)) to 7-aminomethyl-7-deazaguanine (preQ\(_1\)), a late step in the biosynthesis of queuosine A [113]. The reduction of a nitrile is unprecedented in biology, and the enzyme from *B. subtilis* has been characterized and a chemical mechanism for this enzyme-catalyzed reduction has been proposed [114,115]. The expression and characterization of queF from *E. coli* K-12 (EcoNR) has also been demonstrated to reduce its natural nitrile substrate preQ0 at 37 °C and pH 7 to the corresponding amine product preQ1 [116]. A nitrile reductase from *Geobacillus kaustophilus* has been cloned, expressed, and characterized and a range of common nitriles that have been tested as substrates showed a narrow substrate range for the wild-type enzyme [117]. Mutants were investigated regarding the natural substrate preQ0 as well as a range of preQ0-like substrates, whereby a distinct substrate dependence of the wild-type enzyme activity was observed and two nonnatural preQ0-like substrates could be reduced to their corresponding amino compounds. Selected nitrile reductions to amines are shown in Figure 1.7 (see Chapter 2 for further details).

### 1.6 Biocatalytic Deoxygenation Reactions

A selection of biocatalytic deoxygenation reactions is shown in Figure 1.8. The reducing power of baker’s yeast in an ethanol–water mixture and sodium hydroxide at 60°C has been found effective for the rapid and selective reduction of a series of N-oxides like aromatic and heteroaromatic N-oxide compounds [118]. DMSO reductase from *Rhodobacter sphaeroides* f. sp. *denitrificans* catalyzed the (S)-enantioselective reduction of various sulfoxides and enabled the resolution of racemic sulfoxides for the synthesis of (R)-sulfoxides with >97% ee [119,120]. Purified dimethyl sulfoxide reductase from *Rhodobacter capsulatus* resolved a racemic mixture of methyl p-tolyl sulfoxide by catalyzing the reduction of (S)-methyl p-tolyl sulfoxide and gave enantiomerically pure (R)-methyl p-tolyl sulfoxide in 88% yield, while whole cells of *E. coli,*
Proteus mirabilis and Proteus vulgaris reduced the same sulfoxide with opposite enantioselectivity [121]. The resolution of racemic alkylaryl sulfoxides and thiosulfates by dimethyl sulfoxide reductase from the anaerobic bacterium Citrobacter braakii gave the corresponding enantiopure alkylaryl sulfoxides and thiosulfates [122].

Recombinant ribonucleoside triphosphate reductase from Lactobacillus leichmannii has been used for the 2′-deoxygenation of ATP to prepare 2′-deoxyadenosine-triphosphate with a high degree of conversion and high yield using 1,4-dithio-DL-threitol as artificial reducing agent for the ribonucleoside triphosphate reductase [123]. Good biocatalytic 2′-deoxygenation with the same enzyme has also been...

Figure 1.7 Selected biocatalytic reductions of nitriles to amines [101].
observed for the other ribonucleoside-5'-triphosphate substrates CTP, GTP, ITP, and UTP [124].

1.7 Emerging Reductive Biocatalytic Reactions

Newly discovered biocatalytic reductions are emerging as interesting alternative reactions to well-known chemical reductions (Figure 1.9). Interesting benzoyl coenzyme A reductases (BCR) from facultatively anaerobic bacteria, which catalyze
ATP-dependent dearomatization reaction of aromatic rings to cyclohexa-1,5-diene-1-carboxyl CoA compounds analogous to Birch reductions, have been investigated with respect to the stereochemical course and exchange reactions and the findings indicate that BCR forms the trans-dienyl CoA product [125]. The findings support the proposed Birch reduction mechanism of BCR [125], opening the emerging area of biocatalytic Birch reductions. A new tungsten-containing class II benzoyl coenzyme A reductase from Geobacter metallireducens has been discovered, which catalyzes the fully reversible ATP-independent dearomatization of benzoyl-CoA to
cyclohexa-1,5-diene-1-carboxyl-CoA (dienoyl-CoA) at extremely low redox potential [126,127]. The tetrahydroxynaphthalene reductase is another enzyme of interest for dearomatization reactions and shows a broad substrate range [128].

In the area of biocatalytic reductive cyclizations, the interesting plant-derived iridoid synthase generating the bicyclic monoterpene ring has been discovered, which uses the linear 10-oxogeraniol as substrate instead of the geranyl diphosphate used by all known terpene cyclases [129]. A very interesting novel diphosphate-independent terpene cyclase from *Zymomonas mobilis* has been discovered, which catalyzes the cyclization of the nonactivated citronellal to isopulegol [130], and squalene–hopene cyclase can be converted by a single amino acid exchange into citronellal cyclases [131].

1.8 Reaction Engineering for Biocatalytic Reduction Processes

Since the reductant will be oxidized in the biocatalytic reduction process, the required reducing equivalents should either be inexpensive or otherwise an adequate regeneration system for the reductant has to be utilized. The development of the most suitable reaction conditions for new enantioselective reductions is decisive and starts first with the route selection and basic design, and then needs further experimental verification on a suitable scale as well as an adequate and meaningful analytical methodology, which is a prerequisite for rapid and straightforward reaction engineering. The development of a fast and simple batch process with complete substrate conversion is illustrated by the reaction engineering of the two-step reduction of dehydrocholic acid (DHCA) to 12-keto-ursodeoxycholic acid (UDCA) as the key enzymatic steps in the preparation of UDCA [132].

The analysis of reaction enantioselectivity for asymmetric reductions is key for the process development and an interesting combined use of high-performance liquid chromatography and circular dichroism has led to an efficient procedure for alcohol dehydrogenase-catalyzed asymmetric reduction of 1-phenyl-2-propyn-3-trimethylsilyl-1-one [133].

The bottleneck of substrate and product toxicity to the microbial cells used for the reduction has been overcome by the use of a polymeric resin to both supply substrate to and remove the product from the reaction mixture and therefore the microbial cells in the biocatalytic reduction of 3,4-methylene-dioxyphenyl acetone by *Zygosaccharomyces rouxii* to the corresponding (S)-3,4-methylene-dioxyphenyl isopropanol in >95% isolated yield and with >99.9% enantiomeric excess [134]. This substrate feed and product recovery (SFPR) design made it possible to increase concentration from 6 to 40 g/l and to achieve the reaction, product isolation, and resin recycling within a single piece of equipment at an overall reactor productivity of 75 g/l/day.

Another potential bottleneck to be overcome is the inherent equilibrium problem associated with the coupled substrate approach to biocatalytic carbonyl reduction and *in situ* product removal allowed the isolation of the pure (S)-2-bromo-2-cyclohexen-1-ol in 88% yield and with 99.8% enantiomeric excess [135].
The combination of a chemoselective enzymatic reduction step with another second enzymatic reaction is another opportunity to overcome limitations, for example, in the case of the enantioselective reduction of prochiral unsaturated aldehydes by coupling a reduction step with an isolated ene reductase (OYE 2 or OYE3) together with an oxidation step with HLADH in a cascade system, which allowed both yields and enantioselectivities to be improved [136].

1.9 Summary and Outlook

The use of biocatalysts for reduction reactions in organic chemistry at the laboratory scale as well as at the industrial production scale has found its prominent place as a valuable synthetic toolbox able to compete with the best other synthetic methodologies available over the last decades. The development of strategies for new reductive biocatalytic reaction chemistry will benefit from the interdisciplinary interaction and at the same time moving the disciplinary frontiers and interfaces between chemistry, biology, and biotechnology [137–139], between the molecular and engineering sciences [140], between the analytical and preparative technologies and perspectives. The future of biocatalytic asymmetric reduction reactions and reductive biotransformations looks very promising due to the tremendous scientific and technological progress and the inherent chirality of the biocatalysts [141]. The analysis of protein functions requires the synthesis or isolation of pure enzyme substrates in order to perform the biocatalytic reactions and measure enzyme activities. The diverse approaches for the discovery of novel reductive enzyme functions share the requirement of meaningful, robust, and sensitive analytical methodologies and will benefit from the standardization of quantitative and reproducible measurements of reductive substrate-to-product conversions and their reporting in publications [142]. The development and production of highly selective and stable biocatalysts for reductions, which can also be used for a larger group of substrates, is instrumental for the expanding adoption of biocatalytic reaction steps by the organic chemists. The rich diversity of Nature’s small molecules and their biosynthesis provide inspirations for a variety of reduction reactions in monomer biosynthesis, coupling reactions, and tailoring reactions [143].

Reaction engineering and product recovery are equally important and green chemistry will continue to be a useful central design framework for the translation of this new knowledge into daily industrial practice of fine and specialty chemical production [144]. The key element for the success of enzymatic reductions in green production methods is the continuous process improvement, functional group tolerance, and chemoselectivity of the particular reduction reaction due to the mild reaction conditions, the implementation in fine chemical production and the intensified inclusion in organic, green, and sustainable chemistry, catalysis, and industrial biotechnology [145,146]. Since the chemical production is highly complex, diverse, and based on a variety of scientific and technological disciplines, detailed analyses of existing process challenges in certain industrial production areas and
research perspectives for green production methodologies are useful [147], but the exciting developments in bioreductions and reductive biotransformations for organic chemistry will certainly shape the arsenal of industrial reduction technologies.

References


References


