Part VI
Miscellaneous Topics in Homogeneous Hydrogenation

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43
Transition Metal-Catalyzed Regeneration of Nicotinamide Cofactors

Stephan Lütz

This chapter is dedicated to the memory of Prof. Dr. E. Steckhan (1943–2000).

43.1
Introduction

Among the many reactions that biocatalysts can bring about [1, 2], redox reactions are of special interest for the synthesis of chiral compounds, including hydroxy acids [3], amino acids [4, 5], steroids [6] or alcohols [7–12] from prochiral precursors. The biocatalysts involved in these reactions belong to the class of oxidoreductases (E.C.1) and are dependent on so-called coenzymes or cofactors [13]. These cofactors can act as hydrogen, oxygen or electron-delivery systems. Nature has developed an amazing molecular machinery for this transfer of redox equivalents, using enzyme-bound (e.g., FMN, FAD or PQQ) or freely dissociated (e.g., NAD/H, NADP/H) molecules (Fig. 43.1).

The most important coenzymes in synthetic organic chemistry [14] and industrially applied biotransformations [15] are the nicotinamide cofactors NAD/H (**3a/8a**, Scheme 43.1) and NAD(P)/H (**3b/8b**, Scheme 43.1). These pyridine nucleotides are essential components of the cell [16]. In all the reactions where they are involved, they serve solely as hydride donors or acceptors. The oxidized and reduced form of the molecules are shown in Scheme 43.1, the redox reaction taking place at the C-4 atom of the nicotinamide moiety.

Formally, in its oxidized state the cofactor NAD^+ is charged negatively due to the two phosphate groups; the positive charge denotes quaternization of the nitrogen. It is noteworthy that from the reduced form only the 1,4-NAD(P)H instead of the 1,6-NAD(P)H is enzyme-active, which imposes some restrictions on the regeneration systems in terms of the selectivity.

Cofactor regeneration is a necessary prerequisite for an *in-vitro* application of oxidoreductase enzymes, as the cofactors are too expensive to be used in stoichiometric amounts (Fig. 43.2) [17, 18].

Furthermore, too-high levels of cofactors can even act as inhibitors for the production enzyme; thus, a low level of the coenzyme and constant regenera-

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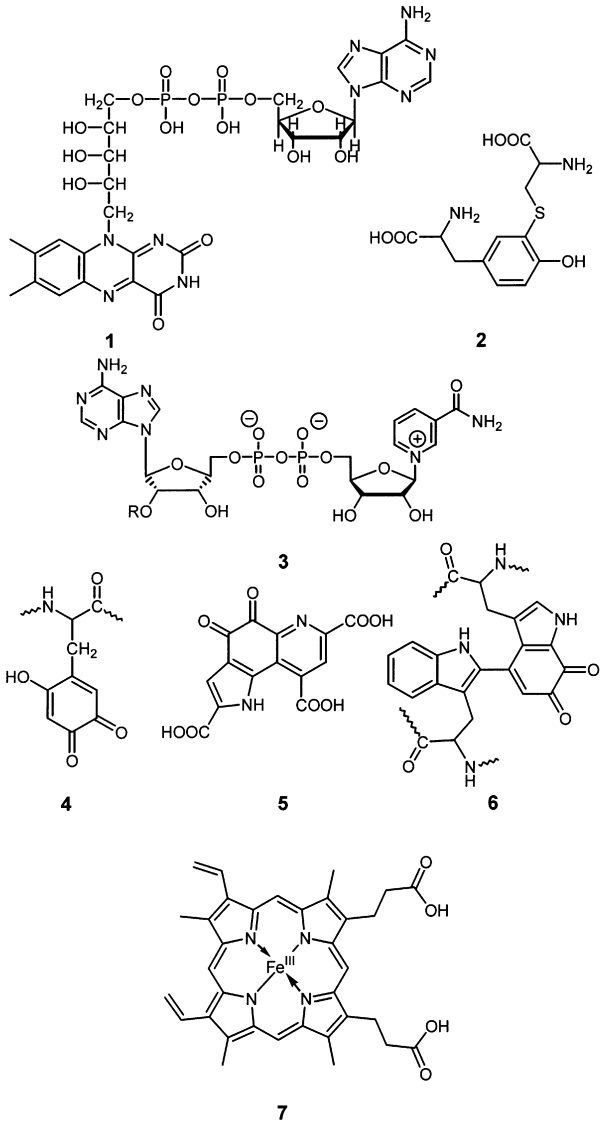
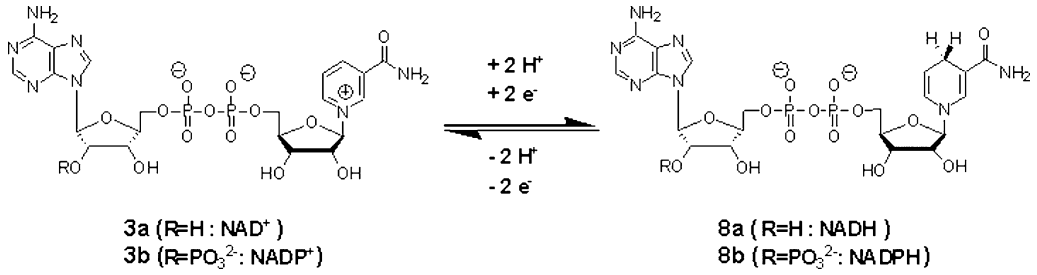


Fig. 43.1 Prosthetic groups in oxidoreductases (**1**: flavin adenine dinucleotide (FAD); **2**: thio-tyrosine; **3a** (R = H): nicotinamide adenine dinucleotide; **3b** ($R = PO_3^{2-}$): nicotinamide adenine dinucleotide phosphate ($NADP^+$); **4**: 6-hydroxy-DOPA; **5**: methoxanthine (pyrroloquinoline quinine; PQQ); **6**: tryptophan-tryptophan quinine).

tion of the desired oxidation state is necessary. In principle, both reactions which are implied in Scheme 43.1 – reduction and oxidation – are of interest in synthetic applications, but this chapter will focus only on the reduction reaction. The most important enzymatic reactions, in which regeneration of the nicotinamide cofactors are used, are alcohol dehydrogenase (ADH)-catalyzed [19], in



Scheme 43.1 Oxidized (left, NAD(P)$^+$, **3a**/**3b**) and reduced (right, NAD(P)H, **8a**/**8b**) forms of nicotinamide cofactors.

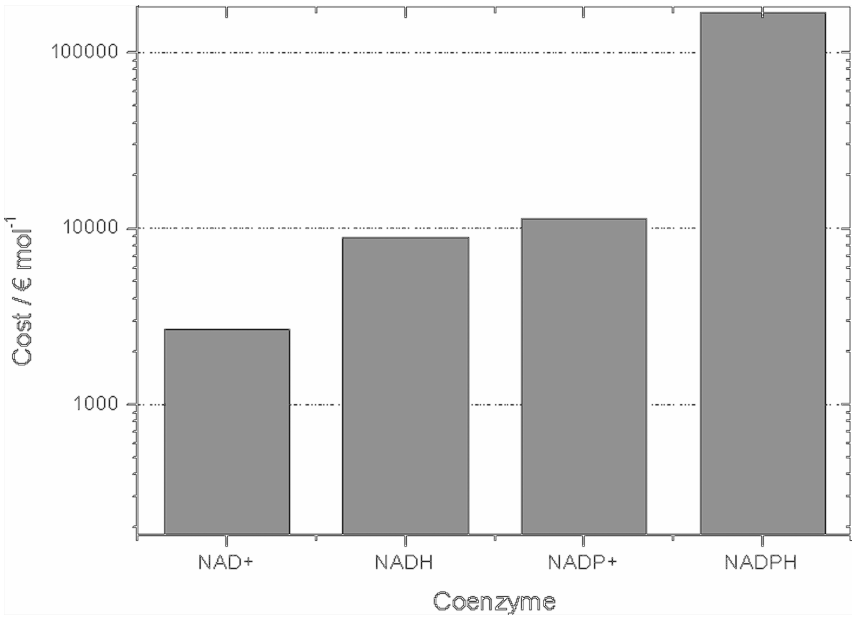
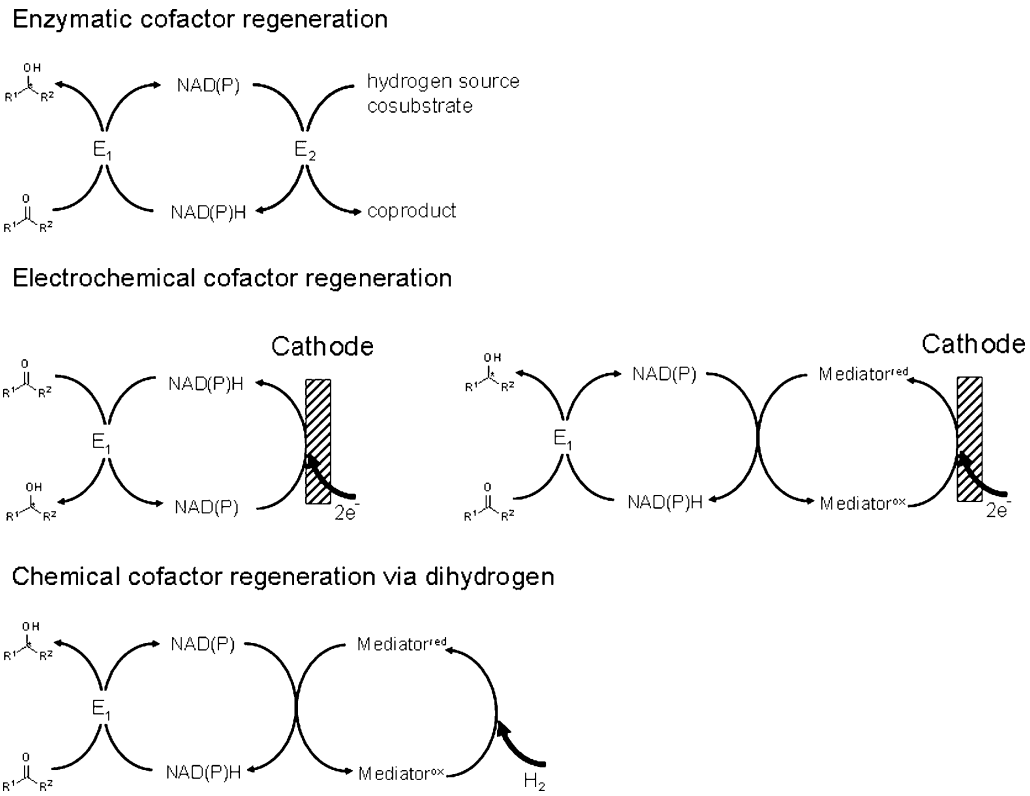


Fig. 43.2 Costs of nicotinamide cofactors (Source: Jülich Fine Chemicals, 2003).

which the reduced cofactor is the direct hydride source to convert a ketone into an alcohol, and monooxygenase-catalyzed biotransformations [20], where molecular oxygen is the final electron acceptor: one oxygen atom is selectively transferred to the substrate while the other is reduced by the cofactor to form water. Here, only ADH-catalyzed reactions will be shown as examples, but the regeneration systems also apply to monooxygenases.

The principal strategies of cofactor regeneration – namely the enzymatic, chemical and electrochemical approach – are presented in Scheme 43.2 and have been reviewed recently [17, 21–23]. This chapter does not intend to be exhaustive; rather, it focuses on the systems where a transition-metal complex and



Scheme 43.2 Principal strategies of cofactor regeneration (E_1: production enzyme; E_2: regeneration enzyme).

molecular hydrogen are involved, and includes a brief overview of enzymatic systems for comparison.

43.2 Enzymatic Cofactor Regeneration

Enzymatic cofactor regeneration can be subdivided into two categories: the enzyme-coupled approach, where two different enzymes are used (one for the production reaction, and one for the regeneration reaction); and the substrate-coupled approach, where one and the same enzyme is used for both production and regeneration ($E_1 = E_2$). The most convenient and commonly used enzymatic regeneration systems are summarized in Table 43.1.

A recently developed method uses a hydrogenase from *Pyrococcus furiosus* to regenerate reduced cofactors, using molecular hydrogen as reducing agent. This very promising approach is the first example of a biocatalytic hydrogenation of

Table 43.1 Enzymatic cofactor regeneration.

Regeneration reaction	Cofactor	Regeneration enzyme
$HCOOH/CO_2$	NAD	Formate dehydrogenase
Isopropanol/Acetone	NADP	ADH
Glucose/Gluconic acid	NAD/NADP	Glucose dehydrogenase

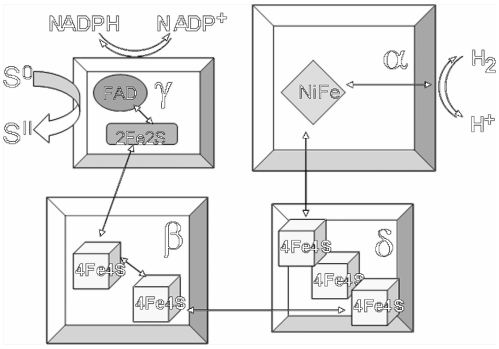


Fig. 43.3 The four subunits of *Pyrococcus furiosus* hydrogenase I involved in cofactor hydrogenation.

cofactors, and has been applied practically on a laboratory scale [24, 25]. Turnover frequencies (TOF) of 28 to 44 h^{-1} have been achieved for this reaction.

The active center of the enzyme consists of four subunits (Fig. 43.3) [26, 27], and the heterolytic cleavage of molecular hydrogen takes place at the α-subunit containing a nickel-iron center. The electrons are then channeled via several iron-sulfur-clusters to the γ-subunit, where either sulfur or cofactor reduction can occur.

To circumvent the cofactor regeneration problem, redox biotransformations are also carried out in whole cells – for example, baker's yeast [28, 29] or engineered *Escherichia coli* cells [30] – using the intracellular cofactor pool and inherent or recombinant regeneration systems.

43.3 Electrochemical Cofactor Regeneration

Electrochemical cofactor reduction can be achieved by direct reduction of the cofactor at the electrode surface, or indirectly by using a mediator molecule to shuttle electrons between the electrode and the cofactor. For details on the direct approach the reader is referred elsewhere [31, 32], since here no transition-metal complexes are involved. One point to be considered in the direct approach is the issue of selectivity. Whereas direct cofactor oxidation can be successfully achieved, special care must be taken to produce enzyme active reduced cofactors by direct electrolysis.

Several dyes or transition-metal complexes can be used as redox mediators in indirect electrolyses. Pentamethylcyclopentadienyl-rhodium(bipyridine) complexes $[Cp^*Rh^{III}(bpy)(H_2O)]^{2+}$ **9** [33], which were pioneered and intensively studied by Steckhan et al. [34–36], are very versatile catalysts for the reduction of cofactors.

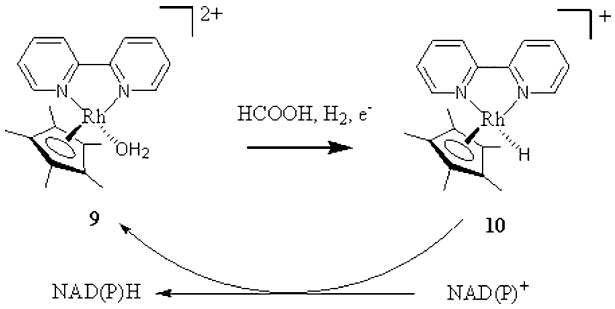
Complex **9** (Scheme 43.3) can be reduced by different redox equivalents to the active rhodium(I) species **10**; namely, by electrons, formate [37, 38], and hydrogen. This hydrido complex then transfers the hydride ion onto the nicotinamide. In electrochemical applications, TOFs in the range of 5 to 11 h^{-1} have been reported [31, 39]. It is noteworthy that this complex accepts NAD^+ and $NADP^+$ as substrates with the same efficiency and almost exclusively produces the 1,4-reduced cofactor (selectivity >99%).

For application in an electrochemical enzyme membrane reactor, polymer-supported derivatives of **9** have been synthesized, which could be retained by ultrafiltration membranes and were thus retained within the electroenzymatic reactor [31, 40].

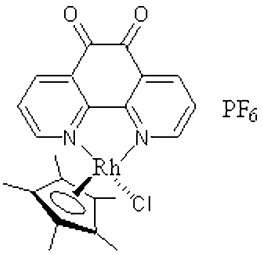
The unmodified complex can be applied in very dilute concentrations allowing total turnover numbers (TONs), or a substrate (NAD(P)) to catalyst (rhodium complex) ratio of up to 400 [41]. This efficiency was due to the design of a three-dimensional electrode, which also resulted in an extraordinary space-time yield of the reduced cofactor of up to 1 kg L^{-1} per day.

Several successful examples of coupling this regeneration system to synthesis reactions with different electrochemical reactors have been reported, including ADH and monooxygenase reactions [39, 42, 43].

Recently, the use of pentamethylcyclopentadienyl(1,10-phenanthroline-5,6-dione)chloro rhodium(III) hexafluorophosphate $[(Cp^*)Rh^{III}(phend)Cl]PF_6$, **11** (Fig. 43.4) has been reported for the electrochemical NAD^+ reduction. TONs between 7 and 453 h^{-1} have been achieved by varying pH, temperature and the complex concentrations [44]. This study reveals only preliminary results, so the mechanism of cofactor reduction is not explained; however, due to the structural



Scheme 43.3 Cofactor reduction using the pentamethylcyclopentadienyl rhodium(bipyridine) complex (**9**/**10**).



11

Fig. 43.4 Pentamethylcyclopentadienyl(1,10-phenanthroline-5,6-dione)chloro rhodium(III) hexafluorophosphate (**11**).

resemblance to the bipyridine complex (see Scheme 43.3), the same mechanism can be assumed.

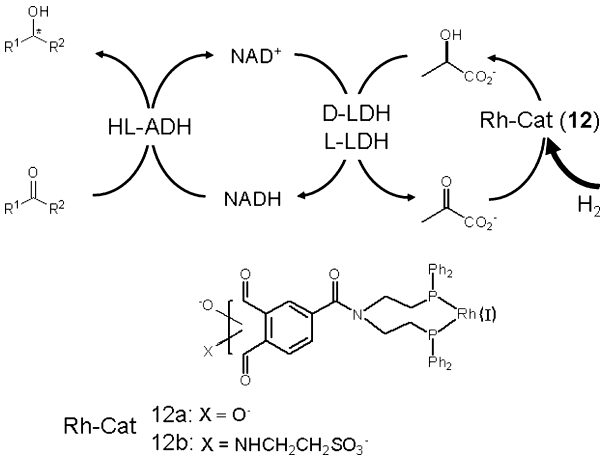
Considering that these two transition-metal complexes are the only ones reported for the electrochemical cofactor reduction, the results are quite promising and show the need for further research in this field to identify new catalysts. In addition to the use of soluble redox mediators in electrochemical cofactor regeneration, modified electrodes have also been used. Details on these systems can also be found in the above-mentioned reviews [31, 32].

43.4
Chemical Cofactor Regeneration

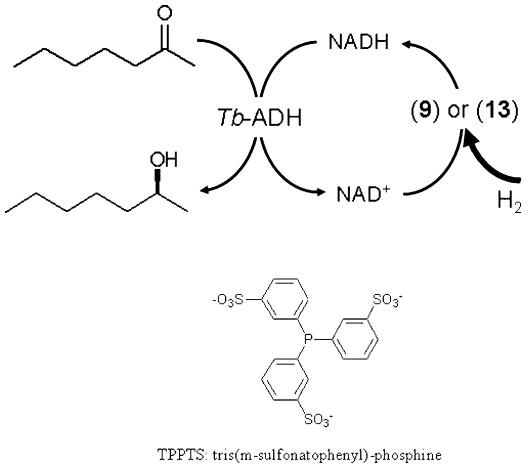
The first example of a chemical cofactor reduction utilizing hydrogen dates back to the 1980s, and is a hybrid approach [45].

Using two types of specially synthesized rhodium-complexes (**12a**/**12b**), pyruvate is chemically hydrogenated to produce racemic lactate. Within the mixture, both a D- and L-specific lactate dehydrogenase (D-/L-LDH) are co-immobilized, which oxidize the lactate back to pyruvate while reducing NAD^+ to NADH (Scheme 43.4). The reduced cofactor is then used by the producing enzyme (ADH from horse liver, HL-ADH), to reduce a ketone to an alcohol. Two examples have been examined. The first example is the reduction of cyclohexanone to cyclohexanol, which proceeded to 100% conversion after 8 days, resulting in total TONs (TTNs) of 1500 for the Rh-complexes **12** and 50 for NAD. The second example concerns the reduction of (±)-2-norbornanone to 72% *endo*-norbornanol (38% ee) and 28% *exo*-norbornanol (>99% ee), which was also completed in 8 days, and resulted in the same TTNs as for the first case.

Besides the electrochemical application, the (Cp*)Rh(bpy)-complex **9** can also be used to reduce cofactors with hydrogen. In a recent study it was compared with ruthenium complex **13** $[RuCl_2(TPPTS)_2]_2$ (TPPTS: tris(*m*-sulfonatophenyl)-phosphine; Scheme 43.5). Both complexes were used to regenerate the cofactors in the reduction of 2-heptanone to (*S*)-2-heptanol, catalyzed by an ADH from *Thermoanaerobium brockii* (*Tb*ADH) [46, 47]. The TON for both catalysts was 18.



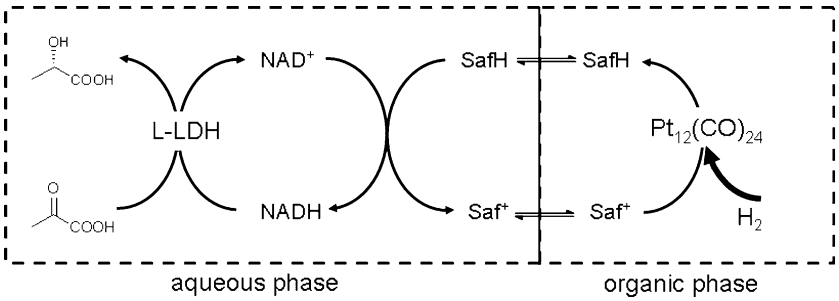
Scheme 43.4 The first hybrid organometallic/enzymatic cofactor regeneration using hydrogen.



Scheme 43.5 Direct hydrogenation of cofactors with transition-metal complexes in an enzymatic synthesis.

A different approach is the combination of a Pt-carbonyl-cluster with a special dye, Safranine O (Saf^+; 3,7-diamino-2,8-dimethyl-5-phenylphenazinium) in an aqueous/organic two-phase system [48]. The dye is reduced in the organic phase and subsequently, in a type of phase-transfer catalysis, it reduced the cofactor in the aqueous phase. In this example L-LDH is used as a production enzyme, reducing pyruvate to L-lactate (Scheme 43.6). Complete conversion was obtained within 48 h, the mixture containing pyruvate, NAD^+ and the Pt-cluster catalyst in a 600 : 10 : 1 molar ratio. The TOF for NAD^+ was 15 h^{-1}.

These three systems are the only ones reported in the literature for achieving cofactor reduction utilizing molecular hydrogen and transition-metal complexes.



Scheme 43.6 Cofactor reduction using a Pt-carbonyl-cluster/dye system.

43.5
Other Chemical Cofactor Regeneration Procedures

Regeneration of the oxidized form of the cofactors, while not within the frame of this chapter, is needed for several biotransformations (e.g., oxidative kinetic resolution of diols). In these procedures, transition-metal complexes have also been applied. For this task, $Ru(phend)_3$ complex and derivatives thereof can be used, either with oxygen or in an electrochemical procedure [49–51].

A number of photochemically or photoelectrochemically activated transition-metal complexes have also been used, both for oxidation and reduction of the nicotinamide cofactors. Among these complexes is the aforementioned Cp*Rh(bpy)-complex **9** [52, 53]. For details of these systems or other regeneration procedures using special dyes, the reader is referred to other reviews on coenzyme regeneration [17, 21–23].

43.6
Conclusions and Outlook

Until now, only a few versatile, selective and effective transition-metal complexes have been applied in nicotinamide cofactor reduction. The TOFs are well within the same order of magnitude for all systems studied, and are within the same range as reported for the hydrogenase enzyme; thus, the catalytic efficiency is comparable. The most versatile complex Cp*Rh(bpy) (**9**) stands out due to its acceptance of NAD^+ and $NADP^+$, acceptance of various redox equivalents (formate, hydrogen and electrons), and its high selectivity towards enzymatically active 1,4-NAD(P)H.

With biocatalysis becoming increasingly accepted in synthetic organic chemistry on both the laboratory and industrial scale, there is a huge need for new complexes that can utilize electrons or hydrogen as redox equivalents in cofactor reduction. These redox equivalents are very inexpensive, readily available, and produce no side products, which in turn significantly facilitates the downstream processing of products.

Nevertheless, today enzymatic regeneration procedures are more conveniently applied. The enzymes are available commercially (e.g., [18]). Although more expensive than hydrogen or electrons, the redox equivalents for the enzymatic regeneration procedures (formic acid, glucose or isopropanol) are – in view of the very high cofactor prices – economically as feasible as hydrogen and electrons, and the side products are volatile (CO_2, acetone) or highly polar (gluconate) and can be easily separated from the desired product of the biotransformation.

Depending on the development of new regeneration systems, the choice of the ideal system might well depend on the specific synthetic application.

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Abbreviations

ADH	alcohol dehydrogenase
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
LDH	lactate dehydrogenase
NAD(P)	nicotinamide adenine dinucleotide (phosphate)
PQQ	pyrroloquinoline quinone
TOF	turnover frequency
TON	turnover number
TTN	total turnover number

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