Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 51 (2008) 57–72
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Review

Electroenzymatic synthesis

Christina Kohlmann, Wolfgang Märkle, Stephan Lütz *

Institute of Biotechnology 2, Research Centre Jülich, 52425 Jülich, Germany

Received 24 May 2007; received in revised form 24 August 2007; accepted 3 October 2007
Available online 10 October 2007

In memoriam Prof. Dr. E. Steckhan (1943–2000).

Abstract

Bioelectrochemistry is a well-established and reliable tool for analytical purposes, such as for the determination of glucose concentration in the blood of diabetes patients or in various other biosensors. The combination of electrochemistry and enzymology can also be used for the synthesis of chiral compounds catalysed by oxidoreductases. In this case, electrons instead of chemical compounds serve as redox equivalents for enzymatic redox reactions. This review will present the state of research for electroenzymatic syntheses and identify the advantages and drawbacks of this method.

Keywords: Cofactors; Electrochemistry; Oxidoreductases; Cofactor regeneration; Biocatalysis

Contents

1. Introduction

The use of enzymes in organic synthesis is very promising [1,2], as enzymes show remarkable chemo-, regio- and stereoselectivities. Moreover, in most cases no side reactions occur and therefore downstream processing can be simplified. About 150 industrial processes are known so far, where enzymes are used in the production of fine chemicals [3].

While using oxidoreductases for synthesis reactions, there is always a need for a source or sink for the electrons involved, i.e. a redox equivalent. This function is fulfilled by freely dissociated cofactors like nicotinamide adenine dinucleotide (NAD(H)) or nicotinamide adenine dinucleotide phosphate (NADP(H)) as well as enzyme-bound coenzymes such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) or heme (Fig. 1). As these cofactors/coenzymes are very expensive, there is need for methods to regenerate the cofactors involved in order to make synthesis with oxidoreductases economically feasible. Moreover, the cofactor in the contrary redox state might be inhibitory so constant adjustment of the right redox state is essential for

* Corresponding author. Tel.: +49 2461 614388; fax: +49 2461 613870.
E-mail address: s.luetz@fz-juelich.de (S. Lütz).

1381-1177/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2007.10.001

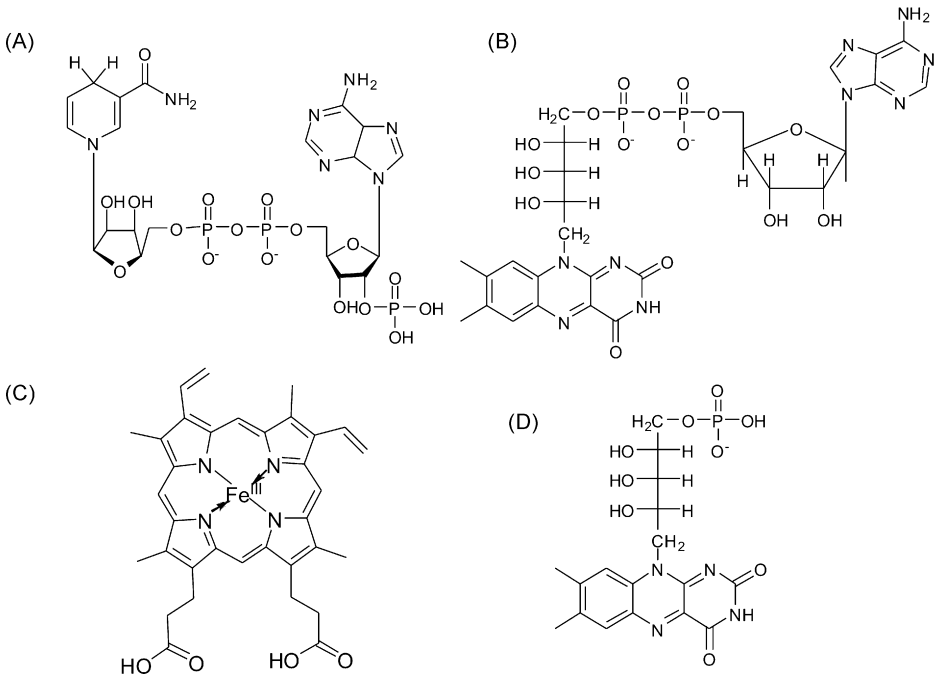

Fig. 1. Cofactors and prosthetic groups (A) NADPH, (B) FAD, (C) heme, (D) FMN.

a successful application of oxidoreductases in organic synthesis.

Several efficient methods have been developed for cofactor recycling [4], they include: enzyme-coupled systems, substrate-coupled-systems [5] and various chemical methods (transition-metal catalyses [6], photochemical, electrochemical) as well as combined methods.

While working with isolated nicotine-amide-dependent enzymes, different methods have been developed for cofactor regeneration. In enzyme-coupled regeneration methods, a second enzyme is applied that converts a cosubstrate to a coproduct while converting the cofactor back into the desired form [7].

Another approach uses the enzyme applied in the synthesis reaction for the regeneration reaction, too. This method is called substrate-coupled cofactor regeneration. Problems may occur if the coproduct formed is harmful for the applied biocatalyst and engineering concepts therefore have to be developed to overcome this problem. Again downstream processing can be more difficult when a cosubstrate and a coproduct have to be separated.

If flavin-dependent enzymes are used, regeneration can simply be done with oxygen. Unfortunately, hydrogen peroxide is formed during this reaction, a substance which is known to inactivate various enzymes. The hydrogen peroxide formed can be destroyed by applying catalase, but then again a second enzyme is required.

Electrochemistry in particular is a promising tool for the regeneration of the different cofactors and coenzymes. The combination of isolated enzymes with electrochemical reactions is well established in biosensor techniques for analytical purposes. In electrochemical biosensors, the biocatalyst is usually bound onto the electrode surface [8–10]. As this arrangement is useful for analytical applications, it is in most cases lacking in long-term stability for synthetic applications and the current density is low. Only few examples therefore exist where electrochemistry and enzyme catalysis are used in preparative synthesis and the majority of these examples deal with indirect electron transfer via a mediator. However, several advantages of electrochemical cofactor regeneration can be identified: the supply of redox equivalents is basically mass-free since only electron transfer reactions are involved. No cosubstrate is required and, therefore, no coproduct is formed. The use of a second enzyme can be avoided. Furthermore, electrons are among the cheapest redox equivalents available [11].

Some drawbacks have to be considered nevertheless: Specially designed bioreactors (i.e. electrochemical cells) are required for the synthesis reactions. Additionally, expertise in both biotechnology and electrochemistry is needed to establish these systems.

The following review deals with enzymatic synthesis reactions where the cofactors or coenzymes involved are regenerated electrochemically or the required cosubstrates are generated electrochemically. Since this type of research is published in a variety of journals covering enzymology, electrochemistry and organic synthesis, it is sometimes difficult to obtain all the experimental details. Wherever possible in this review we try to use standardised units to overcome this problem and make a comparison of procedures easier.

2. Electroenzymatic synthesis reactions

2.1. NAD(P)$^+$-dependent oxidation reactions

Syntheses where the cofactor NAD(P)$^+$ has to be regenerated to its oxidised state can be carried out with direct, indirect and enzyme-coupled electrochemical cofactor regeneration (Fig. 2). Direct regeneration means that the species to be regenerated

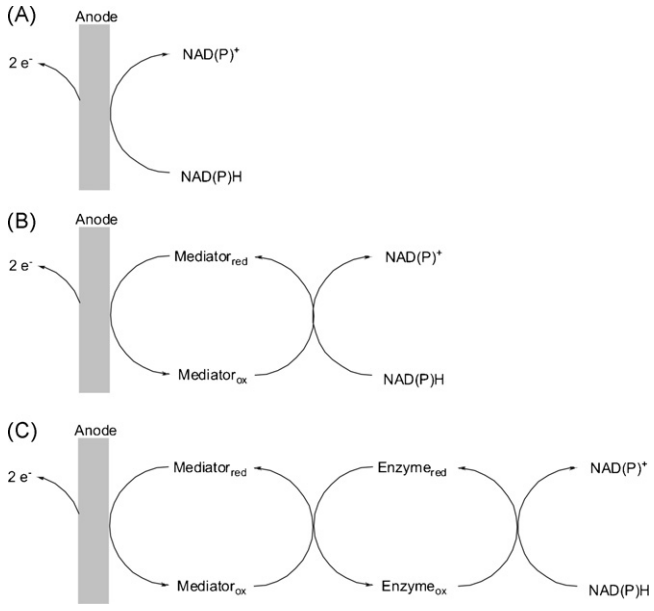

Fig. 2. $NAD(P)^+$-dependent oxidation reactions: (A) direct electrochemical regeneration, (B) indirect electrochemical regeneration, (C) enzyme-coupled electrochemical regeneration.

itself reacts at the electrode. Indirect regeneration means that another substance (a so-called mediator, Fig. 3) acts as an electron shuttle between the electrode and the cofactor used. If a second enzyme is used as the electron shuttle the process is called enzyme-coupled electrochemical cofactor regeneration. For the regeneration of $NAD(P)^+$ directly at an anode, high overpotentials of at least 900 mV vs. the saturated calomel electrode (SCE) [12] are required. Therefore, this method can only be used for reactions with substrates that are oxidation-stable to avoid side reactions.

One example of the successful application of the direct regeneration of NAD^+ is the synthesis of D-gluconolactone from D-glucose by glucose dehydrogenase (Table 1, process 1) [13]. In a plug flow reactor, the working and auxiliary compartments were separated by a membrane. With the setup used in this experiment, oxidation of NADH at an imposed potential lower than 0 mV vs. SCE was reported. A product formation of 13 g/L was possible at a potential of 700 mV vs. SCE.

To avoid high overpotentials, various chemical redox agents can be used as electron carriers. Steckhan and coworkers investigated different types of phenanthrolinedione complexes for this purpose. *meso*-5,6-Dihydroxy-methyl-7-oxabicyclo [2.2.1]hept-2-ene was synthesised from *meso*-5,6-dihydroxy-methyl-7-oxabicyclo[2.2.1]hept-2-ene by an alcohol dehydrogenase (ADH) using 1 μmol of *N*-methyl-1,10-phenanthrolinium-5,6-dione tetrafluoroborate ($PDMe(BF_4)_2$) as mediator leading to 74% conversion and 30 turnovers per hour for the mediator (Table 1, process 2) [14].

A ruthenium complex of phenanthrolinedione ($Ru(PD)_3(ClO_4)_2$) was applied in the conversion of cyclohexanol to cyclohexanone. With 25 U of alcohol dehydrogenase from horse liver (HLADH), 0.5 mM NAD^+, 0.1 mM mediator, 10 mM substrate and a potential of 100 mV vs. Ag|AgCl 75 % conversion was achieved (Table 1, process 3) [15]. The mediators $PDMe^{2+}$ and $Ru(PD)_3{}^{2+}$ were also used in the synthesis of tetrahydro-3H-isobenzofuranone (Table 1, process 4) [14,16] leading to a turnover number of $35\,h^{-1}$ for the mediator.

Another phenantroline complex suitable for the regeneration of NAD^+ is iron 3,4,7,8-tetramethyl phenantroline ($Fe(tmphen)_3$). Its efficiency was investigated in the synthesis of 2-hexenal by ADH from yeast (Table 1; process 5) [17] and 2-butenal by *Thermoanaerobium brockii* ADH (Table 1,

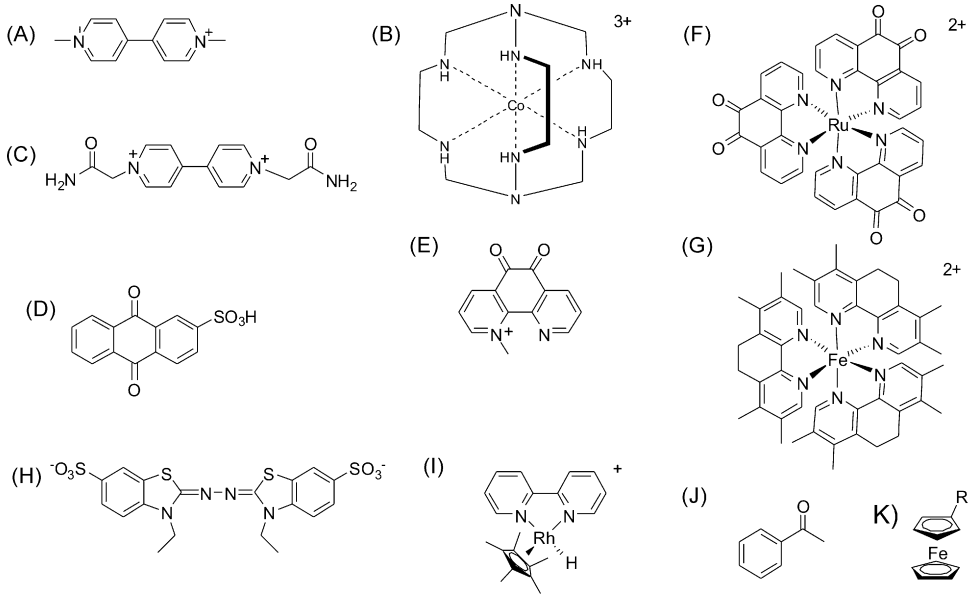

Fig. 3. Substances used as electron shuttles in electroenzymatic synthesis: (A) methyl viologen MV, (B) cobalt sepulchrate CoSep, (C) carboxamidomethyl viologen CAV, (D) anthraquinone 2-sulfonate AQ-2-S, (E) *N*-methyl-1,10-phenanthroline-5,6-dione, (F) tris(phenanthroline-5,6-dione)Ru(II), (G) tris(3,4,7,8-phenanthroline)Fe(II) $Fe(tmphen)_3$, (H) 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonate ABTS, (I) (2,2′-bipyridyl)(pentamethylcyclopentadienyl) rhodium complex, (J) acetophenone, (K) ferrocene derivatives.

Table 1
Synthesis reactions with electrochemical regeneration of $NAD(P)^+$

Process	Enzyme	Substrate	Product	Cofactor	Mediator	Results	Literature
1	Glucose dehydrogenase (GDH, E.C. 1.1.99.10)	D-Glucose	D-Glucono-δ-lactone	NAD^+/H	None	13 g L^{-1},$ttn_{cofactor}$ >10,000	[13]
2	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	*meso*-5,6-Dihydroxy-methyl-7-oxabicyclo [2.2.1]hept-2-ene	4,10-Dioxa-tricyclo[5.2.1.02,6]dec-8-en-3-one	NAD^+/H	PDMe $(BF_4)_2$	Conversion 74%, $tof_{mediator}$ 30 h^{-1}	[14]
3	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	Cyclohexanol	Cyclohexanone	NAD^+/H	$Ru(PD)_3(ClO_4^-)_2$	Conversion 75%	[15]
4	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	*meso*-3,4-Dihydroxy-methylcyclohexene	3a,4,7,7a-Tetrahydro-3H-isobenzofuran-1-one	NAD^+/H	PDMe $(BF_4)_2$	Conversion 99.5%, $tof_{mediator}$ 35 h^{-1}	[14,16]
5	Alcohol dehydrogenase from yeast (YADH, E.C. 1.1.1.1)	2-Hexen-1-ol	2-Hexenal	NAD^+/H	$Fe(tmphen)_3$	1.77 mM, $ttn_{cofactor}$ 18, $ttn_{mediator}$ 36, current efficiency 90	[17]
6	Alcohol dehydrogenase from *Thermoanaerobium brockii* (TBADH, E.C. 1.1.1.1)	2-Butanol	2-Butanon	$NADP^+$/H	$Fe(tmphen)_3$	4.1 mM, $ttn_{cofactor}$ 41, $ttn_{mediator}$ 82, current efficiency 95%	[17]
7	Glycerol dehydrogenase from *Cellulomonas* sp. (GDH, E.C. 1.1.1.6)	*rac*-Phenylethane-1,2-diol	(*S*)-Phenylethane-1,2-diol	NAD^+/H	ABTS		[18]
8	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	*meso*-3,4-Dihydroxy-methylcyclohexene	3a,4,7,7a-Tetrahydro-3H-isobenzofurane-1-on	NAD^+/H	ABTS	Yield 93.5%, e.e. >99.5	[19]
9	Glucose dehydrogenase (GDH, E.C. 1.1.1.47)	D-Glucose	Gluconic acid	NAD^+/H	3,4-Dihydroxy-benzaldehyde	$ttn_{cofactor}$ 100	[20]
10	6-Phosphogluconate dehydrogenase from pig heart (E.C. 1.1.1.44)	6-Phosphogluconate	Ribulose 5-phosphate	$NADP^+$/H	CAV, AMAPOR	Conversion 98% in 2.5 h	[21]
11	Isocitrate dehydrogenase from pig heart (E.C. 1.1.1.41)	(2*R*,3*S*)-Isocitrate	2-Oxoglutarate	$NADP^+$/H	CAV, AMAPOR	13,000 mmol kg^{-1} h^{-1} after 3.8 h (e.e. >99%)	[21]
12	Isocitrate dehydrogenase from pig heart (E.C. 1.1.1.41)	(2*R*,3*S*)-Isocitrate	2-Oxoglutarate	$NADP^+$/H	A-Q-2-S, AMAPOR	14,000 mmol kg^{-1} h^{-1} after 3.6 h (e.e. >99%)	[21]
13	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	Cyclohexanol	Cyclohexanone	NAD^+/H	Pd derivative		[22]
14	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1.)	Cyclohexanol	Cyclohexanone	NAD^+/H	Ferrocene + diaphorase		[23]

process 6) [17]. In divided cell reactors with a potential of 0.63 V vs. Ag|AgCl current efficiencies of ∼90% and higher were achieved. For the synthesis of 2-hexenal, the total turnover number for the cofactor was 18 and for the mediator 36. In the synthesis of 2-butenal, total turnover numbers of 41 for NAD^+ and 82 for $Fe(tmphen)_3$ were achieved.

The mediator 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonate (ABTS) was used in the synthesis of (*S*)-phenylethane-1,2-diol by glycerol dehydrogenase (Table 1, process 7) [18] and tetrahydro-3H-isobenzofuranone by HLADH (Table 1, process 8) [19]. The oxidative resolution of racemic phenylethane-1,2-diol was carried out in an electrochemical membrane reactor yielding enantiopure (*S*)-diol, whereas the synthesis of tetrahydro-3H-isobenzofuranone was performed in a quasi-divided electrochemical cell leading to 93.5% conversion with an enantiomeric excess (e.e.) >99.5%.

A reactor with glucose dehydrogenase and 3,4-dihydroxybenzaldehyde as mediator immobilised on a carbon felt electrode for the synthesis of gluconic acid was reported by Manjon et al. (Table 1, process 9) [20]. A divided cell reactor was used and operated at 0.7 V vs. Ag|AgCl and 0.2 V vs. Ag|AgCl. As expected, higher potentials led to faster glucose consumption, nevertheless total turnover numbers of about 100 for the cofactor resulted from both experiments.

It is also possible to combine indirect regeneration of $NAD(P)^+$ with an enzymatic regeneration step. For example, the oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate with 6-phosphogluconate dehydrogenase was carried out using the mediator carboxamidomethyl viologen (CAV) in combination with an artificial mediator accepting pyridine nucleotide oxidoreductase (AMAPOR) (Table 1, process 10) [21]. In a reaction containing 20 mM substrate, 3 mM CAV, 0.5 mM $NADP^+$, 15 U of 6-phosphogluconate-dehydrogenase and 20 U AMAPOR a yield of 98% was obtained in 2.5 h. The applied potential was −200 mV vs. SCE.

The synthesis of (*2R*,*3S*)-isocitrate was carried out by the resolution of isocitrate racemates via decarboxylation of (2*S*,3*R*)-isocitrate to 2-oxoglutarate by an isocitrate dehydrogenase. The required cofactor $NADP^+$ was regenerated by AMAPOR and either CAV (Table 1, process 11) [21] or anthraquinone 2-sulphonate AQ-2-S (Table 1, process 12) [21]. Both mediators lead to comparable results; for CAV space time yields of 13,000 mmol product per kg catalyst and hour and for AQ-2-S space time yields of 1400 mmol product/kg catalyst and hour were obtained. For substrate concentrations of up to 400 mM of the racemate, full conversion of (2*S*,3*R*)-isocitrate was achieved leading to an e.e. of higher than 99% for (*2R*,*3S*)-isocitrate.

The conversion of cyclohexanol into cyclohexanone by *horse liver* ADH was combined to a regeneration approach using quinone and diaphorase (Table 1, process 13) [22]. Full conversion of the substrate was achieved in a setup containing 2 mmol cyclohexanol, 0.1 mM NAD^+, 0.5 μmol HLADH, 0.1 mM mediator and 10 U mL^{-1} of diaphorase. The electrolysis was carried out using glassy carbon working electrodes and a potential of −100 mV vs. SCE.

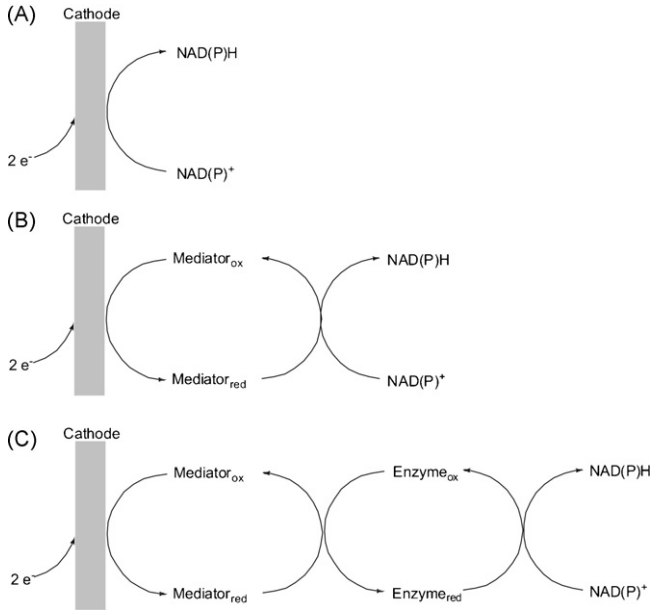

Fig. 4. NAD(P)H-dependent reduction reactions, (A) direct electrochemical regeneration, (B) indirect electrochemical regeneration, (C) enzyme-coupled electrochemical regeneration.

Oxidation of cyclohexanol to cyclohexanone was also performed by using a ferrocene/diaphorase/HLADH immobilised electrode (Table 1, process 14) [23]. For electrode preparation aminoferrocene and 2-aminoethylferrocene were bound to a graphite felt electrode coated with a thin polyacrylic acid film. In the next step, the electrode was treated with diaphorase and finally with alcohol dehydrogenase. After this preparation, the electrode was successfully applied for the oxidation of NADH to NAD^+.

2.2. NAD(P)H-dependent reduction reactions

Theoretically direct, indirect and indirect enzyme coupled processes are possible for the electrochemical regeneration of NAD(P)H (Fig. 4). The direct reduction of $NAD(P)^+$ is a two-step reaction; for the first step a potential of ∼−1.2 V vs. SCE is necessary to transfer one electron to $NAD(P)^+$ forming a radical species [24]. Afterwards this radical can be protonated and a second electron can be transferred (potential values vary from ∼−1.7 V to −2.0 V vs. SCE) [24–27]. However, the radicals formed in the first reduction step can dimerise leading to enzymatically inactive side products. Moreover, protonation is not selective; besides the desired 1,4-NAD(P)H, enzymatically inactive 1,6-NAD(P)H is likely to occur. After a few regeneration cycles no enzyme-active 1,4-NAD(P)H is left, so that direct regeneration is not normally used in practice.

In two examples, direct regeneration of NADH is enabled by using very small amounts of the cofactor. It is assumed that with low concentrations of the cofactor the dimerisation reactions are negligible. One example is the reduction of pyruvate to D-lactate by D-lactate dehydrogenase (Table 2, process 15) [28]. Cofactor regeneration takes place at a cholesterol-modified gold amalgam electrode. With this set-up a turnover number

Table 2
Synthesis reactions with electrochemical regeneration of NAD(P)H

Process	Enzyme	Substrate	Product	Cofactor	Mediator	Results	Literature
15	D-Lactate dehydrogenase (D-LDH, E.C. 1.1.1.28)	Pyruvate	D-Lactate	NAD^+/H	None	Conversion 72%, 18.2 mM in 21 h, $ttn_{cofactor}$ 1400	[28]
16	L-Glutamate dehydrogenase from bovine liver (GDH, E.C. 1.4.1.3)	α-Ketoglutarate	L-Glutamate	NAD^+/H	None	Conversion 100%, $ttn_{cofactor}$ 3300	[29]
17	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	Cyclohexanone	Cyclohexanol	NAD^+/H	$[Rh(bpy)_3]^{2+}$	Conversion ~26%, $ttn_{cofactor}$ 2.9, $ttn_{mediator}$ 1.2	[30]
18	D-Lactate dehydrogenase from *Staphylococcus epidermidis* (D-LDH, E.C. 1.1.1.28)	Pyruvate	D-Lactate	NAD^+/H	$[Cp^*Rh(bpy)Cl]^+$	14 mM, e.e. 93.5%, $ttn_{cofactor}$ 14, $ttn_{mediator}$ 7, current efficiency 67%	[31]
19	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	4-Phenyl-2-butanone	(*S*)-4-Phenyl-2-butanol	NAD^+/H	Cp*Rh(bpy)L	Conversion 70%, e.e. 65% (*S*-product)	[12]
20	Alcohol dehydrogenase from *Rhodococcus* sp. (S-ADH, E.C. 1.1.1.2)	4-Phenyl-2-butanone	(*S*)-4-Phenyl-2-butanol	NAD^+/H	Cp*Rh(bpy)L	Conversion 76%, e.e. 77% (*S*-product)	[12]
21	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	Cyclohexanone	Cyclohexanol	NAD^+/H	Cp*Rh(bpy)L	Conversion 92 % in 42 days	[32]
22	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	Cyclohexanone	Cyclohexanol	NAD^+/H	Cp*Rh(bpy)L	Conversion 100 % in 3 days	[32,33]
23	Alcohol dehydrogenase from *Lactobacillus brevis* (LbADH, E.C. 1.1.1.1)	Acetophenone	(*R*)-Phenylethanol	$NADP^+$/H	Cp*Rh(bpy)L	Conversion 98%, e.e. >99.9%, 13.5 g L^{-1} d^{-1}, ttn_{enzyme} 75,000, $ttn_{cofactor}$ 35, $ttn_{mediator}$ 35, current efficiency 55%	[34]
24	Alcohol dehydrogenase from *Lactobacillus brevis* (LbADH, E.C. 1.1.1.1)	Acetophenone	(*R*)-Phenylethanol	$NADP^+$/H	Cp*Rh(bpy)L	Conversion 93%, e.e. >98%, 9.0 g L^{-1} d^{-1}, ttn_{enzyme} 21,000 $ttn_{cofactor}$ 12, $ttn_{mediator}$ 55, current efficiency 93%	[34]
25	Alcohol dehydrogenase from *Lactobacillus brevis* (LbADH, E.C. 1.1.1.1)	Acetophenone	(*R*)-Phenylethanol	$NADP^+$/H	Cp*Rh(bpy)L	Conversion 98%, e.e. >99.9%, 2.6 g L^{-1} d^{-1}, ttn_{enzyme} 5000, $ttn_{cofactor}$ 64, $ttn_{mediator}$ 64 current efficiency 45%	[34]
26	Alcohol dehydrogenase (ADH, E.C. 1.1.1.1)	Cyclohexanone	Cyclohexanol	NAD^+/H	MV^{2+}, diaphorase	Conversion 100%, current efficiency 97.8	[36]
27	Alcohol dehydrogenase (ADH, E.C. 1.1.1.1)	2-Methyl-cyclohexanone	(1*S*,2*S*)-(+)-2-Methylcyclohexanol	NAD^+/H	MV^{2+}, diaphorase	Conversion 48.9%, e.e. >99%, $ttn_{mediator}$ 91, current efficiency 98.6%	[36]
28	Alcohol dehydrogenase (ADH, E.C. 1.1.1.1)	3-Methyl-cyclohexanone	(1*S*,3*S*)-(−)-2-Methylcyclohexanol	NAD^+/H	MV^{2+}, diaphorase	Conversion 51.7%, e.e. 93.1%, $ttn_{mediator}$ 94, current efficiency 96.5%	[36]
29	D-Lactate dehydrogenase from *Leuconostoc mesenteroides* (D-LDH, E.C. 1.1.1.28)	Sodium pyruvate	D-Lactate	NAD^+/H	MV^{2+}, diaphorase	Conversion 80%	[37]
30	Benzoylformate dehydrogenase from *Enterococcus faecalis* (BFR)	Benzoylformate	(*R*)-Mandelate	NAD^+/H	MV^{2+}, diaphorase	40 mM in 30 h, $ttn_{cofactor}$ 133	[38]

31	Benzoylformate dehydrogenase from *Enterococcus faecalis* (BFR)	Benzoylformate	(*R*)-Mandelate	NAD^+/H	FAD + LipDH	47.5 mM in 18 h, $ttn_{cofactor}$ 158	[38]
32	D-Lactate dehydrogenase (D-LDH, E.C. 1.1.1.28)	Pyruvate	D-Lactate	NAD^+/H	MV^{2+} + LipDH	Conversion 94% in 9 days, e.e. 94%, ttn_{enzyme} 3.5×10^7, $ttn_{cofactor}$ 940, ttn_{LipDH} 5.4×10^5, current efficiency $104 \pm 10\%$	[39]
33	Alcohol dehydrogenase from horse liver (HLADH, E.C. 1.1.1.1)	Cyclohexanone	Cyclohexanol	NAD^+/H	MV^{2+} + LipDH	Conversion 65%	[33]
34	D-Lactate dehydrogenase (D-LDH, E.C. 1.1.1.29)	Pyruvate	D-Lactate	NAD^+/H	MV^{2+} + FDR	Conversion 90% in 14 days, e.e. 94%, ttn_{enzyme} 2.2×10^7, $ttn_{cofactor}$ 900, ttn_{FDR} 7.3×10^6, current efficiency $103 \pm 10\%$	[39]
35	Glutamate dehydrogenase (GluDH, E.C. 1.4.1.3)	α-Ketoglutarate	L-Glutamate	$NADP^+$/H	MV^{2+} + FDR	Conversion 100% in 7 days, ttn_{enzyme} 1.1×10^7, $ttn_{cofactor}$ 1000, ttn_{FDR} 7.5×10^6, current efficiency $105 \pm 10\%$	[39]
36	Glutamate dehydrogenase from beef liver (GluDH, E.C. 1.4.1.3)	Oxoglutarate	(*S*)-Glutamate	$NADP^+$/H	MV^{2+}, AMAPOR	29 mol kg^{-1} h^{-1}, $ttn_{cofactor}$ 29,000	[21]
37	Glutamate dehydrogenase from beef liver (GluDH, E.C. 1.4.1.3)	Oxoglutarate	(*S*)-Glutamate	NAD^+/H	MV^{2+}, AMAPOR	7 mol kg^{-1} h^{-1}, $ttn_{cofactor}$ 29,000	[21]
38	Glutamate dehydrogenase from beef liver (GluDH E.C. 1.4.1.3)	Oxoglutarate	(*S*)-Glutamate	$NADP^+$/H	CoSep, AMAPOR	1 mol kg^{-1} h^{-1}	[21]
39	Alcohol dehydrogenase from *Thermoanaerobium brockii* or equine liver (ADH, E.C. 1.1.1.1 or E.C. 1.1.1.2)	Various ketones and alcohols	Alcohols	$NADP^+$/H or NAD^+/H	Acetophenone		[40]
40	Alcohol dehydrogenase from *Thermoanaerobium brockii* or equine liver (ADH, E.C. 1.1.1.1 or E.C. 1.1.1.2)	Various ketones and alcohols	Alcohols	$NADP^+$/H or NAD^+/H	MV^{2+} + FNR or MV^{2+} + diaphorase		[40]
41	L-Glutamate dehydrogenase from bovine liver (GDH, E.C. 1.4.1.2)	α-Ketoglutarate	L-Glutamate	NAD^+/H	Hydrogenase		[41]

for NADH of ~1400 and a conversion of ~72% was achieved. The other example deals with the synthesis of L-glutamate from α-ketoglutarate by a L-glutamate dehydrogenase (Table 2, process 16) [29]. Conductive vanadium-silica gels were added to the reaction medium to increase the conductivity of the reaction medium. Complete conversion of the substrate and a total turnover number (ttn) of 3300 for the cofactor were obtained (ttn = amount of product divided by amount of catalyst or else the mediator or cofactor).

To overcome the problems of direct electrochemical regeneration of NAD(P)H a mediator meeting the following criteria has to be applied [12]:

1. The mediator must transfer two electrons or one hydride ion in one step.
2. The electrochemical activation of the mediator must be possible at potentials less negative than −0.9 V vs. SCE (at more negative potentials the direct reduction of $NAD(P)^+$ takes place).
3. The mediator must not transfer the electrons to the substrate.
4. Only enzymatically active 1,4-NAD(P)H must be formed.

One of the first substances that met all four requirements was the (2,2′-bipyridyl)rhodium complexe (Rh(bpy)). One example where this mediator is used to regenerate NADH is the reduction of cyclohexanone to cyclohexanol catalysed by an alcohol dehydrogenase (Table 2, process 17) [30]. 0.25 mmol of $[Rh(bpy)_3]^{2+}$, 0.1 mmol of NAD^+ and 1.12 mmol cyclohexanol were dissolved in a tris(hydroxymethyl)aminomethane (TRIS) buffer (pH 9.0). With this system, 2.9 cycles were achieved for NAD regeneration and 1.2 cycles for mediator regeneration. The small number of regeneration cycles is thought to result from a passivation of the cathode, caused by a layer of $[Rh(bpy_2)(H_2O)_2]Cl$ or $[Rh(bpy_2)(OH)_2]Cl$ formed on the electrode surface.

The next generation of rhodium mediators was developed a few years later. It was possible to improve the performance of the mediators by using the pentamethycyclopentadienyl (Cp*) as ligand. High regioselectivity is characteristic for this type of mediator; 1,4-NAD(P)H is formed with a selectivity of >99 %. The first synthetic application was the conversion of pyruvate to D-lactate by D-lactate dehydrogenase (Table 2, process 18) [31]. A solution of 1 mM $[Cp(Me)_5Rh(bpy)Cl]Cl$, 2 mM NAD^+, 20 mM substrate and 1300 U enzyme was electrolysed at −0.6 V vs. Ag|AgCl yielding 14 mM D-lactate (e.e. 93.5%) and turnover numbers of 14 for the mediator and 7 for the cofactor.

This type of mediator has found widespread applications. Steckhan et al. investigated the reduction of 4-phenyl-2-butanone to (*S*)-4-phenyl-2-butanol by horse liver alcohol dehydrogenase (Table 2, process 19) [12] and by an alcohol dehydrogenase from *Rhodococcus* sp. (Table 2, process 20) [12] As mediator Cp*Rh(4-ethoxy-methyl-2,2′-bpy)L and several types of water-soluble polymer-bound versions of the mediator were used and applied in batch reactors as well as in an electrochemical enzyme membrane reactor.

Bergel and co-workers applied the mediator $[Cp(Me)_5$ Rh(bpy)Cl]Cl in a dialysis membrane electrochemical reactor (D-MER, with a dialysis membrane consisting of regenerated cellulose) (Table 2, process 21) [32] and in an ultrafiltration dialysis membrane electrochemical reactor (UF-MER, with a ultrafiltration membrane made of cellulose) (Table 2, process 22) [32,33], to keep the catalyst close to the carbon working electrode. By using these two different reactors and 100 mM cyclohexanone as substrate a conversion of 92% in 42 days, or 100% in 3 days, was achieved. For the D-MER setup, 0.5 mM rhodium complex, 1 mM NAD^+ and 23 U of HLADH were applied and in the UF-MER approach 1 mM Rh complex, 1 mM NAD^+ and 73 U of HLADH.

Recently, Hildebrand et al. reported the synthesis of (*R*)-phenylethanol catalysed by the alcohol dehydrogenase from *Lactobacillus brevis* with regeneration of NADPH by Cp*Rh(bpy) (Table 2, process 23)[34]. Excellent e.e.'s of over 99.9% as well as high volumetric productivities of 14 g L^{-1} d^{-1} were reported by employing a 200 mL batch reactor with a carbon felt as the working electrode, a platinum grid in a dialysis sack as the counter electrode and a reference electrode of Ag|AgCl. Enzyme stabilisation by the addition of bovine serum albumin yielded total conversion and total turnover numbers of ttn (ADH) = 75,000. Cofactor and mediator showed total turnover numbers of ttn = 35.

The application of immobilised enzyme [35] to the same reactor type led to higher turnover numbers of the mediator (ttn = 55) while showing comparable productivity (Table 2, process 24) [34]. Furthermore, the current efficiency was slightly improved. Once again, high enantiomeric excesses were reported (e.e. = 98%).

The first cofactor regeneration with Cp*Rh(bpy) in an aqueous-organic two-phase system was also reported by Hildebrand et al. (Table 2, process 25) [34]. In a reaction setup similar to the one described above, methyl *tert*-butyl ether was added as the organic phase. In this way, the mediator and cofactor were decoupled from the substrate, resulting in higher turnover numbers (ttn = 64). A product solution of 180 mM (*R*)-phenylethanol with an e.e. > 99.9% was obtained, thus clearly indicating the potential of combining the concept of two-phase systems with electroenzymatic synthesis.

As it is very difficult to find an electrochemical redox catalyst that fulfils all requirements for regenerating NAD(P)H effectively, attempts have been made to regenerate the cofactor indirectly by coupling with a second enzymatic reaction. While this alleviates the problem of dimer formation it loses the potential advantage associated with electroenzymatic synthesis of having no need for a second regenerating enzyme. Several examples can be found where methyl viologen (MV^{2+}) in combination with diaphorase, lipoamide dehydrogenase (LipDH), ferredoxin $NADP^+$ reductase (FNR) or so-called AMAPORS acts as electron shuttle to regenerate NAD(P)H.

Regeneration of NADH with MV^{2+} together with diaphorase was used in the alcohol dehydrogenase catalysed reduction of cyclohexanone (Table 2, process 26) [36], 2–methylcyclohexanone (Table 2, process 27) [36] and 3–methylcyclohexanone (Table 2, process 28) [36]. MV^{2+}, diaphorase, NAD^+ and ADH were immobilised on the surface of the electrode. During the electrolysis a potential of −0.8 V

vs. SCE was applied. Under these conditions a yield of >99% and a current efficiency of 97.6% were obtained for the synthesis of cyclohexanol. For the synthesis of (1*S*,2*S*)-(+)-2-methylcyclohexanol, a yield of 48.9% was achieved with an e.e. of > 99%, a turnover of 91 for MV^{2+} and a current efficiency of 98.6%. For (1*S*,3*S*)-(−)-2-methylcyclohexanol, a yield of 51.7% with an e.e. of >93.1%, a turnover of 94 for MV^{2+} and a current efficiency of 96.5% was obtained.

The regeneration system MV^{2+}/diaphorase is also used in combination with NAD^+ and D-lactate dehydrogenase (Table 2, process 29) [37]. In this reaction, pyruvate is reduced to D-lactate. By adding 1.5 U mL^{-1} diaphorase, 0.2 mM MV^{2+} and 0.3 mM NAD^+ as well as 50 mM pyruvate to the solution and performing electrolysis at −0.7 V vs Ag|AgCl, it was possible to achieve a conversion of roughly 80%.

Also the synthesis of (*R*)-mandelic acid from benzoylformate catalysed by benzoylformate dehydrogenase was investigated with regeneration by MV^{2+} and diaphorase (Table 2, process 30) [38] as well as with FAD and lipoamide dehydrogenase (LipDH, Table 2, process 31) [38]. Regeneration with MV^{2+} and diaphorase led to a conversion of 80% in 30 h and the total turnover number for NAD^+ was 133. The FAD-LipDH system provided much better results; with this regenerating system a conversion of 95% in 18 h and a turnover number of 158 for the cofactor was obtained.

The combination of MV^{2+} with LipDH was used for the synthesis of D-lactate by D-lactate dehydrogenase (Table 2, process 32) [39]. At a tungsten electrode, a potential of −0.72 V vs. SCE was applied. In a batch experiment, a conversion of 81% was measured with an e.e. of 94% and a turnover number of 940 for NAD.

The mediator MV^{2+} together with LipDH has also been tested in a continuous process. Bergel et al. applied this regeneration system in their D-MER together with an ADH for the synthesis of cyclohexanol from cyclohexanone (Table 2, process 33) [33]. Electrolysis was carried out at −0.7 V vs. SCE. During this synthesis the enzyme reaction between NAD^+ and the mediator seemed to be the rate-limiting step as the conversion could be increased from 26% to 65% with the further addition of LipDH.

Together with ferredoxin reductase (FDR), MV^{2+} was used for the synthesis of D-lactate from pyruvate by D-lactate dehydrogenase (Table 2, process 34) [39]. Turnover numbers of 900 for NADH, 2.2×10^7 for D-lactate dehydrogenase and 7.3×10^6 for FNR were achieved. The application of the same regeneration system for the production of L-glutamate from α-ketoglutarate by L-glutamate dehydrogenase (Table 2, process 35) [39] led to turnover numbers of 1000 for NADPH, 1.1×10^7 for L-glutamate dehydrogenase and 7.5×10^6 for FNR. Also complete conversion was obtained.

AMAPORs were used in combination with MV^{2+} (Table 2, processes 36 and 37) [21] or alternatively with cobalt sepulchrate (CoSep) for the synthesis of (*S*)-glutamate (Table 2, process 38) [21]. In TRIS buffer (pH 7.3) 100 mM 2-oxoglutarate, 250 mM ammonium acetate, 3 mM methyl viologen and 36 U of glutamate dehydrogenase were dissolved and electrolysed at a potential of −729 mV vs. SCE. 29 mol product $kg_{catalyst}^{-1}\,h^{-1}$ was produced with 0.5 mM $NADP^+$ and 50 mg wet cells of *C. thermoalceticum*, whereas for 0.5 mM NAD^+ and 100 mg of wet cells a productivity of 7 mol product $kg_{catalyst}^{-1}\,h^{-1}$ was obtained. Conversion was between 95% and 99% in various experiments. Using CoSep instead of MV^{2+} the performance dropped to only 3.5% of the productivity reached before so that the use of MV^{2+} seems to be beneficial.

As acetophenone can be reduced directly to the racemic mixture of the corresponding alcohol at a potential of ∼−0.8 V vs. Ag|AgCl, it can be used for indirect enzyme-coupled NADPH regeneration without adding a second enzyme. The alcohol formed can be oxidised by the ADH; consuming $NAD(P)^+$ and therefore leading to NADPH. This approach has been tested in the synthesis of different alcohols leading to e.e. values of >99%, but only with a very small conversion of around 10% (Table 2, process 39) [40]. The same substrates have also been used for synthesis reactions with MV^{2+} and ferredoxine reductase or diaphorase (Table 2, process 40) [40]. Conversion was doubled by these regeneration systems and the current efficiencies were increased.

Hydrogenase from *Alcaligenes eutrophus* was used to regenerate NADH in the reduction of α-ketoglutarate to L-glutamate by L-glutamate dehydrogenase (Table 2, process 41)[41]. In a 5 mL cylindrical divided cell, electrolysis was carried out at a potential of −0.7 V vs. SCE. The working and auxiliary electrodes were made of platinum. By using this setup, it was possible to achieve complete conversion of 300 mM substrate with a turnover frequency of ∼200 h^{-1}.

2.3. Oxidative regeneration in flavin-dependent oxidations

In enzymatic oxidations with flavo enzymes, the cofactor flavin adenine dinucleotide can be regenerated by molecular oxygen. The main drawback of this method is the formation of hydrogen peroxide, which may drastically decrease enzyme activity and stability [42]. An approach which can be used to overcome this problem is the addition of catalase to remove the hydrogen peroxide [43,44]. Electrochemistry provides an elegant anaerobic method for cofactor regeneration without using catalase. However, the large size of the biomolecules and, in most cases, the location of the active site of the enzyme deep inside the protein layer hampers direct electron transfer [45]. Thus, for an effective electron transfer from the FAD to the electrode, it is necessary to use mediators as electron shuttles.

Ferrocene derivatives are often used as mediators for the oxidative regeneration of FAD-dependent oxidations (Fig. 5). For example, ferroceneboronic acid was used in the production of *p*-hydroxybenzaldehyde by oxidation of *p*-cresol with

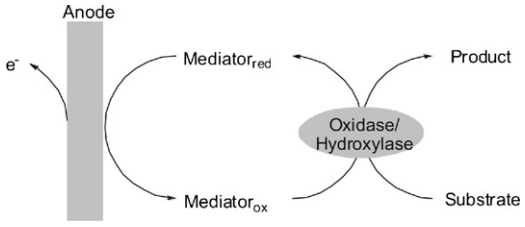

Fig. 5. Oxidative regeneration in flavin-dependent oxidations.

Table 3
Synthesis reactions with oxidative regeneration of flavin-dependent enzymes

Process	Enzyme	Substrate	Product	Mediator	Results	Literature
42	*p*-Cresol methylenehydroxylase from *Pseudomonas alcaligenes u.a.* (PCMH, E.C. 1.17.99.1)	*p*-Methylphenol	*p*-Hydroxy benzaldehyde	Azurine ferrocene boric acid	Conversion 85%	[46]
43	*p*-Cresol methylenehydroxylase from *Pseudomonas putida* (PCMH, E.C. 1.17.99.1)	*p*-Methylphenol	*p*-Hydroxy benzaldehyde	PEG-ferrocene	Conversion 84% in 17 h, ttn_{enzyme} 130,000, $ttn_{mediator}$ 66, current efficiency 100%	[47]
44	*p*-Cresol methylenehydroxylase from *Pseudomonas putida* (PCMH, E.C. 1.17.99.1)	*p*-Methylphenol	*p*-Hydroxy benzaldehyde	PEG-ferrocene a,w,bis-methylferrocene polyethyleneglycol (20,000)	Conversion 100%, ttn_{enzyme} 400,000, $ttn_{mediator}$ 500	[48]
45	*p*-Cresol methylenehydroxylase from *Pseudomonas putida* (PCMH, E.C. 1.17.99.1)	*p*-Ethylphenol	(*S*)-1-(4-Hydroxyphenyl)-ethanol	PEG-ferrocene	Conversion ~70%, e.e. 88%	[48]
46	Ethylphenol methylenehydroxylase from *Pseudomonas putida* EPMH	*p*-Ethylphenol	(*R*)-1-(4-Hydroxy phenyl)ethanol	PEG-ferrocene a,w,bis-methylferrocene polyethylene glycol (20,000)	Conversion 100%, e.e. 93% (optical purity) and 99% (GC), respectively	[48]
47	Galactose oxidase from *Fungus fusarium* NRRL 2903 (GOase, E.C. 1.1.3.9)	L-Xylitol	L-Xylose	PEG-ferrocene a,w,bis-methylferrocene polyethylene glycol (20,000)	7.4 mM in 3 weeks, ttn_{enzyme} 208,720, $ttn_{mediator}$ 15, current efficiency 34.6%	[49]
48	Glycerine-3-phosphate oxidase from *Pediococcus* sp. (E.C. 1.1.3.21)	Glycerine-3-phosphate	Dihydroxyacetone phosphate	PEG-ferrocene	90 mM	[12]
49	Crude enzyme extract from *Escherichia coli*	Succinate	Fumarate	None	~0.8 mM in 6 h	[50]
50	Crude enzyme extract from *Saccharomyces cerevisiae*	Xylitol	Xylose	None	~0.55 mM in 10 h	[50]

p-cresolmethyl hydroxylase (PCMH) (Table 3, process 42) [46]. The reaction was carried out in a two-compartment cell with a gold working electrode. A yield of up to 85% was obtained. A similar electroenzymatic reaction with polyethylene glycol (PEG)-bound ferrocene as mediator at a graphite foil yielded 84% *p*-hydroxybenzaldehyde (Table 3, process 43) [47].

FAD regeneration by electrochemical means was also included in continuous processes with *in situ* product removal [12,48,49]. An electrochemical cell with a carbon felt working electrode was connected to an enzyme membrane reactor forming a so-called electrochemical enzyme membrane reactor (EEMR). The product is removed via an ultrafiltration membrane, while the enzyme is held back in the flow cycle. As the mediator size is similar to that of the educts and products, it has to be bound to a homogeneously soluble polymer to ensure that it remains in the reaction cycle. With this setup, an electroenzymatic oxidation of *p*-cresol to *p*-hydroxybenzaldehyde was performed with *p*-cresol methylenehydroxylase as biocatalyst (Table 3, process 44) [48]. The reaction was carried out in a galvanostatic mode and after 50 h a steady state with almost quantitative conversion of *p*-cresol was reached. Total turnover numbers of 400,000 for the enzyme and 500 for the mediator were achieved. In the same reactor, the substrate 4-ethylphenol was converted by *p*-cresol methylenehydroxylase, leading to (*S*)-1-(4-hydroxyphenyl)-ethanol with 88% e.e. (Table 3, process 45) [48]. Hydroxyacetophenone from the oxidation of benzylic alcohol was found as a by-product. The process was also investigated with 4-ethylphenol methylenehydroxylase (EPMH) (Table 3, process 46) [48]. The (*R*)-enantiomer was obtained in 99% e.e. and the ketone by-product formation decreased to 10%.

Galactose oxidase was used for the oxidation of xylitol to L-xylose with regeneration by ferrocene (Table 3, process 47) [49]. Rapid loss of enzyme activity was obtained in batch electrolyses due to denaturation at the counter electrode and the influence of shear forces. Therefore, a fixed-bed reactor with immobilized galactose oxidase was coupled with an electrochemical flow-through cell. 7.4 mM of xylitol was converted within 3 weeks leading to a ttn of >200,000 for the enzyme.

Another flavo enzyme, L-glycerin-3-phosphate oxidase (GPO), was used in the batch electrolysis of L-glycerin-3-phosphate forming dihydroxyacetone phosphate (DHAP) (Table 3, process 48) [12]. Polymer-bound ferrocene served as the mediator. The DHAP formed was used for *in situ* C–C-coupling with propanal in the presence of fructose-1,6-diphosphate aldolase.

An example of an electrochemical regeneration of FAD without using a mediator is given for the use of a catalytic electrode [50]. Graphite powder, inorganic binder and 3% Cu(II) ion were mixed and fixed on a plate. The counter electrode was fabricated in the same manner with Fe(II) ions instead of copper ions. The reactions were performed in a two-compartment cell with 2 V cell voltage. Within this setup, succinate was oxidised to fumarate (Table 3, process 49) [50] and xylitol to xylose (Table 3, process 50) [50] with crude enzyme extract from *Escherichia coli* and *Saccharomyces cerevisiae*, respectively.

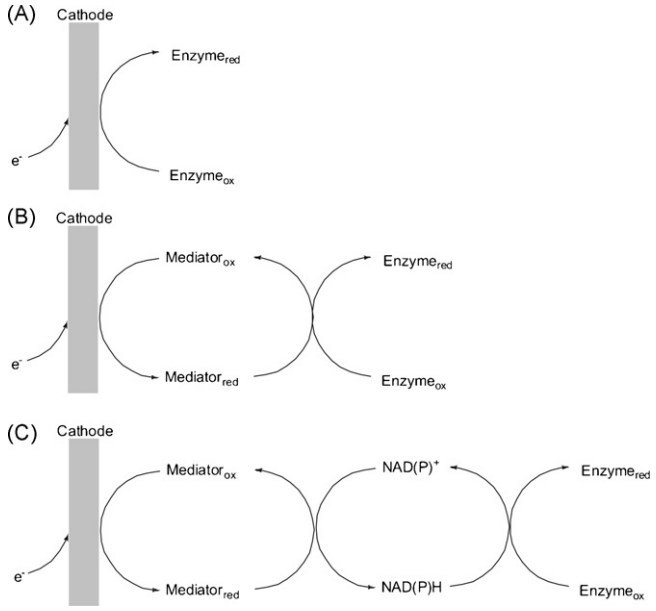

Fig. 6. Reductive regeneration in flavin-dependent oxidations, (A) direct electrochemical regeneration, (B) indirect electrochemical regeneration, (C) enzyme-coupled electrochemical regeneration.

2.4. Reductive regeneration in flavin-dependent oxidations

Monooxygenases are versatile biocatalysts for oxidation reactions. Hydroxylations, epoxidations, heteroatom oxidations and Baeyer-Villiger oxidations can be performed with a broad range of substrates [51]. The oxidation proceeds via transfer of an oxygen atom to the substrate, so that molecular oxygen is essential as cosubstrate. The second atom of O_2 is reduced by the cofactor, leading to H_2O. Thus, the cofactor is regenerated by providing reducing equivalents, like NAD(P)H [52]. Due to the high cost of stoichiometric amounts of these cofactors, electrochemistry is an attractive tool for the supply of electrons. The enzymes involved can be regenerated directly at a cathode, or with the help of a mediator, or by regeneration of the cofactor involved (Fig. 6).

A couple of electroenzymatic syntheses dealing with this problem have been reported. For the electroenzymatic ω-hydroxylation of lauric acid (Table 4, processes 51 and 52) [53,54], a fusion protein was used where a cytochrome P450 monooxygenase is linked to rat NADPH-P450 reductase and a reconstitution system with an engineered monooxygenase and added NADPH-P450 reductase. Two electrons are required to activate P450 for monooxygenation. The usual electron donor for this system is NADPH. The above mentioned proteins are able to accumulate two or more electrons donated by an electrochemical mediator, in this case cobalt(III)sepulchrate trichloride [53]. 1,1′-Dicarboxycobaltocene was used in a similar way for the monooxygenation of lauric acid with cytochrome P450 BM3 [54]. This worked not only with the holoprotein but also with the heme domain alone. However, due to rapid oxidation of the reduced mediator by dioxygen, the reaction is not very efficient.

The flavin-dependent 2-hydroxybiphenyl-3-monooxygenase catalyses the specific *o*-hydroxylation of α-substituted phenol

Table 4
Synthesis reactions with reductive regeneration of flavin-dependent enzymes

Process	Enzyme	Substrate	Product	Cofactor	Mediator	Results	Literature
51	Fusion protein rFP450 [mRat4A1/mRatOR]L1 (rFP4504A1)	Lauric acid	ω-Hydroxy lauric acid	FAD^+/H_2	Cobalt(III)sepul-chrate trichloride	0.65 nmol min^{-1} mL^{-1}	[53]
52	Cytochrome P450 BM3 from *Bacillus megaterium* (BM3)	Lauric acid	ω-Hydroxy laureate	FAD^+/H_2	1,1′-Dicarboxy-cobaltocene	ttn_{enzyme} 224	[54]
53	2-Hydroxybiphenyl-3-monooxygenase from *Pseudomonas azelaica* (HbpA)	2-Hydroxybiphenyl	2,3-Dihydroxybiphenyl	FAD^+/H_2 $NADP^+/H$	[Cp*Rh(bpy)Cl]Cl	204 mg h^{-1} L^{-1}, $tof_{mediator}$ 11 h^{-1}	[55]
54	Styrene monoxygenase (only $FADH_2$-dependent oxygenase component) from *Pseudomonas* sp. VLB 120 (StyA)	Styrene and derivatives	(*S*)-Epoxidised styrene derivatives	FAD^+/H_2	None	0.074–0.222 mMh^{-1}, e.e. 98.1–99.9%	[56]
55	Pyruvate dehydrogenase complex (PDH)	Acetyl coenzyme A	Pyruvate	FAD	MV^{2+}	0.98 μmol in 100 h	[57]
56	D-Amino acid oxidase from porcine kidney (D-AAO, E.C. 1.4.3.2)	Pyruvic acid	D-Alanine	FAD^+/H_2	1-Aminopropyl-1′-methyl-4,4′-dipyridinium iodide	8.9 mM in 10 h, e.e. >99%, $ttn_{mediator}$ 36,000, current efficiency ~97%	[58]
57	L-Amino acid oxidase from *Crotalus adamanteus* venom (L-AAO, E.C. 1.4.3.2)	Phenylpyruvic acid	L-Phenylalanine	FAD^+/H_2	1-Aminopropyl-1′-methyl-4,4′-dipyridinium iodide	8.5 mM in 10 h, e.e. >99%	[58]

derivatives. An approach with indirect electrochemical regeneration of NADH used a [Cp*Rh(bpy)Cl]Cl complex as mediator (Table 4, process 53) [55]. A drawback of this system was the formation of hydrogen peroxide by the reaction of the hydridorhodium complex with molecular oxygen and even O_2 reduction at the carbon-felt cathode. The productivity of the electroenzymatic reaction (204 mg L^{-1} h^{-1}) reached about 50% of the biotransformation process by using either whole cells or isolated enzymes with enzymatic cofactor regeneration.

Direct unmediated electron transfer to FAD without inclusion of the NADH regeneration cycle was performed in the styrene monooxygenase catalysed epoxidation of styrenes (Table 4, process 54) [56]. By electrochemical means, the number of enzyme reactions involved in this system was reduced from three (regeneration of NADH, transfer of reducing equivalents from NADH to FAD, and the epoxidation reaction) to the actual product formation reaction. A series of (*S*)-epoxidised styrenes were produced with e.e. >98% at rates from 0.074 to 0.222 mM h^{-1}.

The pyruvate dehydrogenase complex (PDC) decarboxylates pyruvate to acetyl-coenzyme A. The reverse reaction, formation of pyruvate, was performed by electroenzymatic fixation of CO_2 in acetyl-coenzyme A with PDC (Table 4, process 55) [57]. Methyl viologen served as mediator. Pyruvate production stagnated at a certain time due to the formation of coenzyme A, which inhibited the reaction. By adding phosphotransacetylase and acetyl phosphate coenzyme A was transferred to acetyl-coenzyme A and the pyruvate production was five times higher. At best, 0.98 μmol pyruvate was formed in 100 h.

An approach with enzyme and mediator immobilised on a glassy carbon electrode was performed for the asymmetric electrosynthesis of amino acids (Table 4, processes 56 and 57) [58]. The FAD-dependent enzyme amino acid oxidase oxidises amino acids to imino acids, which consecutively hydrolyse to the corresponding alpha-keto acids. It was possible to reverse the reaction by using 1-aminopropyl-1′-methyl-4,4′-dipyridinium iodide as mediator immobilised on the electrode together with the enzyme. If a potential is applied at which the mediator is permanently in its reduced state, the FAD will be provided in its reduced form, too. This forces the amino acid oxidase to function as a reducing agent for imino acids. With this approach, D-alanine and L-phenylalanine were produced from pyruvic acid and phenylpyruvic acid with >99% e.e. and yields of around 30% within 10 h.

2.5. Electrochemical reactant supply

Peroxidases are not dependent on NAD(P) or FAD cofactors, but contain other prosthetic groups where the redox reaction takes place, including vanadate or heme. To bring about the reaction and regenerate the prosthetic group to the correct redox state they require a cosubstrate, like hydrogen peroxide. Although necessary for the reaction, in some cases these cosubstrates are harmful for the enzymes. Especially, the porphyrin unit in heme peroxidases is very sensitive to oxidative degradation by hydrogen peroxide and therefore the presence of the oxidant leads to inactivation of the enzyme.

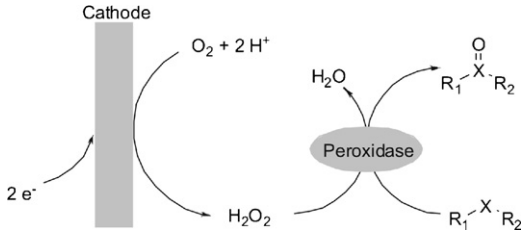

Fig. 7. Electrochemical cosubstrates supply.

There are many publications dealing with reaction engineering that try to avoid high concentrations of the cosubstrates in order to overcome this limitation. For example, one method investigated is sensor-controlled dosing [59], but depending on the quality of the sensor, it is impossible to avoid high local concentrations of the oxidant. Moreover, the addition of diluted hydrogen peroxide continuously increases the reaction volume. Another approach is the *in situ* synthesis of hydrogen peroxide by a chemical reductant or a second enzyme [60]. The general drawbacks of these methods are the formation of a coproduct in stoichiometric amounts and the difficulty of adjusting the rate of hydrogen peroxide generation for every single enzymatic reaction to avoid accumulation of the cosubstrates.

The electrochemical reactant supply is an interesting method for the controlled addition of hydrogen peroxide (Fig. 7). Cathode materials that can be used for the generation of the oxidant via the reduction of dissolved oxygen are mercury, gold and carbon [61], since with most other materials the formation of water predominates. Due to safety risks while working with mercury and the high cost of gold electrodes; carbon is the favoured electrode material. This procedure avoids the formation of by-products and influences on the reaction volume. A further advantage is that the rate of hydrogen peroxide formation can be adjusted by simply changing the potential.

Electrochemical cosubstrates supply was used for the conversion of 2,4,6-trimethylphenol to 3,5-dimethyl-4-hydroxybenzaldehyde by horse radish peroxidase in a divided cell batch reactor and a flow cell reactor (Table 5, processes 58 and 59) [62]. The regioselectivity of the oxidation depended on the applied potential; nevertheless a mixture of at least four different products was always formed. The same enzyme and the same principle was also used for the synthesis of *N*-methylaniline from *N*,*N*-dimethylaniline (Table 5, process 60) [63].

Also the synthesis of veratraldehyde from veratryl alcohol by lignin peroxidase with *in situ* electrogeneration of hydrogen peroxide has been reported (Table 5, process 61) [64]. For this approach, the anode and cathode were divided by a cation exchange membrane and the cathode compartment was continuously aerated with oxygen. The cathode consisted of reticulated vitreous carbon with a platinum plate as the anode.

A lot of research has been done on reactions with chloroperoxidase, as this versatile enzyme is known to be very sensitive to hydrogen peroxide. In a first attempt, the chlorination of barbituric acid to 5-chlorobarbituric acid was investigated (Table 5, process 62) [65]. The reactor setup consisted of an electrochemical cell in which oxidation of H_2O to O_2 is followed by the generation of H_2O_2 and a hollow-fibre module in which the biocatalysis takes place. The product was retained in an anion exchanger. After complete conversion 96% of the product was recovered from the column.

As chloroperoxidase is known to oxidise various sulfides enantioselectively, *in situ* generation of hydrogen peroxide was applied to the synthesis of various sulfoxides, e.g., (*R*)-methylphenylsulfoxide (Table 5, process 63) [66], (*R*)-methyl *p*-tolylsulfoxide (Table 5, process 64) [67], (*R*)-methoxyphenyl methyl sulfoxide (Table 5, process 65)[67] and *N*-MOC(methoxycarbonyl)-L-methionine methyl ester sulfoxide (Table 5, process 66) [67]. Best results were achieved for the synthesis of (*R*)-methylphenylsulfoxide. A space-time yield of $30\,g\,L^{-1}\,d^{-1}$ was obtained in a batch setup consisting of a carbon felt cathode fixed on stainless steel and an anode compartment formed by a platinum wire in a dialysis sack. The product had an e.e. of 98.5%. Using a three-dimensional electrolysis cell, a productivity of $104\,g\,L^{-1}\,d^{-1}$ was achieved (Table 5, process 67) [68].

2.6. Paired electrolysis

Electrolysis usually deals with the conversion of a substrate to a product or an intermediate at an electrode (working electrode). At the second electrode, called the counter or auxiliary electrode, a sacrificial reaction takes place to close the power circuit, without leading to a useful product. In paired electrolysis, both cathode and anode act as working electrodes [69]. The products formed at the two electrodes can be the same or different and they can originate from one or more substrates [16].

An interesting example of paired electrolysis is the combination of electrochemical cofactor regeneration with a product formation reaction at the counter electrode. The main potential of this method is the enzymatic redox-resolution of a racemate, leaving one enantiomer unreacted, with the electrochemical racemisation of the product from the enzymatic reaction. Deracemisation can be achieved with a theoretical yield of 100% of the desired enantiomer or even the inversion of configuration of an enantiopure compound.

The approach of combining an enzymatic reaction with paired electrolysis was performed in the synthesis of malate from lactate (Table 6, process 68) [70]. In the anodic half-cell reaction, lactate is oxidised at a RuO_2/Ti electrode forming pyruvate. This intermediate migrates through an anion exchange membrane into the cathode compartment, where the enzymatic reaction takes place. Malic enzyme catalyses the CO_2 fixation in pyruvate leading to malate. The cofactor NADPH is regenerated electrochemically at the cathode with methyl viologen as mediator and ferredoxin-$NADP^+$-reductase. Around 8 μmol of malate was produced in 20 h with this setup, the limiting steps were the enzymatic reaction and the transport of pyruvate through the separating membrane.

Another example of combining electrochemical cofactor regeneration with electrogeneration of the substrate was the conversion of L-lactate into D-lactate by an L-dehydrogenase catalysed oxidation of the L-enantiomer into pyruvate, followed

Table 5
Synthesis reactions with electrochemical cosubstrates supply

Process	Enzyme	Substrate	Product	Results	Literature
58	Horse radish peroxidase (HRP, E.C. 1.11.1.7)	2,4,6-Trimethylphenol	Mixture of substances	40% of 3,5-dimethyl-4-hydroxybenzyl alcohol, traces of 3,5-dimethyl-4-hydroxybenzaldehyde and 2,6-dimethylbenzoquinone	[62]
59	Horse radish peroxidase (HRP, E.C. 1.11.1.7)	2,4,6-Trimethylphenol	Mixture of substances	68% of 3,5-dimethyl-4-hydroxybenzyl alcohol, 3% 3,5-dimethyl-4-hydroxybenzaldehyde, 3% 4-(4-hydroxy-3,5-dimethyl-benzyloxy)-2,4,6-trimethylcyclohex-2,5-dienone	[62]
60	Horse radish peroxidase (HRP, E.C. 1.11.1.7)	*N*,*N*-Dimethylaniline	*N*-Methylaniline	Conversion 90%	[63]
61	Lignine peroxidase from *P. chrysosporium* (LiP)	Veratryl alcohol	Veratraldehyde	Not available	[64]
62	Chloroperoxidase from *Calariomyces fumago* (CPO, E.C. 1.11.1.10)	Barbituric acid	5-Chlorobarbituric acid	Conversion >96%, ttn_{enzyme} 500,000, current efficiency >90%	[65]
63	Chloroperoxidase from *Calariomyces fumago* (CPO, E.C. 1.11.1.10)	Thioanisole	(*R*)-Methylphenylsulfoxide	30 g L^{-1} d^{-1}, e.e. 98.5%, ttn_{enzyme} 95,000, current efficiency 65.6%	[66]
64	Chloroperoxidase from *Calariomyces fumago* (CPO, E.C. 1.11.1.10)	Methyl *p*-tolyl sulfide	(*R*)-Methyl *p*-tolyl sulfoxide	Conversion 76%, e.e. 93%, ttn_{enzyme} 58,900	[67]
65	Chloroperoxidase from *Calariomyces fumago* (CPO, E.C. 1.11.1.10)	1-Methoxy-4-(methylthio)benzene	(*R*)-Methoxyphenyl methyl sulfoxide	Conversion 83%, e.e. 99%, ttn_{enzyme} 64,400	[67]
66	Chloroperoxidase from *Calariomyces fumago* (CPO, E.C. 1.11.1.10)	N-MOC-L-methionine methyl ester	N-MOC-L-methionine methyl ester sulfoxide	Conversion 60%, dr 81:19 (*R*), ttn_{enzyme} 700	[67]
67	Chloroperoxidase from *Calariomyces fumago* (CPO, E.C. 1.11.1.10)	Thioanisole	(*R*)-Methylphenyl sulfoxide	104 g L^{-1} d^{-1}, e.e. 98.5%	[68]

Table 6
Paired electrolysis synthesis reactions

Process	Enzyme	Substrate	Product	Cofactor	Mediator	Results	Literature
68	Malic enzyme (ME, E.C. 1.1.1.40)	Lactate (pyruvate)	Malate	$NADP^+/H$	MV	~8 μmol in 20 h	[70]
69	L-Lactate dehydrogenase (L-LDH, E.C. 1.1.1.27)	L-Lactate	D-Lactate	NAD^+/H	Direct	Conversion ~97%, 15 mmol L^{-1} d^{-1}, $ttn_{cofactor}$ 200, $tof_{cofactor}$ 6.3 h^{-1}	[71]
70	L-Alanine dehydrogenase from *Bacillus subtilis* (L-Ala DH, E.C. 1.4.1.1)	L-Alanine	D-Alanine	NAD^+/H	Direct	10 mM in 140 h	[72]

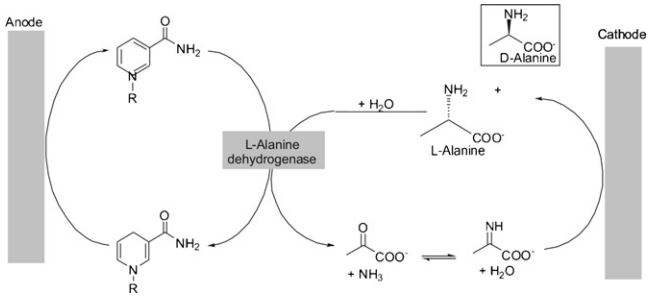

Fig. 8. Paired electrolysis.

by a cathodic reduction of the pyruvate at a mercury cathode, forming racemic D,L-lactate (Table 6, process 69) [71]. Again, the L-enantiomer undergoes the enzymatic reaction and a continuous oxidation–reduction cycle proceeds. The anode was used for NAD^+ regeneration. The anodic and the cathodic reaction were carried out in two separate electrochemical cells. It was possible to achieve a conversion of 97% with this setup.

In the same way, D-alanine was produced from L-alanine with L-amino acid dehydrogenase (Table 6, process 70) [72] (Fig. 8). A drawback of this reaction is the instability of the imino acid, which easily hydrolyses to the α-keto acid. Thus, selective reduction of the imino acid at a mercury cathode was necessary to avoid formation of the α-hydroxy acid by reduction of the keto compound. The unfavourable keto-imine equilibrium leads to a low concentration of the imino acid in the solution, hence the electrochemical conversion to the amino acid was very slow. Due to this fact, the overall process needed a long time, in fact after 20 days, only 60% of D-alanine was produced.

3. Summary and outlook

The current state of the art in the field of research of electroenzymatic syntheses is presented. The vast majority of the processes use electrochemistry as a tool for cofactor regeneration in enzymatic syntheses with oxidoreductases. In nearly all cases, direct electron transfer between electrode and cofactor, which would be the preferable reaction with respect to speed and downstream processing (no additional reagent necessary), was not applied due to drawbacks like very slow electron transfer kinetics or degradation of either cofactor or substrate. Instead, mediators were used as electron shuttles between electrode and cofactor. A variety of different mediator types, such as metal complexes or aromatic compounds, have been investigated and engineered to improve the reaction.

Despite all efforts at improving mediator performance and cell design, the productivity of the majority of electroenzymatic processes is rather low. Moreover, the final product concentration is often too low for a practical synthesis [73]. The heterogeneous electrochemical reaction decelerates the overall reaction. These two obstacles can be successfully addressed by means of reaction engineering. Acceleration of the electrochemical reaction is in principle relatively easy to achieve by an intelligent cell design with a large electrode surface/volume ratio. In three-dimensional cells, productivities of up to 1 kg L^{-1} d^{-1} of reduced cofactor have been achieved [74]. But other parameters like the salt concentration (conductivity) or the mediator concentration have to be carefully adjusted with respect to the enzyme reaction performance [65]. Low final product concentrations can be circumvented by using an aqueous-organic two-phase system to overcome the solubility limitations for hydrophobic substances in the water phase [34].

Apart from cofactor regeneration systems, electrochemistry provides an elegant method for controlled cosubstrate supply. For bioconversions with peroxidases, which require H_2O_2 as cosubstrate, several successful examples can be found with *in situ* electrogeneration of the hydrogen peroxide. Control of the potential was used as a convenient tool for adjusting the production rate.

Paired electrolysis, which combines electrochemical cofactor regeneration with reactant supply, is in principle a very smart concept and proof of concept was demonstrated. However, productivities are still low.

In fact, it will still take some time before electroenzymatic processes can be applied on an industrial scale. Improving the reaction productivity is the main challenge in order to benefit from the advantages of electrochemistry in biocatalysis. Nearly all the processes described were performed without focusing on optimising productivity by considering electrochemical cell design. Thus, reactor concepts with enhanced mass transfer like three-dimensional cells [74] have by no means been exhausted for electroenzymatic processes and offer a chance for improved performance.

Acknowledgements

The authors thank Prof. Dr. C. Wandrey for his ongoing support.

References

[1] K. Faber, M.C.R. Franssen, Trends Biotechnol. 11 (1993) 461–470.
[2] K. Drauz, H. Waldmann, Enzyme Catalysis in Organic Synthesis, VCH, Weinheim, 1995.
[3] A. Liese, K. Seelbach, C. Wandrey, Industrial Biotransformations, WILEY-VCH, Weinheim, 2000.
[4] R. Wichmann, D. Vasic-Racki, Technology Transfer in Biotechnology: From Lab to Industry to Production, vol. 92, 2005, pp. 225–260.
[5] Z. Findrik, D. Vasic-Racki, S. Lütz, T. Daussmann, C. Wandrey, Biotechnol. Lett. 27 (2005) 1087–1095.
[6] S. Lütz, in: J.G. de Vries, C.J. Elsevier (Eds.), The Handbook of Homogeneous Hydrogenation, vol. 3, Wiley-VCH Verlag GmbH, Weinheim, 2006, pp. 1471–1482.
[7] W. Hummel, M.R. Kula, Eur. J. Biochem. 184 (1989) 1–13.
[8] E.I. Iwuoha, M.R. Smyth, Biosens. Bioelectron. 18 (2003) 237–244.
[9] E. Katz, V. Heleg-Shabtai, I. Willner, H.K. Rau, W. Haehnel, Angew. Chem. Int. Ed. 37 (1998) 3253–3256.
[10] Z. Zhang, A.-E.F. Nassar, Z. Lu, J.B. Schenkman, J.F. Rusling, J. Chem. Soc., Faraday Trans. 93 (1997) 1769–1774.
[11] C. Wandrey, Chem. Rec. 4 (2004) 254–265.
[12] E. Steckhan, Top. Curr. Chem. 170 (1994) 83–111.
[13] J. Bonnefoy, J. Moiroux, J.M. Laval, C. Bourdillon, J. Chem. Soc. Faraday Trans. 84 (1988) 941–950.
[14] G. Hilt, B. Lewall, G. Montero, J.H.P. Utley, E. Steckhan, Liebigs Ann. Recl. (1997) 2289–2296.
[15] G. Hilt, E. Steckhan, J. Chem. Soc. Chem. Commun. (1993) 1706–1707.
[16] E. Steckhan, T. Arns, W.R. Heineman, G. Hilt, D. Hoormann, J. Jorissen, L. Kroner, B. Lewall, H. Putter, Chemosphere 43 (2001) 63–73.
[17] J. Komoschinski, E. Steckhan, Tetrahedron Lett. 29 (1988) 3299–3300.
[18] D. Degenring, I. Schroder, C. Wandrey, A. Liese, L. Greiner, Org. Process Res. Dev. 8 (2004) 213–218.
[19] I. Schroder, E. Steckhan, A. Liese, J. Electroanal. Chem. 541 (2003) 109–115.
[20] A. Manjon, J.M. Obon, P. Casanova, V.M. Fernandez, J.L. Ilborra, Biotechnol. Lett. 24 (2002) 1227–1232.
[21] M. Schulz, H. Leichmann, H. Gunther, H. Simon, Appl. Microbiol. Biotechnol. 42 (1995) 916–922.
[22] S. Itoh, H. Fukushima, M. Komatsu, Y. Ohshiro, Chem. Lett. (1992) 1583–1586.
[23] Y. Kashiwagi, T. Osa, Chem. Lett. (1993) 677–680.
[24] J.N. Burnett, A.L. Underwood, Biochemistry 4 (1965) 2060–2064.
[25] R.F. Powning, C.C. Kratzing, Arch. Biochem. Biophys. 66 (1957) 249–251.
[26] T. Kono, Bull. Agric. Chem. Soc. Jpn. 21 (1957) 115–120.
[27] T. Kono, S. Nakamura, Bull. Agric. Chem. Soc. Jpn. 22 (1958) 399–403.
[28] S.H. Baik, C. Kang, I.C. Jeon, S.E. Yun, Biotechnol. Tech. 13 (1999) 1–5.
[29] E. Siu, K. Won, C.B. Park, Biotechnol. Prog. (2006).
[30] R. Wienkamp, E. Steckhan, Angew. Chem. Int. Ed. 21 (1982) 782–783.
[31] R. Ruppert, S. Herrmann, E. Steckhan, Tetrahedron Lett. 28 (1987) 6583–6586.
[32] K. Delecouls-Servat, R. Basseguy, A. Bergel, Chem. Eng. Sci. 57 (2002) 4633–4642.
[33] K. Delecouls-Servat, R. Basseguy, A. Bergel, Bioelectrochemistry 55 (2002) 93–95.
[34] F. Hildebrand, S. Lütz, Tetrahedron: Asymmetry 18 (2007) 1187–1193.
[35] F. Hildebrand, S. Lütz, Tetrahedron: Asymmetry 17 (2006) 3219–3225.
[36] Y. Kashiwagi, Y. Yanagisawa, N. Shibayama, K. Nakahara, F. Kurashima, J. Anzai, T. Osa, Electrochim. Acta 42 (1997) 2267–2270.
[37] Y.W. Kang, C. Kang, J.S. Hong, S.E. Yun, Biotechnol. Lett. 23 (2001) 599–604.
[38] M.H. Kim, S.E. Yun, Biotechnol. Lett. 26 (2004) 21–26.
[39] R. DiCosimo, C.H. Wong, L. Daniels, G.M. Whitesides, J. Org. Chem. 46 (1981) 46224623.
[40] R. Yuan, S. Watanabe, S. Kuwabata, H. Yoneyama, J. Org. Chem. 62 (1997) 2494–2499.
[41] J. Cantet, A. Bergel, M. Comtat, Enzym. Microb. Technol. 18 (1996) 72–79.
[42] V. Massey, J. Biol. Chem. 269 (1994) 22459–22462.
[43] E.-M. Trost, L. Fischer, J. Mol. Catal. B: Enzym. 19–20 (2002) 189–195.
[44] S. Butó, L. Pollegioni, L. D'Angiuro, M.S. Pilone, Biotechnol. Bioeng. 44 (1994) 1288–1294.
[45] F.A. Armstrong, H.A.O. Hill, N.J. Walton, Acc. Chem. Res. 21 (1988) 407–413.
[46] H.A.O. Hill, B.N. Oliver, D.J. Page, D.J. Hopper, J. Chem. Soc. Chem. Commun. (1985) 1469–1471.
[47] M. Frede, E. Steckhan, Tetrahedron Lett. 32 (1991) 5063–5066.
[48] B. Brielbeck, M. Frede, E. Steckhan, Biocatalysis 10 (1994) 49–64.
[49] A. Petersen, E. Steckhan, Bioorg. Med. Chem. 7 (1999) 2203–2208.
[50] S. Jeon, I.H. Jin, B.I. Shin, D.H. Sang, Park, J. Microbiol. Biotechnol. 15 (2005) 281–286.
[51] F. Hollmann, K. Hofstetter, A. Schmid, Trends Biotechnol. 24 (2006) 163–171.
[52] D.M. Ziegler, Trends Pharmacol. Sci. 11 (1990) 321–324.
[53] K.M. Faulkner, M.S. Shet, C.W. Fisher, R.W. Estabrook, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 7705–7709.
[54] A.K. Udit, F.H. Arnold, H.B. Gray, J. Inorg. Biochem. 98 (2004) 1547–1550.
[55] F. Hollmann, A. Schmid, E. Steckhan, Angew. Chem. Int. Ed. 40 (2001) 169–171.
[56] F. Hollmann, K. Hofstetter, T. Habicher, B. Hauer, A. Schmid, J. Am. Chem. Soc. 127 (2005) 6540–6541.
[57] S. Kuwabata, N. Morishita, H. Yoneyama, Chem. Lett. (1990) 1151–1154.
[58] S. Kawabata, N. Iwata, H. Yoneyama, Chem. Lett. (2000) 110–111.
[59] K. Seelbach, M.P.J. van Deurzen, F. van Rantwijk, R.A. Sheldon, U. Kragl, Biotechnol. Bioeng. 55 (1997) 283–288.
[60] F. van de Velde, F. van Rantwijk, R.A. Sheldon, J. Mol. Catal. B: Enzym. 6 (1999) 453–461.
[61] D. Pletcher, Acta Chem. Scand. 53 (1999) 745–750.
[62] P.N. Bartlett, D. Pletcher, J. Zeng, J. Electrochem. Soc. 146 (1999) 1088–1092.
[63] J.K. Chen, K. Nobe, J. Electrochem. Soc. 140 (1993) 299–303.
[64] K. Lee, S.H. Moon, J. Biotechnol. 102 (2003) 261–268.
[65] C. Laane, A. Weyland, M. Franssen, Enzyme Microb. Technol. 8 (1986) 345–348.
[66] S. Lütz, E. Steckhan, A. Liese, Electrochem. Commun. 6 (2004) 583–587.
[67] C. Kohlmann, S. Lütz, Eng. Life Sci. 6 (2006) 170–174.
[68] S. Lütz, K. Vuorilehto, A. Liese, Biotechnol. Bioeng. 98 (2007) 525–534.
[69] H. Pütter, in: O. Hammerich (Ed.), Org. Electrochem., Marcel Dekker, New York, 2001, pp. 1259–1308.
[70] S. Kuwabata, S. Watanabe, H. Inoue, H. Yoneyama, Denki Kagaku 64 (1996) 1080–1083.
[71] A.E. Biade, C. Bourdillon, J.M. Laval, G. Mairesse, J. Moiroux, J. Am. Chem. Soc. 114 (1992) 893–897.
[72] A. Anne, C. Bourdillon, S. Daninos, J. Moiroux, Biotechnol. Bioeng. 64 (1999) 101–107.
[73] R. Ruinatscha, V. Höllrigl, K. Otto, A. Schmid, Adv. Synth. Catal. 348 (2006) 2015–2026.
[74] K. Vuorilehto, S. Lütz, C. Wandrey, Bioelectrochemistry 65 (2004) 1–7.