Biocatalytic Oxidation of Primary and Secondary Alcohols

Wolfgang Kroutil*, Harald Mang, Klaus Edegger, Kurt Faber

Department of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz, Austria
Phone: (+43)-316-380-5350, fax: (+43)-316-380-9840, e-mail: Wolfgang.Kroutil@uni-graz.at

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Abstract: Driven by the immaturity of many organic oxidation reactions and the necessity for 'green' chemical processes, biocatalytic redox processes are being investigated with increasing intensity in order to tap the full potential of the excellent chemo-, regio- and enantioselectivity of enzymes. Despite their unmatched advantage in view of environmental aspects, the requirement of cofactors and the availability of redox enzymes able to tolerate high concentrations of organic (co)substrates sets limitations. However, during the past years, an increasing number of applications to the bio-oxidation of primary and secondary alcohols with novel redox enzymes have been developed. This review gives an overview on the different methods and their potential and limits.

Keywords: alcohol dehydrogenase; alcohol oxidation; biocatalysis; monooxygenase; oxidase; oxidoreductase; peroxidase

1 Introduction

Oxidation of alcohols to form carbonyl compounds is one of the most fundamental and important processes in synthetic organic chemistry. Although a variety of methods and reagents have been developed, they all suffer from the general difficulty to transport redox equivalents (electrons or electron-bearing species) between reactants and oxidants. Traditional alcohol oxidation has been performed with heavy metal reagents commonly based on Mn and Cr,[1,2] the majority of which are required in molar amounts; a number of methods could be turned catalytic, for instance, hydrogen-transfer reactions (Ru, Rh, Ir),[3] and Oppenauer oxidations (Al, Zr, lanthanides).[4] On the other hand, metal-free alcohol oxidation (generally denoted as the Swern and Pfitzner–Moffat protocols) is based on, e.g., dimethyl sulfoxide as oxidant in the presence of an 'activating' reagent, such as *N*,*N*′-dicyclohexylcarbodiimide, an acid anhydride or acid halide.[5,6] Although the latter methods avoid the use of heavy metals, they usually depend on moisture-sensitive oxidants and environmentally undesirable reaction media, such as chlorinated solvents. Bearing in mind that the desired oxidation only requires the formal transfer of two hydrogen atoms, their overall atom economy is disastrous. More elegant methods employ molecular oxygen as oxidant in chemocatalytic aerobic oxidations.[7,8]

In the search for alternatives, several biocatalytic methods for alcohol oxidation have been developed, which hold a great potential with respect to environmental compatibility and catalytic efficiency. Quite a number of reviews related to the biocatalytic oxidation of alcohols have been published during the last few years.[9–14]

This review features the biooxidation of primary and secondary alcohols to the corresponding aldehydes and ketones, respectively. For the sake of clarity, the paper is subdivided according to the type of enzyme used. For the oxidation of alcohols, two types of oxidoreductases have been employed most frequently – dehydrogenases and oxidases – while peroxidases and monooxygenases

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Wolfgang Kroutil (born in 1972 in Graz, Austria) received his undergraduate training in chemistry at the University of Technology in Graz (Austria) and completed his graduate studies in Exeter (UK) and Graz. After his Ph. D. he collected two years of industrial experience in the biocatalysis research group at Syngenta (formerly Novartis CP) in Basel (Switzerland) and in the R&D department of Krems Chemie Chemical Services (Austria). In 2000 he became assistant professor in the research group of Prof. Faber at the Department of Chemistry at the Karl-Franzens-University Graz.

Harald Mang, born 1976 in Knittelfeld (Steiermark/Austria), studied chemistry at the Karl-Franzens University in Graz, where he received his M.Sc. under the supervision of Prof. Kurt Faber in 2002. Since 2002 he is doing his Ph. D. thesis focused on selective oxidation reactions with peroxidases/oxidases as catalysts under the supervision of W. Kroutil/K. Faber.

Klaus Edegger, born 1977 in Graz/Austria visited the higher technical school for chemical engineering in Wels/Upper Austria. He studied chemistry at the Karl-Franzens University in Graz and graduated in 2002 in the group of Prof. Faber. In his Ph. D. thesis he focuses on redox reactions with ADHs under the supervision of W. Kroutil/K. Faber.

Kurt Faber, born 1953 in Klagenfurt (Carinthia/Austria), studied chemistry at the Karl-Franzens University in Graz, where he received his Ph. D. in 1982. From 1982–1983 he moved to St. John's (Canada) for a post-doc and continued his career at the University of Technology (Graz), where he became associate Prof. in 1997. The following year he was appointed full Prof. at the University of Graz, where he is heading his research group devoted to the use of biocatalysts for the synthetic transformation of non-natural compounds. He was a visiting scientist at University of Tokyo (1987/1988), Exeter University (1990), University of Trondheim (1994) and Stockholm University (2001).

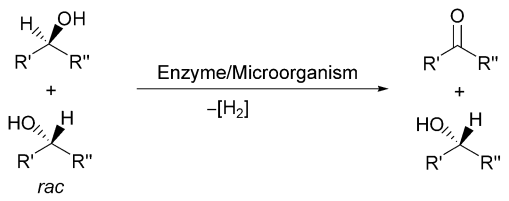
were employed to a lesser extent. Oxidative whole-cell transformations with the enzyme involved being unknown, are treated separately. Finally, combinations of biocatalytic and chemical oxidation methods are described in the last chapter.

2 Stereochemical Consequences

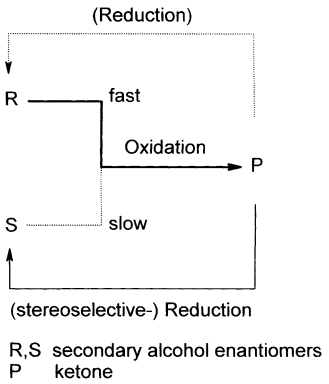
Due to the intrinsic asymmetry of enzymes, the oxidation of chiral *rac-sec*-alcohols always leads to a kinetic resolution, where one enantiomer out of the racemate is transformed faster than its mirror image counterpart (Scheme 1). As a result, the non-reacting enantiomer can be obtained in 50% theoretical yield in high ee. If the ketone is the favoured product, complete oxidation of the racemate is desired, which is impeded by the fact that the oxidation of the second enantiomer generally proceeds at a reduced reaction rate, which requires extended reaction times to reach completion. The latter depends on the enantioselectivity (E value) of the enzyme. Quite frequently, the second enantiomer is not transformed at all within a reasonable time.

After all, the benefit of the high chemo-, regio- and enantioselectivity of enzymes turns into a drawback when non-(enantio)-selective oxidation is required.

In order to circumvent the 50% yield limitation of a single enantiomer in kinetic resolution, a deracemisation protocol has been proposed, which allows the complete transformation of a *rac-sec*-alcohol into a single stereoisomeric product in 100% theoretical yield (Scheme 2). Thus, the enantioselective oxidation of enantiomer R is combined with a (stereoselective)



Scheme 1. Oxidative kinetic resolution of a chiral *sec*-alcohol.



Scheme 2. Deracemisation of secondary alcohols *via* cyclic oxidation/reduction.

reduction of the intermediate ketone P. Overall, one enantiomer (S) remains untouched, while the other one (R) is subjected to a stereo-inversion.[15] Depending on the system, the reduction step may either proceed in a stereoselective fashion (e.g., by using an alcohol dehydrogenase, bold reduction arrow), which completes the deracemisation within two steps. Alternatively, non-stereoselective reduction requires multiple oxidation/reduction cycles.[16] However, with the exception of whole-cell transformations (where the type of enzymes involved and the exact redox mechanism remain unclear), such deracemisation processes[17] were only achieved in a stepwise manner.

3 Alcohol Dehydrogenases

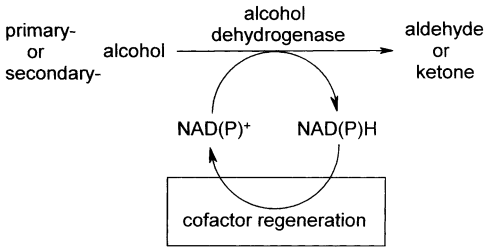
The vast majority of alcohol dehydrogenases (ADHs) which oxidise *prim*- and *sec*-alcohols require $NAD(P)^+$ as cofactor, which is relatively unstable and expensive when used in molar amounts. A good deal of research has been devoted to the development of $NAD(P)^+$ regeneration techniques to ensure that only catalytic amounts are used furnishing economic processes (Scheme 3). Due to the small significance of quinoprotein dehydrogenases for preparative-scale organic transformations, these enzymes are not included in this review.

3.1 Regeneration of $NAD(P)^+$

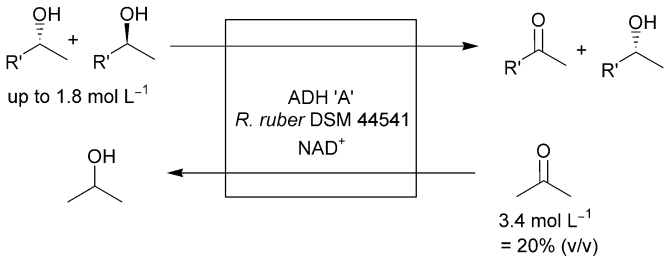
The regeneration of $NAD(P)^+$ from NAD(P)H constitutes an oxidation reaction involving the transfer of two electrons and a proton from the cofactor onto a suitable acceptor. Due to the fact that the equilibrium of nicotinamide-dependent dehydrogenase reactions lies heavily in favour of reduction, the regeneration of $NAD(P)^+$ is more complicated than that of NAD(P)H. Most commonly used acceptors are carbonyl functions, molecular oxygen or the anode of an electrochemical cell. The characteristics of various regeneration methods are summarised in Table 1.

The most widely applied methods for $NAD(P)^+$ regeneration are the 'coupled enzyme' and the 'coupled substrate' approach, whereby a carbonyl compound, such as a ketone or an aldehyde is used as co-substrate/acceptor. Due to the similarity of the redox pairs of substrate/product and co-substrate/co-product, these reactions are often close to equilibrium making it more difficult to shift the reaction towards the desired direction.

With respect to simplicity the 'coupled substrate' approach is favoured, since only a single enzyme is needed. However, the fact that a carbonyl compound is needed in excess to shift the reaction towards oxidation represents a serious hurdle and thus preparative-scale examples are rare.[18,19] Only recently, we reported on an NAD^+-dependent alcohol dehydrogenase from *Rhodococcus ruber* DSM 44541, which accepts acetone as co-substrate for NAD^+ regeneration (producing 2-propanol as co-product) and at the same time performs the desired alcohol oxidation (Scheme 4).[20,21] Most importantly, the enzyme is remarkably stable towards elevated co-substrate concentrations allowing us to use acetone for cofactor regeneration at 20% v/v with a whole-cell preparation and up to 50% v/v with the partially purified enzyme.[22,23] Elevated acetone concentrations not only shift the equilibrium entirely towards oxidation, but also increase the solubility of lipophilic substrates, thus ensuring enhanced reaction rates.



Scheme 3. Cofactor recycling for alcohol oxidation employing $NAD(P)^+$ dependent dehydrogenases.



Scheme 4. Simultaneous NAD^+ regeneration and preparative alcohol oxidation by an ADH from *R. ruber*.

Table 1. General methods for NAD(P)$^+$-regeneration.

Method	Enzyme	Oxidant	Co-product	Auxiliary reagent	Preparative significance[e]
Coupled substrate	1× dehydrogenase[a]	ketone/aldehyde	alcohol	none	++
Coupled enzyme	2 dehydrogenases[b]	ketone/aldehyde	alcohol or amine[c]	(NH_3)[c]	++
NAD(P)H oxidation	NAD(P)H oxidase	O_2	H_2O or H_2O_2	none	+
Flavin oxidation	FMN reductase	O_2	H_2O_2	none	+
Bayer–Villiger oxidation	Baeyer–Villiger monooxygenase	O_2	H_2O	ketone[d]	–
Direct electrochemical	None	'anode'	e^-	none	–
Indirect electrochemical	None	'anode'	e^-	Mediator	–
Diaphorase/indirect electrochemical	diaphorase	'anode'	e^-	Mediator	–
Photochemical	None	Light hν	none	Mediator	–

[a] Single dehydrogenase transforms substrate → product and co-substrate → co-product.
[b] Second dehydrogenase transforms co-substrate → co-product.
[c] NH_3 is only needed for the reductive amination of a ketone to the corresponding amine (e.g., α-ketoglutarate to L-glutamate).
[d] Ketone serving as substrate for Baeyer–Villiger oxidation may be identical to the product from alcohol oxidation.
[e] Preparative applicability indicated as ++ = wide, + = significant and – = marginal.

Table 2. Dehydrogenases for coupled enzyme NAD(P)+ regeneration.

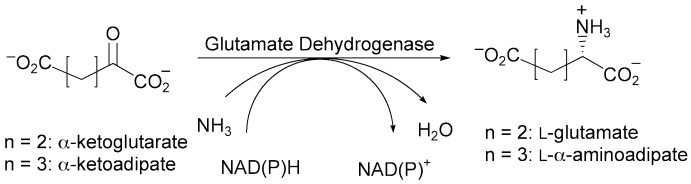
Regeneration enzyme	Co-substrate/ co-product	Specific activity [U/mg]	Stability	Cofactor
YADH[a]	Acetaldehyde/ethanol	300	Low[c]	NAD^+
TBADH[b]	Acetone/2-propanol	30 – 90	Thermostable	$NADP^+$
Lactate DH	Pyruvate/lactate	1000	High	NAD^+
Glutamate DH	α-Ketoglutarate/L-glutamate	40	High	NAD^+ or $NADP^+$

[a] Yeast alcohol dehydrogenase.
[b] *Thermoanaeribium brockii* alcohol dehydrogenase.
[c] O_2 sensitive.

3.1.1 Coupled Enzyme Approach

In order to circumvent possible problems of the coupled substrate approach (equilibrium, enzyme inhibition and deactivation caused by elevated concentrations of the carbonyl compound serving as redox co-substrate), NAD(P)$^+$ regeneration has been 'decoupled' from substrate oxidation by using a second dehydrogenase. The drawback of this approach results from the necessity to use a second enzyme, whose optimal reaction conditions may significantly differ from those of the ADH responsible for substrate oxidation. Furthermore, two-enzyme kinetics are more complex and exclusive enzyme specificities for substrate and co-substrate transformation are needed.

In Table 2 the commonly used dehydrogenases for NAD(P)$^+$ regeneration in coupled enzyme protocols are listed. Overall, the redox equivalents emerging from alcohol oxidation are transferred to an aldehyde or ketone as terminal electron acceptor yielding the corresponding alcohol or amino functionality (in presence of NH_3).



Scheme 5. NAD(P)$^+$ regeneration employing glutamate dehydrogenase.

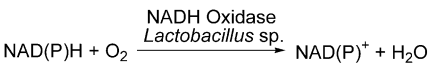
The most widely applied method for the regeneration of NAD(P)$^+$ involves the reductive amination of an α-ketocarboxylic acid to the corresponding

L-amino acid using glutamate dehydrogenase (GluDH) (Scheme 5).[24–26] The system is quite flexible as GluDH accepts both NADH and NADPH as well as α-ketoglutarate or -adipate leading to the formation of high value by-products, L-α-glutamic acid or L-α-aminoadipate, respectively.[27]

On account of the high specific activity (about 300 U/mg) and the low price of yeast alcohol dehydrogenase, YADH has frequently been used as a regeneration enzyme for NAD^+.[28] However, problems arising from enzyme inactivation by ethanol and (particularly) by acetaldehyde used as co-substrate/co-product have limited the applicability of YADH. Attempts to solve this problem by using rather sophisticated gas-membrane techniques were suggested.[29,30] Using lactate dehydrogenase (LDH) to regenerate NAD^+ going in hand with the reduction of pyruvate offers the advantage that LDH is less expensive and exhibits a higher specific activity than GluDH.[31,32] However, the redox potential is less favourable and LDH does not accept $NADP^+$. A thermostable ADH from *Thermoanaerobium brockii* has been used only for the regeneration of reduced cofactor NADPH.

3.1.2 Oxidases for NAD(P)$^+$ Regeneration

In view of its simplicity, the use of the most innocuous and cheapest oxidant – molecular oxygen – for cofactor regeneration would be favourable. Furthermore, the high redox potential of the O_2/H_2O or O_2/H_2O_2 redox couples results in a strong thermodynamic driving force for the regeneration reaction. Since direct oxidation of NAD(P)H by molecular oxygen is very slow,[33] the electron transfer has to be accelerated *via* enzymatic or chemical techniques. For instance, NADH oxidases [e.g., EC 1.6.99.3] have emerged, which are able to oxidise NADH to NAD^+ with simultaneous reduction of O_2 to furnish either H_2O_2 or H_2O.[34–39] Four-electron transfer to render innocuous H_2O is preferred over two-electron reduction yielding H_2O_2, which frequently causes enzyme deactivation even in small amounts. A water-forming NADH oxidase, which is able to utilise not only NADH but also NADPH as substrate with an activity ratio of about 3:1 was isolated from *Lactobacillus sanfranciscensis*.[34] The ability of NAD(P)H oxidase to oxidise both cofactors renders the enzyme an extremely useful catalyst for coupled-enzyme catalysed oxidations (Scheme 6).[40]



Scheme 6. $NAD(P)^+$ regeneration employing oxidases.

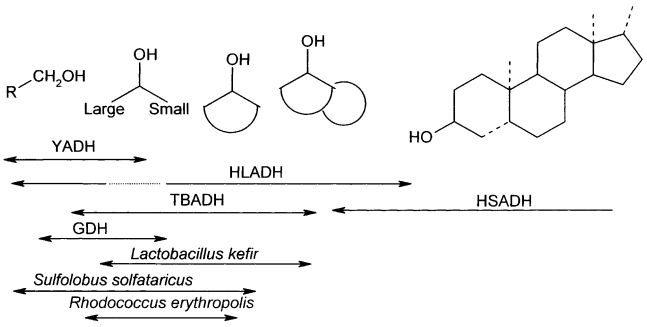
3.1.3 Further Methods

Flavine mononucleotide (FMN) reductase [EC 1.6.8.1] catalyses the hydrogen transfer from NAD(P)H to FMN, yielding the oxidised nictoninamide coenzyme and $FMNH_2$, which spontaneously reacts with molecular oxygen to recycle FMN with concomitant H_2O_2 formation.[41–43] A Baeyer–Villiger monooxygenase (cyclopentanone monooxygenase) was applied to oxidise cyclic ketones which, in turn, were produced by an ADH-catalysed oxidation of substrate alcohols to furnish the corresponding lactones with concomitant regeneration of NAD^+.[44] Another possibility for $NAD(P)^+$ regeneration makes use of electrochemical methods[45] using an anode as terminal electron acceptor. There are three ways for electrochemical oxidation: (i) the (most difficult) direct oxidation,[46] (ii) the indirect electrochemical NAD(P)H oxidation[47–49] and (iii) the diaphorase-accelerated indirect electrochemical oxidation.[50] In practical terms, electrochemical methods play a minor role. Finally, photochemical regeneration[51] of $NAD(P)^+$ *via* light-induced excitation of a mediator was not applied during the last years.

3.2 Sources of Alcohol Dehydrogenases

In principle, virtually all organisms can serve as source for ADHs. However, to date, commonly used, commercially available ADHs originate from horse liver (HLADH) and microorganisms, such as *Thermoanaerobium brockii* (TBADH), baker's yeast (*Saccaromyces cerevisiae,* YADH), *Candida boidinii*, *Candida parapsilosis*, *Rhodococcus erythropolis*, *Lactobacillus brevis*, and *Lactobacillus kefir*.

Redox enzymes attractive for organic synthesis are increasingly derived from thermophilic microorganisms, due to their high operational stability: for instance, an alcohol dehydrogenase from *Thermoanaerobacter*



Scheme 7. Sketch of the substrate spectrum of various ADHs for preparative applications. YADH = yeast ADH; GDH = glycerol dehydrogenase; TBADH = *Thermoanaerobium brockii* ADH; HLADH = horse liver ADH; HSADH = hydroxysteroid ADH.

ethanolius can be used at 50 – 60 °C[52,53] and TBADH is stable at 86 °C.[54]

3.3 ADHs for Preparative Applications and Their Substrate Spectrum

In order to facilitate the choice of the appropriate alcohol dehydrogenase for a given substrate, the preferred substrate sizes of commonly used ADHs are depicted in Scheme 7. Except for sterically demanding alcohols, examples for oxidation for all substrates exist.

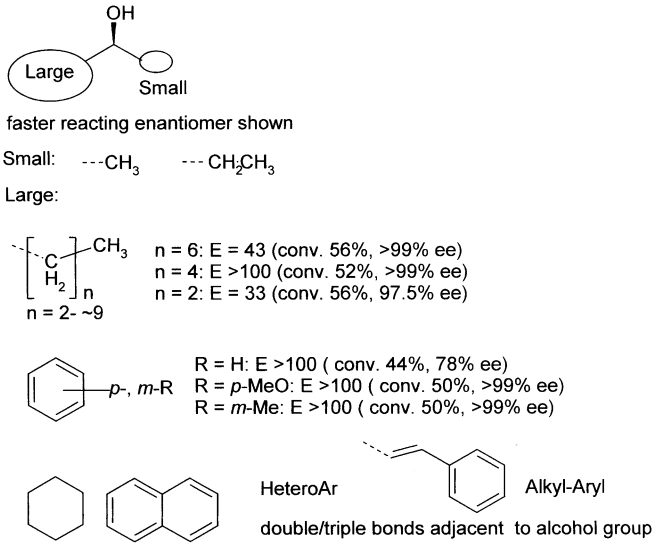
Horse liver alcohol dehydrogenase (HLADH) is one of the most widely used oxidoreductases. The NADH-dependent enzyme exhibits a unique combination of a very broad tolerance for primary and secondary alcohols combined with an almost invariable stereospecificity, which makes HLADH applications highly predictable.[55,56] This enzyme was also applied for the asymmetrisation of prochiral *meso*-diols, due to its ability to discriminate between enantiotopic hydroxy groups.[57] HLADH is a reasonably stable enzyme and exhibits a certain tolerance toward many organic solvents used to solubilise lipophilic substrates;[58] the enzyme is active even in water-saturated organic solvents.[59,60] In combination with HLADH, glutamate dehydrogenase[61,62] and monooxygenases[63] have been commonly used for enzymatic NAD^+ regeneration.

Yeast alcohol dehydrogenase (YADH) is structurally related to HLADH, but it is less stable and shows a low resistance towards organic solvents.[64] Furthermore, its substrate spectrum is limited to primary alcohols and 2-alkanols.[65] *Thermoanaerobium brockii* alcohol dehydrogenase (TBADH) is a thermostable NADP-dependent dehydrogenase, which shows high activity toward (open-chain) secondary alcohols with low activity for primary alcohols.[56] Glycerol dehydrogenase (GDH) was isolated from various bacterial strains, especially from *Schizosaccharomyces pombe*[66] and *Cellulomonas* sp.[67] Oxidation of its natural substrate – glycerol – proceeds selectively at the secondary alcohol function moiety to yield dihydroxyacetone. With few exceptions, the trend for GDH to favour secondary over primary alcohols is seen in many cases: for instance, s*ec*-hydroxy groups of *meso*-diols were selectively oxidised to furnish the corresponding (*S*)-hydroxyketones.[68] An NAD^+-dependent ADH isolated from *Sulfolobus solfataricus* was found to exhibit better thermostability than HLADH together with a distinctive preference for (*S*)-alcohols.[69] The substrate spectrum of the latter enzyme is very broad and encompasses primary linear and branched alcohols as well as linear and cyclic secondary alcohols.[56] Alcohol dehydrogenases isolated from *Rhodococcus erythropolis*,[70] *Pseudomonas* sp. PED and *Lactobacillus kefir*[71,72] accept a broad range of open-chain substrates, including a variety of compounds useful for synthetic chemistry.

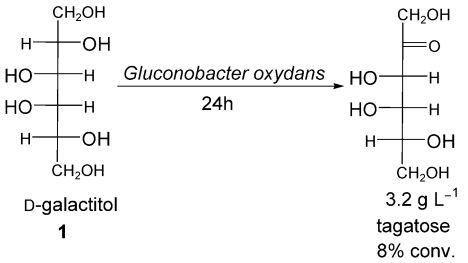
3.4 Whole-Cell Preparations of Alcohol Dehydrogenases

Alcohol oxidations catalysed by whole microbial cells, for which the enzyme(s) involved was/were not characterised are discussed in Section 7. Whole cell transformations with known ADH activity are discussed as follows:

The alcohol dehydrogenase 'ADH-A' from *Rhodococcus ruber* DSM 44541,[22] which can be used for the oxidation of *sec*-alcohols *via* the coupled substrate approach, not only shows a good stability toward the co-substrate acetone (used for cofactor recycling), but also allows remarkably high substrate concentrations of up to 1.8 mol L^{-1}, depending on the substrate.[20] Under non-optimised conditions, the ratio of wild-type cells *versus* substrate was 1:1 (w:w). Besides its exclusive regioselectivity for secondary over primary alcohols, 'ADH-A' preferentially oxidises the (*S*)-alcohol [assuming that the smaller group has the lower Cahn–Ingold–Prelog (CIP) priority], by leaving the (*R*)-alcohol behind. Typical substrate alcohols which are oxidised with high activity and high enantioselectivity have a small group (e.g., methyl, ethyl) and a large group (up to C_{10}) on either side of the carbinol (Scheme 8).[73,74] While cyclohexanol is not oxidised, cyclopentanol is a good substrate. Due to structural limitations within the 'small' group adjacent to the alcohol moiety, 'ADH-A' allows one to differentiate between (ω-1) and sterically more hindered alcohols. Until now, for the ease of handling, this enzyme was only applied as whole-cell preparation from the wild-type strain, however, a clone will be available soon.



Scheme 8. General substrate pattern for 'ADH-A' from *Rhodococcus ruber* DSM 44541. Data given for 'small' = CH_3.



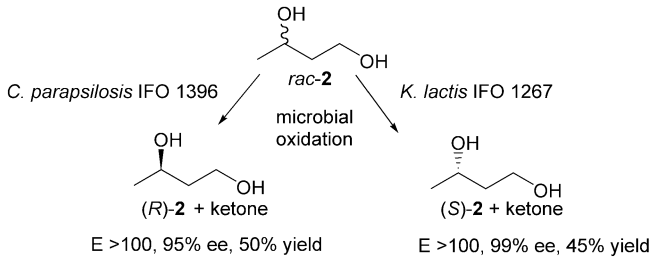
Scheme 9. Synthesis of the sweetener tagatose by microbial alcohol oxidation.

The synthesis of the low-calorie carbohydrate sweetener tagatose from D-galactitol (**1**; Scheme 9) was achieved by regioselective oxidation of the polyol substrate using the wild-type strain of *Gluconobacter oxydans* DSM 2343 as biocatalyst, whereby a sorbitol dehydrogenase was responsible for the reaction of interest (Table 3).[75,76] Although the conversion was very low (7%), the example nicely underlines the exquisite regioselectivity of enzymes, since a only single secondary alcohol function (out of four) beside two primary alcohols had to be oxidised.

Racemic 1,3-butanediol (*rac*-**2**) was resolved *via* microbial oxidative kinetic resolution yielding 1-hydroxy-3-butanone and non-reacted diol in non-racemic form. Depending on the strain used, either enantiomer could be obtained this way: while *Kluyveromyces lactis* IFO 1267 preferentially oxidised the (*R*)-enantiomer by leaving the (*S*)-enantiomer untouched, *Candida parapsilosis* IFO 1396 displayed the opposite enantiopreference (Scheme 10).[78,80]

The applicability of the above-mentioned methods was proven by the large-scale preparation of (*R*)-1,3-butanediol employing whole cells of *Candida parapsilosis* IFO 1396.[78] The reaction mixture contained 258 kg cells, 465 kg water, 7.5 kg of calcium carbonate and 20 kg of *rac*-1,3-butanediol. (*R*)-1,3-Butanediol (3.1 kg) was obtained in a chemical purity of 99% and 94% ee. However, for an efficient production, the ratio of cells/substrate is too high.

A selection of whole-cell biocatalytic alcohol oxidations is summarised in Table 3.



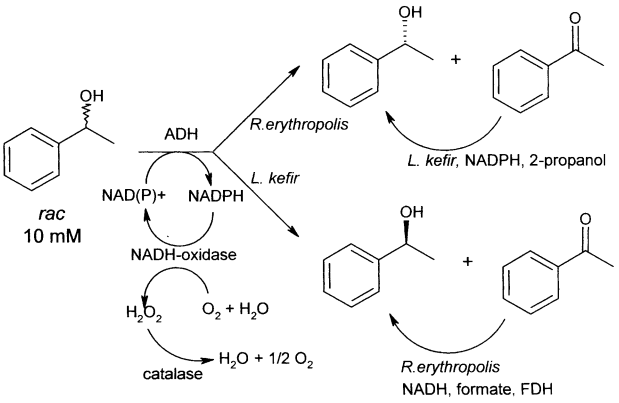
Scheme 10. Microbial resolution using organisms with opposite enantio-preference provides access to both enantiomers.

3.5 Further Applications

A stepwise deracemisation process for secondary alcohols using isolated enzymes was established by Hummel et al. through combination of an (*R*)-specific (NADP-dependent) ADH from *Lactobacillus kefir* and an (*S*)-specific (NAD-dependent) ADH from *Rhodococcus erythropolis*[82] (Scheme 11). In a first step, kinetic resolution *via* enantioselective oxidation yielded 50% of the ketone plus non-reacted alcohol. In a second step, the ketone was asymmetrically reduced with an ADH possessing the opposite stereoselectivity, finally yielding the desired optically pure enantiomer in 100% yield. The synthetic flexibility of this system was demonstrated by the fact that just by switching the order of the ADHs employed, each enantiomer can be obtained at will.

A biofuel cell based on the enzymatic oxidation of methanol to carbon dioxide *via* three steps was developed by Whitesides and coworkers.[83] Thus, in the anodic compartment of the cell, (i) methanol was oxidised to formaldehyde by a methanol dehydrogenase, (ii) formaldehyde was oxidised to formate by formaldehyde dehydrogenase and ultimately (iii) oxidation of formate by formate dehydrogenase furnished CO_2. In each step one molecule of NADH is produced, which transfers its electrons through diaphorase *via* a mediator (viologen) onto the anode. The redox potential showed (– 0.55 V) and was only slightly below that of MeOH/CO_2 (– 0.64 V).

Enzymatic alcohol oxidation by dehydrogenases often suffers from low conversions and slow reaction rates caused by product inhibition. This drawback can be overcome by continuous product extraction by changing the concentrations, while leaving the kinetic parameters unchanged. For this goal it may be favourable to apply a differential circular reactor with continuous product extraction, where only a small amount of product is formed per cycle, while the product is continuously



Scheme 11. Deracemisation of secondary alcohols using isolated ADHs; both enantiomers are accessed by switch of enzymes.

Table 3. Oxidation employing alcohol dehydrogenases in whole cells.

Organism	Substrate	Conversion [%]	Ref.
Gluconobacter oxydans DSM 2343	**1**	~3	[75,76]
Gluconobacter oxydans R	**3**	>90	[77]
Kluyveromyces lactis IFO 1267	**2**	55[a, b] (99% ee)	[78]
Candida parapsilosis IFO 1396	**2**	50[a, c] (95% ee)	[77,79,80]
Mycobacterium sp. NRRL B-3805	cholesterol	51	[81]

[a] 1-Hydroxy-3-butanone was the major product.
[b] The (*R*)- or [c] (*S*)-enantiomer was preferentially oxidised.

extracted using a microporous hydrophobic hollow fibre membrane.[84] Nevertheless, for simple and efficient lab-scale procedures, enzymes being inert towards inhibition phenomena are required.

4 Alcohol Oxidases

4.1 Redox Cycles

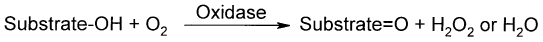
The use of oxidases has the advantage that the cheapest and environmentally most 'friendly' oxidant – molecular oxygen – can be used. Alcohol oxidases [1.1.3.x] catalyse a two- or four-electron transfer onto molecular oxygen,[85] giving hydrogen peroxide (2 e^-) or water (4 e^-) as by-product, respectively (Scheme 12).

In contrast to alcohol dehydrogenases with their loosely bound (and therefore rather sensitive) nicotinamide cofactor, the redox-active prostetic group (e.g., FAD) is tightly bound to the apoprotein and thus more protected. Oxidases tolerate a wide and fairly unspecific range of synthetic electron acceptors ('mediators') instead of molecular oxygen, and thus allow the *in-vitro* regeneration of the redox cofactor. For example, aliphatic alcohol oxidase accepts synthetic acceptors, such as dichlorophenol-indophenol or phenazine methosulfate.[86]

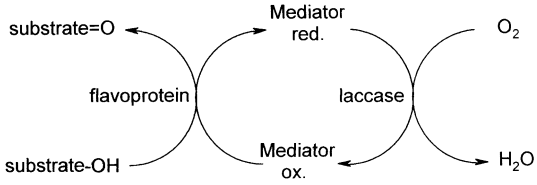
Most enzyme systems are sensitive towards molecular oxygen, which acts through oxidation of essential structural elements of proteins, particularly involving thiol and disulfide residues. This problem may partly be solved by addition of dithiothreitol or 2-mercaptoethanol.[86] In addition, reduction of O_2 generally leads to the formation of hydrogen peroxide (or superoxide anion) as unavoidable by-products. These highly reactive species are toxic for the enzyme. In most cases, partial protection may be achieved by catalytic disproportionation of H_2O_2 into O_2 and water by catalases, which are, however, fairly unstable too (Scheme 13).

Finally, the stability of the desired oxidation product – usually a carbonyl compound – towards spontaneous 'over–oxidation' by molecular oxygen has to be considered: most prominent is the spontaneous radical auto-oxidation of benzaldehyde. The use of an anode in an electrochemical cell as electron sink instead of molecular O_2 was suggested as an elegant solution, however, the method never reached preparative scale, which is most likely due to the limited biocompatibility of the additionally required mediator.[87–89]

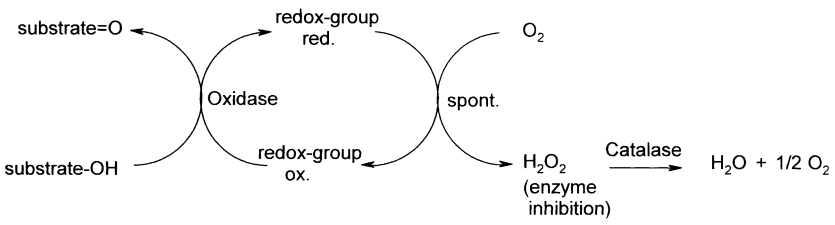
A novel method for cofactor regeneration in oxidase reactions uses an enzymatic (rather than an electrochemical) re-oxidation of mediators *via* laccases (Scheme 14).[90] Advantageously, laccase catalyses a



Scheme 12. Alcohol oxidation catalysed by oxidases.



Scheme 14. Laccase-based re-oxidation of mediators.



Scheme 13. Removal of H_2O_2 from the reaction mixture employing catalases.

four-electron transfer onto the terminal acceptor O_2 by forming innocuous H_2O.

Alcohol oxidases can be conveniently be subdivided according to their preference for primary or secondary alcohols.

4.2 Primary Alcohol Oxidases

FAD-dependent aliphatic alcohol oxidase [EC 1.1.3.13] (e.g., from *Candida boidinii*) catalyses the oxidation of primary alkanols to the corresponding aldehydes by generating H_2O_2 as side product (Table 4).[91,92] Recently, an aliphatic alcohol oxidase was applied as dehydrated enzyme in a gas-solid bioreactor in combination with catalase to avoid enzyme inhibition caused by hydrogen peroxide.[97]

In contrast, aromatic alcohol oxidase [EC 1.1.3.7] has a strong preference for benzylic alcohols bearing various aromatic substituents. Anaerobic regeneration of the enzyme with molar amounts methylene blue was reported.[93,94]

Galactose oxidase [EC 1.1.3.9] oxidises D-galactose to D-galactohexodialdose.[95] The enzyme can be reoxidised aerobically by dioxygen or electrochemically, using mediators like ferrocene.[98] Besides its natural substrate, this oxidase accepts a wide range of primary alcohols substrates, such as glycerol,[99] 2-propyn-1-ol, and dihydroxyacetone. As an example, D/L-threitol was oxidised to yield D-threose *via* a kinetic resolution.[96] Another interesting application of galactose oxidase is the enantioselective oxidation of 3-halo-1,2-propanediols to the corresponding aldehydes.[99] Very recently, galactose oxidase was employed for the transformation of glycerol to L-glyceraldehyde, which in turn was used in a one-pot multi-enzyme synthesis of L-fructose.[100] However, preparative-scale oxidations with galactose oxidase proceed rather slowly and yields are generally low.

4.3 Secondary Alcohol Oxidases

A bacterial secondary alcohol oxidase was found to be involved in the degradation pathway of polyvinyl alcohol (Table 5, Scheme 15).[107,108] The extra-cellular protein produced by *Pseudomonas vesicularis* var. *povalolyticus* PH exhibited strong activity toward secondary alcohols such as 2- and 4-decanol, in addition, cyclohexanol and benzyl alcohol were good substrates.[101] The availability of an oxidase of this type would be of considerable interest to be used in combination with an alcohol dehydrogenase for the development of a deracemisation process as depicted in Scheme 2.

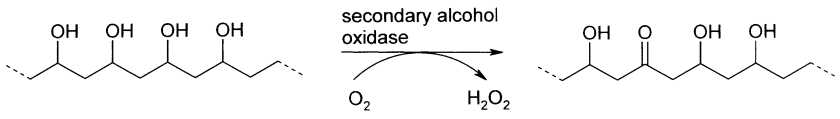
Glucose oxidase GOD [EC 1.1.3.4] is able to transform β-D-glucose to D-glucono-1,5-lactone. Due to its absolute substrate specificity it can only accept glucose and has therefore not found any synthetic application so far. It is widely used as a technical-grade enzyme as an antioxidant in the food industry.[102,109]

Pyranose-2-oxidase P2O [EC 1.1.3.10] from fungal origin oxidises D-glucose at C-2 to the corresponding ketose. Generally, for a substrate of P2O an equatorially orientated 2-hydroxy group in a six-membered pyranoid saccharide is essential. This enzyme has been applied in a synthetic procedure where it is used for the production of D-fructose from D-glucose in the so-called 'Cetus process' (Scheme 16). Thus, D-glucose is first converted to 2-keto-D-glucose by immobilised P2O from *Polyporus obtusus,* followed by chemical hydrogenation to furnish highly pure D-fructose.[110,111] Another application of P2O is the production of 5-keto-D-fructose under aerobic cofactor regeneration.[112] Pyranose-2-oxidase is also used in clinical chemistry for the enzymatic determination of 1,5-anhydro-D-glucitol, an important marker for glycemic control in diabetes patients.[103]

Glycolate oxidase [EC 1.1.3.15] is found in the leaves of many green plants and in mammalian liver. An

Table 4. Alcohol oxidases (AOx) for oxidation of primary alcohols to aldehydes.

Enzyme	Source	Substrate	Co-factor	Sub. conc. [mmol L^{-1}]	Ref.
aliphatic AOx	e.g., *Candida boidinii*	*n*-$C_xH_{2x+1}OH$, x = 1–5, allyl alcohol	FAD	5	[91,92]
aromatic AOx	White-rot fungi	R-C_6H_4-CH_2OH	FAD	2	[93,94]
galactose oxidase	*Dactylium dendroides*	galactose, glycerol, 2-propyn-1-ol, dihydroxyacetone	PQQ	~50	[95,96]

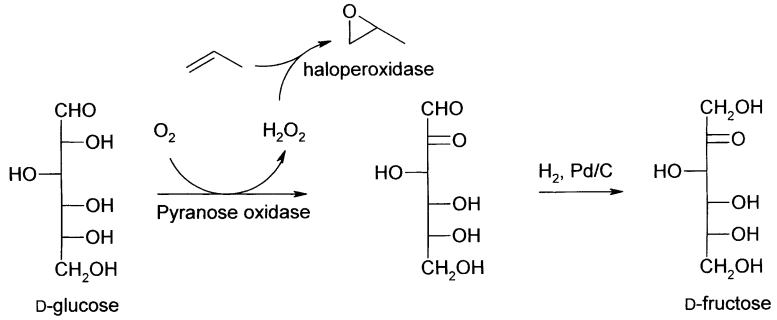


Scheme 15. First degradation step of polyvinyl alcohol by an oxidase.

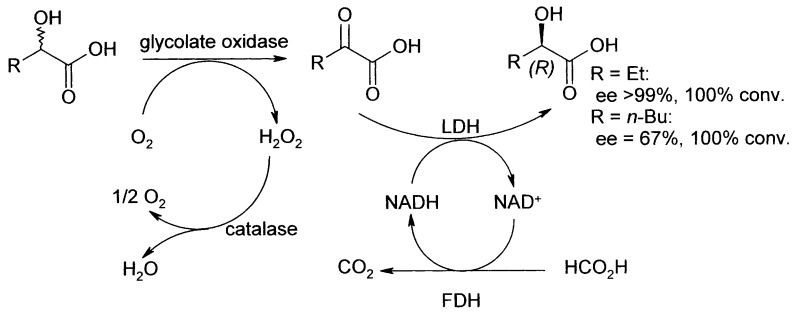
Table 5. Alcohol oxidases (AOx) for oxidation of secondary alcohols to ketones.

Enzyme	Source	Substrates	Co-factor	Sub. conc. [mmol L^{-1}]	Ref.
sec-AOx	*Pseudomonas*	2-hexanol, 2-heptanol, 2-octanol, 4-decanol, cyclohexanol, benzyl alcohol	Fe^{2+}	60 – 100	[101]
glucose oxidase	*Aspergillus niger*	glucose	FAD	d.n.g.	[102]
pyranose-2-oxidase	fungi	pyranoid saccharides + equatorial 2-OH	FAD	11	[103]
glycolate oxidase	plant leaves, mammalian liver	2-hydroxy acids	FMN	1 – 7	[104]
cholesterol oxidase	*Nocardia* sp.	cholesterol	FAD	0.036 – 70	[105]
vanillyl AOx	*Penicillium simplicissimum*	*p*-hydroxybenzyl alcohols	FAD/Flavin	0.3	[106]

d.n.g. = data not given.



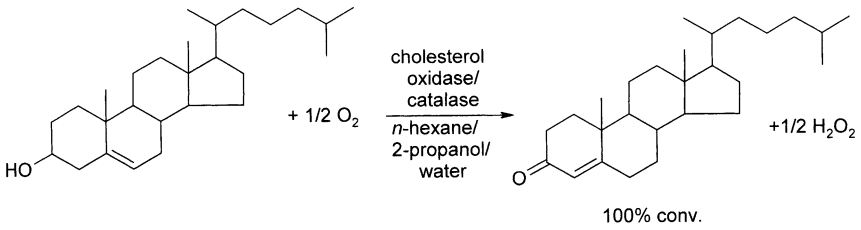
Scheme 16. Cetus process for the production of D-fructose.



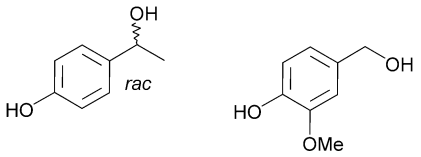
Scheme 17. Glycolate oxidase/LDH catalysed stepwise deracemisation of *rac*-2-hydroxy acids. LDH = lactate dehydrogenase, FDH = formate dehydrogenase.

enzyme isolated from spinach was applied to the enantioselective α-oxidation of various 2-hydroxy acids yielding the corresponding 2-keto acid and the non-converted (*R*)-α-hydroxy acid. A quantitative transformation of racemic 2-hydroxy acids into the (*R*)-stereoisomers using glycolate oxidase in combination with D-lactate dehydrogenase (LDH) and formate dehydrogenase (FDH) was performed in a stepwise manner (Scheme 17) yielding the desired products in high ee ($>99\%$, $R = CH_3$) and yield (100%).[104]

Cholesterol oxidase [EC 1.1.3.6], of which the natural role is the oxidation of cholesterol to cholestenone, is chemically exploited for regioselective oxidations. Since the enzyme is naturally adapted to an extremely lipophilic substrate, it can also be used in biphasic systems or even in neat organic solvents. For example, a preparative-scale oxidation of cholesterol has been achieved in a mixture of *n*-hexane, 2-propanol, and water giving a quantitative yield of cholestenone (Scheme 18).[105]



Scheme 18. Aerobic oxidation of cholesterol by cholesterol oxidase in a ternary solvent system.



Scheme 19. Primary and secondary benzylic alcohols oxidised by vanillyl alcohol oxidase.

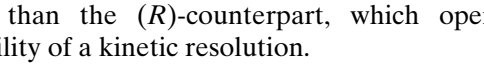
An alcohol oxidase isolated from *Penicillium simplicissimum* showed a strong preference for electron-rich primary and secondary benzylic alcohols and was termed 'vanillyl alcohol oxidase' [EC 1.1.3.38]. Its catalytic mechanism, enzyme structure and biochemical significance as well as its biotechnological potential were investigated in great detail (Scheme 19).[106] For *sec*-benzylic alcohols, the (*S*)-enantiomer is oxidised faster than the (*R*)-counterpart, which opens the possibility of a kinetic resolution.

4.4 Commercial Enzymes

For practical applications it is important to note that an impressive number of oxidases is commercially available, for example, alcohol oxidases from *Pichia pastoris*, *Aspergillus niger*, *Candida boidinii*, and *Hansenula* sp.; glucose oxidase from *Aspergillus niger*; cholesterol oxidase from *Nocardia erythropolis*, *Brevibacterium*, *Streptomyces* sp., *Cellulomonas* sp., and *Pseudomonas* sp.; galactose oxidase from *Dactylium dendroides*, and glycolate oxidase from sugar beet.

5 Peroxidases

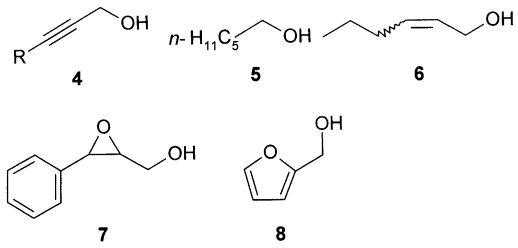
Peroxidases [EC 1.11.1.x] are ubiquitous in nature and are found in plants, microorganisms and animals. They have in common that they accept hydrogen peroxide (or a derivative thereof, such as an alkyl hydroperoxide) as oxidant by producing water as by-product (Scheme 20). Most peroxidases studied to date contain ferric protoporphyrin IX as the prosthetic group and act through a high-valence iron-oxo species.[12,113] Furthermore, selenium- (glutathione peroxidase),[114] manganese- (manganese peroxidase)[115], vanadium peroxidase-[116] and flavin-dependent peroxidases (flavoperoxidase) are known.[117]



Scheme 20. Alcohol oxidation catalysed by peroxidases.

The general dilemma concerning peroxidases is that they are not only rapidly inactivated by the peroxide they depend on, but also by substrate radicals emerging during the catalytic cycle. As a consequence, their practical use is generally impeded by limited productivity.

To date, only a limited number of selective oxidations of primary aliphatic, allylic, propargylic and benzylic alcohols to the corresponding aldehydes employing chloroperoxidase from *Caldariomyces fumago* [EC 1.11.1.10] are known (Table 6) with substrate concentration ranging within 20 and 100 mmol L^{-1}.



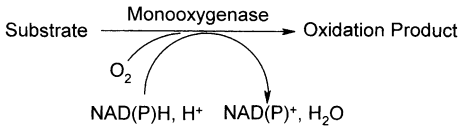
6 Monooxygenases

For preparative-scale biotransformations, monooxygenases are predominantly used for C–H hydroxylation,

Table 6. Primary alcohol oxidation to aldehyde by chloroperoxidase.

Substrate	R	Conc. [mmol L^{-1}]	Conversion [%]	Ref.
4	Me	100	94	[118]
4	Et	100	95	[118]
4	Ph	100	92	[118]
5	–	30	81	[119]
(*E*)-**6**	–	30	99	[119]
(*Z*)-**6**	–	30	95	[119]
7	–	30	46[a]	[119]
8	–	20	92	[120]

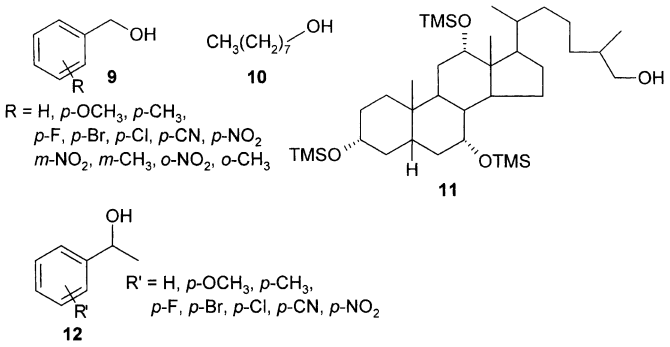
[a] Alcohol **7** showed 45% ee.



Scheme 21. Monooxygenase reaction.

Baeyer–Villiger oxidation, alkene epoxidation and heteroatom (sulfur) oxidation. [121] Although the enzymes accept environmentally benign molecular oxygen as oxidant, its chemical activation is extremely complex. As a consequence, monooxygenases are rather unstable enzymes, some consisting of multiple components, which might be membrane-bound and require cofactors such as NAD(P)H, which require regeneration (Scheme 21).[122]

Due to the complex redox cycle of monooxygenase reactions, whole-cell systems are preferred over isolated enzymes for biocatalytic transformations. In the majority of cases, alcohol oxidation to the corresponding aldehyde or ketone has been observed as an undesired 'over – oxidation' in parallel to the desired the formation of the alcohol by oxidation of a C–H bond. Thus, the use of monooxygenases for alcohol oxidation is limited and therefore just a selection of transformed substrates will be given. A screen of literature data reveals that the oxidation of primary alcohols to the corresponding aldehyde prevails, e.g., substituted benzyl alcohols **9**,[123] but also examples for aliphatic primary alcohols (1-octanol) **10**[124] or the partially protected cholestantetrol derivative **11**[125] are described. As an example for a secondary alcohol, substituted 1-phenylethanols **12** were transformed to the corresponding ketones.[123]



Going in line with the multiple oxidative capabilities of monooxygenases (see above), the oxidation of primary alcohols often does not stop at the aldehyde stage, but proceeds through to the corresponding acid.[126] However, depending on the reaction, careful analysis of the reaction kinetics allowed one to produce 3,4-dimethylbenzaldehyde **10** or acid **11** from the benzylic alcohol **9** as the major product (Scheme 22).

The development of a sturdy monooxygenase biocatalyst and the transfer of a monooxygenase process from laboratory tests to an industry scale is demanding and time-consuming.[127] For example, stepwise oxidation of *trans*-2-butene catalysed by the methane monooxygenase system of whole cells of *Methylosinus trichosporium* gave crotyl alcohol and *trans*-crotonaldehyde in a 2:1 ratio.[128]

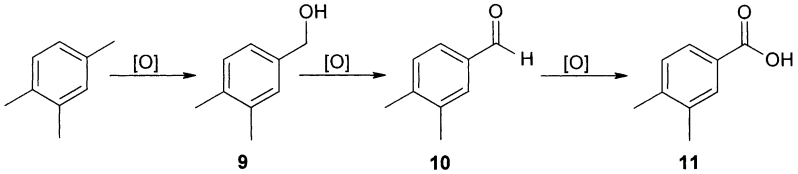
7 Whole-Cell Oxidations (Unknown Enzymes)

Whole-cell oxidation of alcohols, where the actual enzyme system(s) involved is/are not known are summarised in this chapter.

For the whole-cell oxidative kinetic resolution of secondary alcohols a variety of organisms has been used (Table 7). Most organisms were employed under fermenting conditions, which ensures that the majority of metabolic pathways are active, which facilitates further (oxidative) degradation of the formed carbonyl compound, e.g., by Baeyer–Villiger-type reaction. After all, the driving force of life within a fermenting organism is the complete degradation of a carbon source and not the synthesis of a desired carbonyl compound.

In order to maintain the full viability of (fermenting) cells, the substrate concentration employed has to be kept rather low (reported maximum at 100 mmol L^{-1}, see Table 7). Depending on the strain, the substrate spectrum encompasses a wide range of bulky (e.g., 1-phenyl-3-undecanol[141]) as well as sterically less demanding alcohols [e.g., methyl-aryl carbinols,[135,137] 1-(hetero)aryl-2-propanols,[133] aliphatic alcohols[134,141,142]], ols[140,142] and α-hydroxy acids.[129]

Resting cells with a 'sleeping' carbon metabolism in the absence of any source of energy were used for the production of optically active 2-hydroxy-1-indanone.[130] Depending on whether the *cis*- or the *trans*-diastereom-



Scheme 22. Benzylic hydroxylation followed by alcohol and aldehyde oxidations catalysed by xylene monooxygenase of *Pseudomonas putida* mt-2.

Table 7. Whole-cell oxidation of primary (*prim*)/secondary (*sec*) alcohols with unknown enzyme(s) involved.

Organism	*sec*/*prim*	Alcohol	Conc. [mmol L^{-1}]	Cells used	Ref.
Alcaligenes bronchisepticus	*sec*	(substituted) mandelic acid	~20	fermenting	[129]
Arthrobacter sp.	*sec*	1,2-indanediols	up to 100	resting	[130]
Acinetobacter sp.	*prim*	2-phenyl-1-propanol	20–25	fermenting	[131,132]
Bacillus stearothermophilus	*sec*	1-(het)aryl-2-propanols, aliphatic	up to 12	fermenting	[133,134]
Geotrichum candidum	*sec*	alkyl-aryl-carbinols	up to 30	resting, immobilised	[135]
Gluconobacter oxydans	*prim*	aliphatic, benzylic	~21	fermenting	[136]
Nocardia corralina B-276	*sec, prim*	cyclic, benzylic	3–6	fermenting	[137–139]
Pseudomonas aeruginosa	*sec*	1,2-indanediols	up to 100	resting	[130]
Pseudomonas paucimobilis	*sec*	1-(het)aryl-2-propanols	up to 12	fermenting	[133]
Rhodococcus equi IFO 3730	*sec*	cycloalkanols	up to 60	fermenting	[140]
		alkanols, alkenols	up to 60	fermenting	[141]
Yarrowia lipolytica sp.	*sec*	cyclic, aliphatic	up to 20	fermenting	[142]

er of *rac*-1,2-indandiol was used, either (*R*)- or (*S*)-2-hydroxy-1-indanone was obtained upon whole-cell oxidation with *Arthrobacter* sp. 1HE. Resting immobilised cells of *Geotrichum candidum* were successfully employed for the oxidative kinetic resolution of *rac*-1-aryl-alkanols. Various carbonyl compounds, such as cyclohexanone or chloroacetone served as hydrogen acceptors.[135]

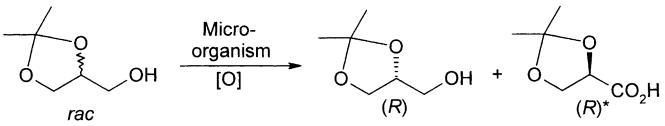
Due to the intrinsic enantiodiscrimination of *sec*-alcohols by oxidative enzymes, the maximum yield of formed ketone plus remaining non-converted secondary alcohol is 50% each with highly enantioselective enzymes. For low to moderate selectivities, the reaction rate slows down at about mid-way, since the activity for the remaining (slower converted) enantiomer is reduced. In cases where the enantiopure alcohol is the desired product, the ketone may be recycled either by non-selective reduction (e.g., $NaBH_4$), or – more elegant – by stereoselective reduction using a different organism possessing a matching *opposite* stereopreference.[134] The problem of enantiodiscrimination is avoided when non-chiral *sec*-alcohols are used instead of a racemate. For instance, cyclohexanol or cyclododecanol were oxidised to the corresponding ketones in 76 and 95%, GC yield, respectively.[140]

The use of whole viable cells implies an unknown number of oxidative enzymes being potentially active. Thus, the outcome of a given reaction not only depends on the strain being used and the particular substrate structure, but also on the biological status of the cells, such as age, growth conditions and growth history, as well as the presence of potential inducers. As a consequence, the predictability of whole-cell alcohol oxidations and the potential product pattern with respect to regio-, chemo- and enantioselectivities is generally low. For instance, no regiodiscrimination between primary and secondary alcohols was observed with *Nocardia corralina* B-276,[137–139] the opposite was found for fermenting cells of *Gluconobacter oxydans* DSM 2343: biooxidation of 1,3-butanediol proceeded with complete chemoselectivity of one primary alcohol group to furnish 3-hydroxybutanal, albeit at low isolated yield (35%).[136] Despite the disadvantages of whole-cell oxidations mentioned above, an impressive number of successful processes has been reported, which are discussed below.

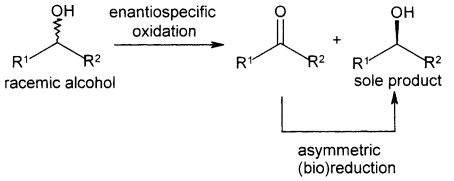
7.1 Application

Various fermenting microorganisms, such as *Rhodococcus* spp., *Corynebacterium* spp., *Nocardia* spp. or *Mycobacterium rhodochrous* were used for the enantioselective oxidation of (*S*)-isopropylideneglycerol to (*R*)-isopropylideneglyceric acid starting from a racemic mixture (Scheme 23). The untouched (*R*)-enantiomer as well as the obtained chiral acid are important chiral C_3-building blocks.[143]

Racemic mixtures of secondary alcohols can be resolved completely by enantiospecific enzyme-catalysed oxidation resulting in one enantiomer of the alcohol and the ketone followed by asymmetric enzyme-



Scheme 23. Microbial oxidative kinetic resolution of *rac*-isopropylidene glycerol. * Switch in CIP-sequence priority.



Scheme 24. Deracemisation of *sec*-alcohols by enantioselective oxidation followed by stereoselective reduction with opposite enantiopreference .

catalysed reduction of the latter (Scheme 24). For this process, either two separate microorganisms for each step may be used,[144–146] or a single microorganism, possessing the desired enzyme activities showing opposite enantiopreference.[147–149]

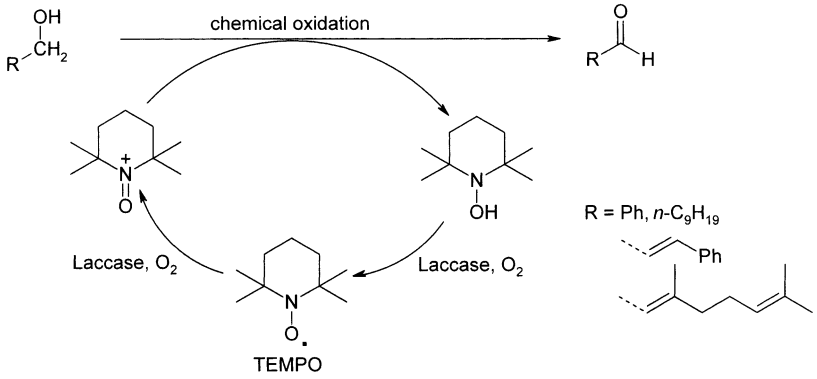
8 Combinations of Biocatalytic and Chemical Methods

An interesting approach to use molecular oxygen as terminal oxidant without the occurrence of undesired hydrogen peroxide as by-product was developed by combination of an organic oxidation method with biocatalysis. Thus, TEMPO (2,2,6,6-tetramethylpiperidin-1-yloxy, free radical) – acting as the actual oxidant in catalytic amounts for the oxidation of a primary alcohol to furnish the corresponding aldehyde – was recycled by using the enzyme laccase [EC 1.10.3.2] from the fungus *Trametes villosa* (viz. *Poliporus pinsitus*) at the expense of molecular oxygen (Scheme 25, Table 8).[150] Laccases can be found in many plants and fungi.[151] In contrast to other oxidases, laccases reduce molecular oxygen to form water. Laccase is commercially available from various higher fungi, such as *Trametes versicolor*, *Agaricus bisporus*, *Trametes* sp., *Agaricus bisporus*, and *Rhus vernificera*.

Especially electronically activated primary (benzylic and allylic) alcohols (Table 8), namely substituted benzyl alcohols, cinnamyl alcohol and geraniol were selectively oxidised to the corresponding aldehydes with good yield (> 90%), while over-oxidation was largely avoided. Other non-activated primary alcohols were oxidised rather slowly. Among the secondary alcohols investigated, only 1-(*p*-methoxyphenyl)-ethanol and 1-(*p*-methoxyphenyl)-1-propanol were oxidised with 85% and 95% yield, respectively. The mechanism of TEMPO[152] and related oxidative mediators, such as ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)[153] were investigated recently.[154] Although the approach looks very promising, any (regio)selectivities will be low in comparison to those exerted by an enzyme, since TEMPO is only interacting with the substrate without the catalytic aid of an enzyme. On the other

Table 8. Substrates oxidised using the combined approach.[150]

Substrate	Product	GC-Yield [%]
Ph-CH_2OH	Ph-CH=O	91
cinnamyl alcohol	cinnamyl aldehyde	94
geraniol	geranial	96
1-decanol	decanal	58
1-(*p*-methoxyphenyl)-ethanol	1-(*p*-methoxyphenyl)-ethanone	85
1-(*p*-methoxyphenyl)-1-propanol	1-(*p*-methoxyphenyl)-1-propanone	95



Scheme 25. Enzymatic recycling of the organic oxidant TEMPO by using O_2/laccase.

hand, for cases where total oxidation of a *rac-sec*-alcohol to the corresponding ketone is desired, the system has the advantage that TEMPO cannot show any enantiodiscrimination. For large-scale applications, optimisation with respect to the substrate concentration (20 mM reported) and the ratio of substrate/mediator (TEMPO, actual value of 10:3) is certainly required.

9 Conclusion

Despite the fact that only few industrial applications for the biooxidation of alcohols have been reported to date, the survey of methods within this review reveals that the potential of enzymes for the chemo-, regio- and enantioselective oxidation of alcohols is largely 'underexploited'. Driven by the increasing need for environmentally benign 'green' oxidative methods, it is expected that more oxidative enzymes showing increased operational stability and improvements in terms of the productivity of the existing methods will be developed in the near future.

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References and Notes

[1] Other metals, such as Ru, Ce, Ag, Pb, Mo, Os and Ni have been used more scarcely.
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