Alcohol Dehydrogenase from *Lactobacillus brevis*: A Versatile Robust Catalyst for Enantioselective Transformations

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Review
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The alcohol dehydrogenase from *Lactobacillus brevis* (*Lb*ADH) is a versatile catalyst for enantioselective reduction of ketones. Its substrate scope is wide with high regio- and enantioselectivity. In this critical review, we have gathered the information available on the substrate scope as well as the applications reported. Quantitative information such as productivity per catalyst, space-time yield (STY), cofactor utilisation, and stability are derived to allow comparison and assessment of practical value.

Key words:
Biocatalysis, alcohol dehydrogenase, *Lactobacillus brevis*, critical review, application

Introduction

The upcoming demand for enantiopure intermediates in the fine chemicals- and pharma-industry makes biocatalysis an increasingly profitable alternative to conventional chemical catalysis/synthesis.[1,2] Also, as the environmental footprint of a process is gaining increased attention, biocatalytic processes come to the focus of the chemical industry. The generally mild reaction conditions (moderate pH, low T, aqueous solution, no heavy metals) which characterise biocatalysis enhance this effect.

Over the years, hydrolases such as Lipase B from *Candida antarctica* (CALB) have been dominating industrial biocatalysis due to their stability and robustness even in the presence of organic solvents and reactants.

The application of alcohol dehydrogenase from *Lactobacillus brevis* (*Lb*ADH) is rising as reflected in the number of publications (Fig. 2), while the number of patent applications for this enzyme is also an indication of its potential[3–19] *Lb*ADH is a robust and versatile enzyme which catalyses the enantioselective reduction of ketones to the corresponding alcohols and requires NADPH ($+H^+$) as reduction equivalent (Fig. 1). Even in the presence of non-conventional reaction media, such as organic solvents, supercritical fluids (scF), or gaseous reactants, *Lb*ADH remains active. The excellent chemo- and enantioselectivity makes *Lb*ADH a valuable tool for the synthesis of chiral building blocks. In most cases exclusively (*R*)-alcohols are formed (typical enantiomeric excess (*ee*)>0.99, see below). The high activity of *Lb*ADH for a broad range of substrates (from simple aromatic ketones and keto-esters to branched acetophenone derivatives, see Fig. 4), is one factor for the ongoing and growing interest for this catalyst. Another factor for the rising interest is that *Lb*ADH is among the few

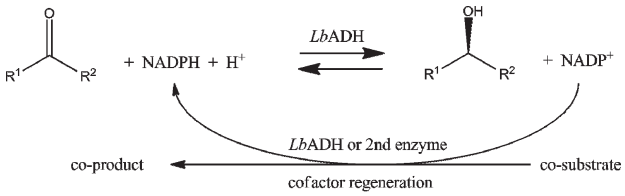

Fig. 1 – *General reaction scheme for the LbADH-catalysed reduction of a ketone to the corresponding R-alcohol and co-factor-regeneration, with $R^2 > R^1$*

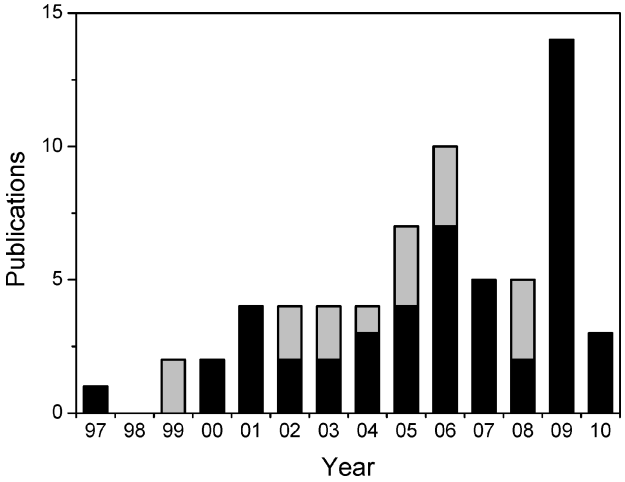

Fig. 2 – *Publications per year since discovery in 1996 (light grey: patents, dark grey: peer reviewed publications)*

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oxidoreductases with high solvent tolerance for monophasic systems with solvent addition as well as in biphasic media.

A number of reviews are available, such as Hummel[20] presenting different enzymes for the synthesis of chiral compounds, Nakamura *et al.*[21] and Daußmann *et al.*[22] for general aspects of producing chiral alcohols, Eckstein *et al.,* Hollmann *et al.,* Wichmann and Vasic-Racki[23–25] for cofactor regeneration and Müller *et al.*[26] for a more detailed insight into the asymmetric reduction of 3,5-dioxocarboxylates and propargylic ketones.

Here, we have gathered the information available on the *Lb*ADH in view of its promising activity and stability. To allow comparability, additional values following the guidelines given in Gardossi *et al.*[27] were calculated from the information as derived by the authors. To enable practical comparison not at least in view of optimisation potential, we focused on space time yield (STY) as productivity per unit volume of the reactor, absolute productivity per catalyst amount used to derive the product, and cofactor utilisation, thereby allowing a shorthand assessment of practical applicability. Occasionally, these rigid definitions led to values given within this review that differ from the ones in the original articles. Sometimes, the necessary data for the calculations could not be derived from the article alone; the corresponding authors were contacted to gather further information. Where possible, PhD theses were consulted for additional information. A table providing an additional overview can be found as electronic supporting information (ESI).

Enzyme technology

The *Lb*ADH was discovered by Hummel and coworkers during a screening in the class of *Lactobacillus* and has close homology to the alcohol dehydrogenase from *Lactobacillus kefir* (*Lk*ADH).[28] Although, the enzymes are closely related in view of amino acid sequence with only 18 residues difference *Lb*ADH is found to be exceptionally more stable than *Lk*ADH, thus a 10-fold higher yield could be achieved by the same purification protocol.[20,28] Its recombinant expression in *Escherichia coli* (*E. coli*) is also highly efficient and convenient purification protocols are available which are possible because of the high robustness the enzyme shows throughout.[3,28] The metabolic role is unknown[28] which, with the increasing significance of metagenomics and other screening strategies, will be more and more common for industrially used enzymes.

The *Lb*ADH is classified as a short chain dehydrogenase/reductase (enzyme class EC 1.1.1.2, CAS 9031-72-5).[29] It is denoted as *R*-selective. This is applicable when the formal Cahn-Ingold-Prelog priorities (CIP) match the steric demand which is coincidentally often but not necessarily the case.[30,31] For the application of *Lb*ADH the most prominent exception are α-halogen substituted ketones where the (*S*)-product is formed but the intrinsic selectivity or side of hydride addition to the prochiral ketone does not change as compared to the non-substituted homologue (see below).[32] The term *R*-selective is most often used to set the enzymes apart from the previously known ADH as with the same substrate the opposite enantiomer is derived. The *Lb*ADH was among the first commercially available dehydrogenases opening up the venue for these enantiomers. It is patented by a non-profit organisation and thus commonly available at reasonable terms as the commercial availability by enzyme suppliers underlines.[3,33] It is industrially applied for the production of ethyl-3*R*-hydroxy-butanoate on a scale of one ton per year (see below).[22,34,35]

The enzyme is a homotetramer[29,36] with molecular mass between 104–107 kDa[20,36] with monomers of 26 kDa[36,37] or 22.5 kDa[28] depending on the source.

The DNA and amino acid sequence was published.[3,20] The high resolution crystal structure for both, the apoenzyme and holoenzyme are also available (wild type: protein data bank code 1NXQ[29] and 1ZK4, mutant G37D: 1ZK4 1ZJY, 1ZJZ, 1ZK0, 1ZK1, 1ZK2, 1ZK3[37]). The high-resolution crystal structure 1ZJY was used as homology model for the computational study of the ADH from *Lactobacillus kefir* reduction of ethanal with deuterated NADPH.[38] An ample discussion of the implications of the active site can be found in Schlieben *et al.*[37] The stereospecificity can be explained by inspection of the three-dimensional model of the active site.[29] Also, in a comparative study the suitability for four diketones was investigated.[39]

The $NADP^+$-dependence of *Lb*ADH is viewed as a drawback and reaction engineering challenge, as compared to NAD^+ the phosphorylated redox cofactor is more expensive and less stable. Therefore, several approaches were taken.[9,14,37,40,41] One strategy was based on crystal structure and analysis of cofactor binding to allow the use of NAD^+. The site-directed mutagenesis to increase NAD^+-affinity was tried first. However, the best mutant apparently still had 50-fold lower affinity compared to the wild type and $NADP^+$ affinity also decreased.[37] Alternatively, mutations were introduced leading to 4-fold higher activity with NADH compared to the wild

type (v_{max} = 80 U $mg_{(protein)}^{-1}$) but the activity of the wild type with NADPH was still 4-fold higher (v_{max} = 355 U $mg_{(protein)}^{-1}$).[40] Whether activity towards a substrate was also affected was not discussed. The *Lb*ADH-mutant was used later in an oxidative kinetic resolution of phenylethanol with oxidative cofactor regeneration by a NADH oxidase from *Lactobacillus brevis*.[41]

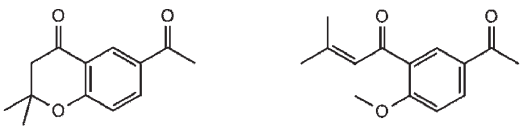
Notably, additives such as organic solvents (miscible and nonmiscible) or ionic liquids are influencing selectivity, activity, and/or half life.[42–46] An aqueous two-phase system with the ionic liquid diethyl-methyl-polyethyleneglycol ammonium chloride (Ammoeng110^{TM}) could be utilised for the extraction of *Lb*ADH. The system was optimised by experimental design.[45] The specific activity was found to be twofold higher in the ionic liquid rich phase. Furthermore, the storage half-life at 30 °C was increased 10-fold from 14 h to 142 h by addition of 30 % (w/w) ionic liquid. In biphasic systems of aqueous buffer and organic solvents storage stability[44,47] and operational stability[43,48] were found to be unusually high with half-life in the range of several hundred hours.

Biotransformations

The application of *Lb*ADH in biotransformation was pioneered by Hummel and coworkers as discussed in Hummel *et al.*[20] The bioorganic potential was exploited earlier on by the group of Müller.[26]

Substrate scope

Substrates that can be converted by *Lb*ADH are shown in Fig. 4 and classified into groups from **1** to **15**. Generally, ketones are converted that have preferential short chain substitution (methyl-, ethyl-) and are not too sterically demanding on the other residue. Notable exceptions are cyclohexanone derivatives (12),[20,49] 2-hydroxy-phenyl-propanone (11),[50,51] and diketones (8, 9, 10, Fig. 3).[39] Interestingly, only 2,3-diketones are reduced to the corresponding diols with high diastereoselectivity for 2,3-diketo-hexane and -heptane to the *syn*-alcohols (2R,3S).[39] This is in accordance with the reduction of 1,2-hydroxy ketones such as 2-hydroxy-phenyl-propanone[50,51] and hydroxy-propanone (13).[3,20] 2,4-Diketones are regioselectively reduced only in 2-position (10).[39]



6-acetyl-2,2-dimethylchroman-4-one

1-(5-acetyl-2-methoxyphenyl)-3-methylbut-2-en-1-one

Fig. 3 – *Diketones used as substrates*[39]

Acetophenone is widely used and also 4-nitro- and 4-ethyl-acetophenone, methyl-naphtyl-ketone, as well as all monosubstituted chloro-acetophenones are accepted with varying activity (11)[3,20,52–54] as is 4-acetylpyridine.[55] Benzaldehyde and propiophenone are accepted with low activity (11).[3,20]

Widely used are also ketoesters such as 2-oxo--ester (3),[3,20] 3-oxo-esters (4),[3,20,53,56–59] 4-oxo-esters (5),[3] and 5-oxo-ester (6).[3] When 3,5-dioxo-esters (7)[60–66] are transformed, the easiest accessible oxo--groups in view of steric hindrance are reduced.

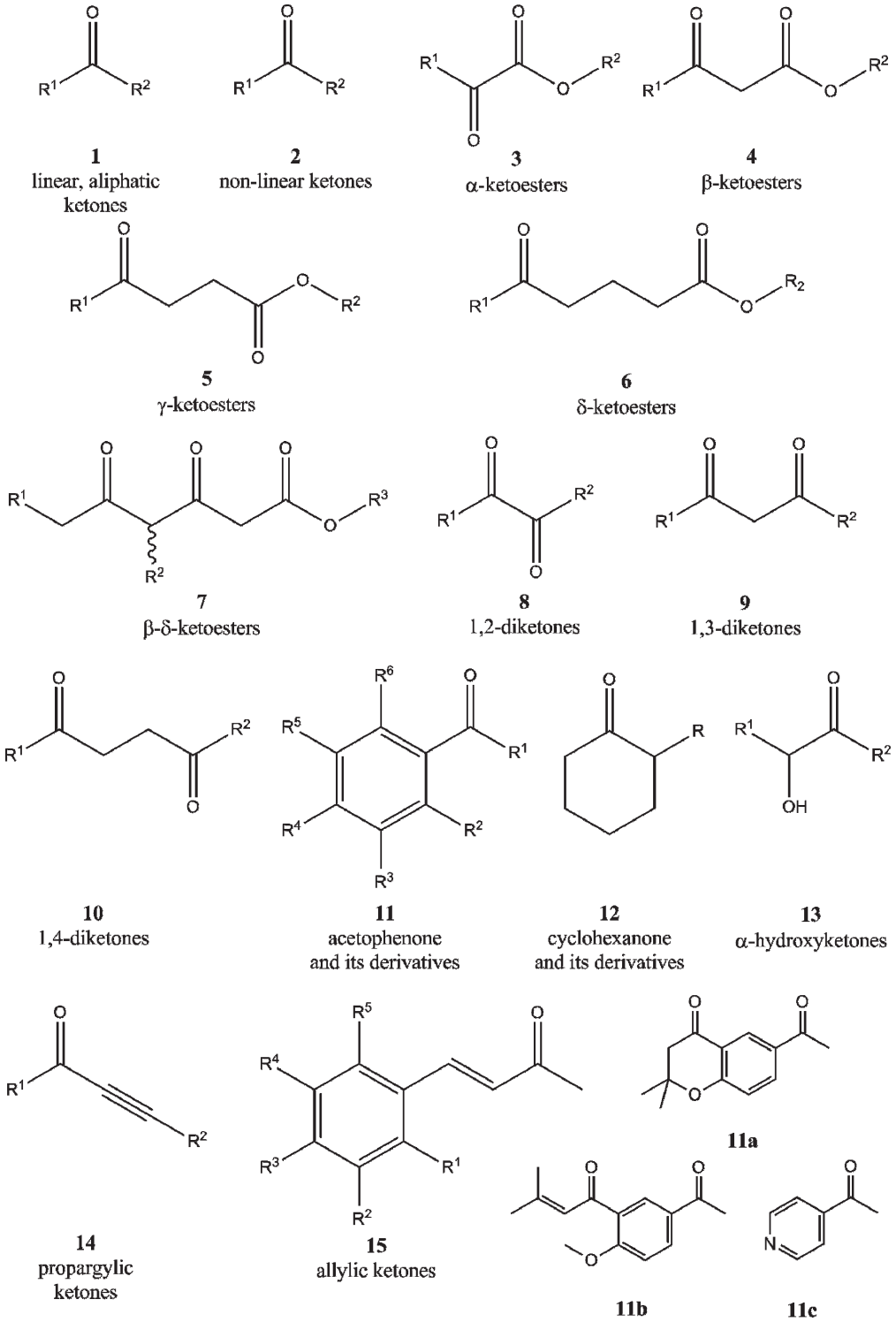
A C-C triple bond in so-called propargylic ketones is also accepted in substrates (14),[11,32,67,68] as well as C-C double bonds in allylic ketones (15).[69]

All aliphatic linear 2-oxo-alkanes from chain--lengths C_3 up to C_{11} (1) are accepted as substrates whereupon the achievable enantiomeric excesses increase with increasing chain length. Butanone gives rise to varying enantioselectivity between $\approx$ 0.32[42] and >0.90.[48] The reduction of 2-pentanone through 2-heptanone is reported without giving enantioselectivity.[70] For the reduction of 2-octanone and 2-nonanone, high enantiomeric excesses are reported.[23,42,48,52,70–72] Partly, solubilisers such as acetonitrile,[42] dioxane,[70] or ionic liquids[45,46] are used with low influence on the enantioselectivity.

Applications

Whole cells of recombinant *E. coli* were applied for the reduction of methyl-3-keto-butanoate (4) with 2-propanol as reducing agent.[57,73] The authors developed a quantitative model for the process based on *in vitro* kinetics and metabolic cofactor concentration with more than 40 model parameters. The regeneration of the cofactor and *Lb*ADH expression were identified as rate limiting. The limitation in view of cofactor regeneration could apparently be circumvented by coexpression of a NAD^+ dependent formate dehydrogenase (FDH) from *Mycobacterium vaccae* N10 along with *Lb*ADH[58] with an approximately doubled productivity per cell mass (40 mmol g_{CDM}^{-1} d^{-1}) *vs.* 290 mmol g_{CWM}^{-1} d^{-1}) (CWM: cell wet mass, CDM: cell dry mass).

Alginate immobilised whole cells were used for transformations of a variety of β-keto esters (4). Immobilisation allowed up to 10 recycles without apparent loss in activity.[59] The higher the cell loading in the immobilisates, the slower the apparent reaction rate or the conversion obtained after a given time. Cell agglomeration and mass transport limitations were discussed briefly by the authors. Recycling improved as after 14 cycles, the conversion with free cells had dropped from more than 0.9 to 0.05, whereas with the immobilised cells conver-



F i g . 4 – *Overview of substrate motifs as converted by LbADH to the corresponding R-ketones, possible residues can be found in the ESI*

sion better than 0.7 was obtained, which is in line with cell recovery. Continuous experiments in a packed bed plug flow reactor could be run for more than 40 h (13 residence times) at STY = 25 g L^{-1} h^{-1} for ethyl-(*R*)-3-hydroxybutyrate. Both in the batch-wise recycling as well as in the continuous reactions, the immobilisation conditions such as pH and ions used in the hardening, affected the stability of the catalyst system.

A whole cell biotransformation with over-expressed *Lb*ADH and FDH from *Mycobacterium vaccae* N10 in *E. coli* for the reduction of 4-chloro--acetophenone (11), ethyl-4-chloroacetoacetate (4), and 1-phenyl-2-chloroethanone (11) was performed

in biphasic systems with 9 and 10 ionic liquids, respectively.[53,54] The authors proposed a selection procedure for the ionic liquid based on testing of the membrane integrity and rating criteria.[53] In the follow-up work, a FDH from *Candida bodinii* was used for the reduction of 2-octanone (1) and 4-chloro-acetophenone (4).[52] The number of ionic liquids was increased to 21. As in the previous work, a bis[(trifluormethyl)sulfonyl]amide (BTA) based ionic liquid gave best yields and was chosen for a 200 mL scale-up to a fed batch with 0.18 kg L^{-1} d^{-1}.

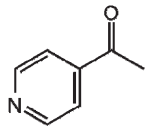
The reduction of *tert*-butyl-6-chloro-3,5-dioxo--hexanoate (7) to *tert*-butyl-6-chloro-5*R*-hydroxy--3-oxo-hexanoate was chosen for optimisation of reaction conditions as the best candidate for statin synthesis among 12 substrates converted by *Lb*ADH.[10,61,62,65] The chemical side reaction of this specific substrate, namely the elimination of HCl giving a stable 5-member furanone, was suppressed by adjusting pH to 5.5 and keeping the substrate concentration low *via* fed batch operation of the stirred loop reactor.[60,66] Alternatively, a biphasic approach with methyl-*tert*-butylether (MTBE) as non-reactive phase was described.[47,61,66] At higher concentrations, this approach gave up to 10-fold higher turnover numbers of the cofactor $NADP^+$. However, selectivity with the competing chemical side reaction dropped to 0.7 with increased substrate concentration. For acetophenone (11) at low concentrations of 1 mmol L^{-1}, a repetitive batch with at least four recycles is described and the enzyme half-life is given as 480 h.[47,66]

The influence of acetonitrile and 1,4-dioxane as cosolvents for the reduction of butanone (1) on enantiomeric excess and half-life was investigated.[42] Half-life was generally reduced with increasing molar fraction of organic cosolvents from 400 h in buffer down to 1.6 h with $x = 0.10$ acetonitrile. Enantioselectivity also depended on the molar fraction of these cosolvents and marginally increased with higher amounts from 0.37 in aqueous buffer to 0.43 with $x = 0.100$ and $x = 0.050$ with acetonitrile and 0.40 at $x = 0.100$ 1,4-dioxane.[42]

To convert hardly water-soluble ketones such as 2-octanone, 3-octanone, 2-nonanone and 2-decanone (1), ionic liquids (IL) were used as solubiliser.[46] For cofactor regeneration glucose dehydrogenase (GDH) catalysed oxidation of glucose was chosen. From an initial set of 10 water-miscible IL, the AMMOENG^{TM}101 was subsequently used. The kinetic characterisation revealed that AMMOENG^{TM}101 activated and stabilised the *Lb*ADH. For all four ketones tested, product inhibition was much lower when 200 g L^{-1} IL was added compared to the pure buffer. The half-life increased from 49 h to 158 h in 200 g L^{-1} IL/buffer-mixture. Optimised batches with cofactor regeneration *via* a glucose dehydrogenase and glucose as co-substrate gave TON_{LbADH} of 842,000, TON_{GDH} of 19,000 and TON_{NADP^+} of 800.

The immobilisation of *Lb*ADH on a commercial amino-epoxy support was optimised aiming for increased stability.[74,75] Immobilisation yielded 0.15 of the activity and half-life of about 20 h at 30 °C similar to the one found in solution, in line with the observation that the enzyme is readily desorbed in a 1 mol L^{-1} sodium chloride solution. However, treatment with glutardialdehyde gave half-life of more than 1000 h with 0.4 activity before treatment. Combination of mercaptoethanol and glutardialdehyde treatment yielded 0.2 of activity with a half-life of 500 h. The process stability was demonstrated in a packed bed plug flow reactor where the immobilised enzyme with combined treatment gave a steady state conversion of 0.6 over more than 1500 h (1 h residence time) for the reduction of acetophenone (11) *via* substrate coupled cofactor regeneration with 2-propanol.

An alternate method for immobilising enzymes or rather *Lb*ADH together with the cofactor is described by.[5,55] Both enzyme and cofactor were absorbed by a superabsorbent polymer, namely Favor®, and dried afterwards. The so prepared catalyst was then used for the enantioselective reduction of acetophenone, 4-acetylpyridine (**11**, Fig. 5) and ethyl-3-oxobutanoate (4). Exclusively the *R*-enantiomer was formed with almost quantitative conversion. The superabsorbed catalyst was easily separated from the reaction mixture and reused four times in a repetitive batch mode. In total, 0.016 mmol product per U were synthesised with $TON_{NADP^+} = 900$.



4-acetylpyridine

Fig. 5 – *4-acetylpyridine used as substrate by*[55]

For the application of whole cells in the continuous synthesis of methyl-(*R*)-3-hydroxybutanoate (4), different cofactor regeneration techniques were tested.[72,73] Cells, over-expressing *Lb*ADH were used in the substrate coupled approach with 2-propanol, or regeneration enzymes like glucose dehydrogenase from *Bacillus megaterium* (GDH together with glucose facilitator GLF from *Zymomonas mobilis*) or formate dehydrogenase (FDH) from *Mycobacterium vaccae* N10 were coexpressed (GDH/GLF,FDH in analogy to[58]). The performance of the so produced cells was subsequently exam-

ined in a stirred loop reactor (Fig. 6) with retention of the cells in a bypass. The operational stability of both of the enzyme coupled approaches turned out to be rather low with deactivation constants of 0.96 d^{-1} (FDH, half-life 17 h) and 0.219 d^{-1} (GDH, half-life 76 h). The deactivation constant using 2-propanol for cofactor regeneration was two orders of magnitude smaller (0.0059 d^{-1}, half-life 2500 h) leading to a stable process for 45 days with a maximum STY of 6 mol L^{-1} d^{-1}. Producing 2-butanol (1) with the same setup gave a 3-fold higher deactivation of 0.016 d^{-1} (half-life >1000 h) with a maximum STY of 4 mol L^{-1} d^{-1}. For methyl-(*R*)-3-hydroxybutanoate the *ee* was >0.99. For 2-butanol no data concerning *ee* is given.

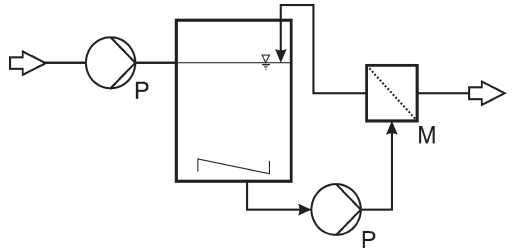


Fig. 6 – *Stirred loop reactor with filtration as used by Schroer et al.,*[72] *P = pump, M = filtration module*

In a two-phase system with MTBE as second phase, using isolated enzymes, the enzyme coupled cofactor regeneration seemed to be favourable.[23] For the reduction of 2-octanone (1), cofactor regeneration with 2-propanol or with GDH/glucose was adopted. In this case, the 2-propanol system was inferior to the GDH/glucose system due to equilibrium constraints. So, a conversion of 75 % was reached with the GDH/glucose system within 1 h, with 2-propanol as reducing agent, the same conversion was reached only after a reaction time of almost 2 h. Also, the authors hint towards repetitive batch application by replacing the organic phase.[23]

If cofactor regeneration is done *via* oxidation of 2-propanol, removal of acetone is always an issue regarding equilibrium constraints. In Eckstein *et al.*[71] 2-octanone (1) was used as substrate with isolated *Lb*ADH. To remove acetone, two two-phase approaches were tested, one with MTBE and one with [BMIM][$CF_3(SO_2)_2N$] as second phase. The partition coefficient for acetone in the IL/buffer system was higher (2.0) than for 2-propanol (0.4) in contrast to the equal partition coefficients for both components in the MTBE/buffer-system with 1.1/1.0, respectively. Thus, the selective extraction of acetone in the IL/buffer-system led to a higher reaction rate than in the MTBE/buffer-system. The conversion was not strongly affected in this case, which was probably due to the 200-fold excess of 2-propanol.[71] Another approach used the same IL, [BMIM][$CF_3(SO_2)_2N$], and MTBE as second phase for *in situ* acetone removal.[76] In this case, the conversion of 1-phenyl-2-propanone (2) was investigated. If MTBE was used as a second phase, the yield was restricted to 24 %, using the ionic liquid [BMIM][$CF_3(SO_2)_2N$] as a second phase, yields of 95 % could be achieved. This fact is due to the different partition behaviour of acetone in the buffer/MTBE and buffer/[BMIM][$CF_3(SO_2)_2N$] system. In the enantioselective reduction of 2,5-hexanedione (5) to the corresponding diol, equilibrium constraints play a key role if the substrate coupled approach is used because two equivalents of acetone are formed. Different non-extractive acetone removal techniques like stripping and pervaporation were tested.[73,76] Without acetone removal, the yield was limited to 55 %. Pervaporation and stripping led to increased yields of 90 and 95 %, respectively. Further stability investigations showed that pervaporation guarantees the highest catalyst stability when compared to stripping and extraction with IL. These findings were later applied for the continuous synthesis of the same target molecule, 2(*R*),5(*R*)-hexanediol.[73,77] A continuous setup was built up with *in situ* acetone removal by pervaporation, leading to a maximum space time yield of 1.4 mol L^{-1} d^{-1} at a maximum yield of 77 %.

In contrast to the above-mentioned findings, in[78] results are reported when reducing butanone (1) to (*R*)-2-butanol using 2-propanol as reducing agent. After 2 h reaction time, generally higher conversions and comparable *ee* were reached when using a MTBE/buffer-system instead of a [PMIM][PF_6]/buffer-system, although the IL showed miscibility with acetone and no miscibility with the reducing agent 2-propanol. An alternative approach was tested with a malate dehydrogenase (MDH) and L-malic acid as reducing agent, but *ee* were lower. Hence, 2-propanol in a MTBE/buffer-system was tested for the continuous production of (*R*)-2-butanol in a minimum volume (2 to 5 mL) biphasic reactor with aqueous buffer and MTBE.[43,48] A space-time yield of 200 mmol L^{-1} d^{-1} with the *ee* starting from ≈ 95 % dropping to ≈ 85 % after 90 h operation is reported.

The production of β-hydroxyesters (4) on an industrial scale was already established by Wacker (Burghausen, Germany).[7,22,34,35,56] In repetitive batch, methyl-3-oxobutanoate was reduced to the corresponding hydroxybutanoate, the cofactor regeneration was achieved by substrate coupled regeneration with 2-propanol. The co-product acetone was removed by reduced pressure and the target product was isolated from the reaction mixture by continuous

extraction with MTBE and subsequent distillation of the solvent. The product-free enzyme-solution was then re-used leading to a TON for the cofactor of 74,000 and a space-time-yield of 92 g L^{-1} d^{-1}.

For the prediction of thermodynamic conversion and yield in various biphasic systems *Lb*ADH was used as model catalyst for the reduction of acetophenone (11) with 2-propanol. An analytical equation was derived to allow prediction of conversion and yield from the equilibrium constant and the partition coefficients, and the implications for maximising them are discussed.[49] The prediction is shown for 8 biphasic systems including two ionic liquids. The lack of data in view of equilibrium constants and possible means of deriving them from alternative sources are further discussed and evaluated.[70] The approach with computational chemistry was developed further.[79,80]

Freeze-dried preparations of *Lb*ADH are active for conversion of gaseous reactants. By optimising immobilisation conditions on coated glass beads with the addition of sucrose, a half-life of 40 days under reaction conditions in a packed bed plug flow reactor was possible.[81] The effect of sucrose on the adsorption isotherms was investigated in detail later showing that sucrose lowers water adsorption per protein as well as the adsorption of acetophenone (11) and isopropanol.[82] Furthermore, the enzyme coated glass beads tended to decrease in protein loading and sinter in the presence of water, as shown by scanning electron microscopy (SEM). In a study centered on yeast ADH (yADH) the influence of pressure during freeze drying was investigated and specific activity after redissolution was found to be up 3-fold higher than of the initial *Lb*ADH preparation at about 40 kPa.[83] The effect of pressure, water activity, cofactor to protein ratio, and temperature on the reduction of acetophenone by an immobilised *Lb*ADH were investigated. *Lb*ADH was applied as lyophilisate on glass beads in a packed bed reactor for the reduction of acetophenone with STY of up to 1 kg L^{-1} d^{-1} at 60 °C calculated on the basis of the packed volume at half-life of 1 day.[84] The authors point out that operational half-life for gas phase reactions cannot be correlated with storage stability.

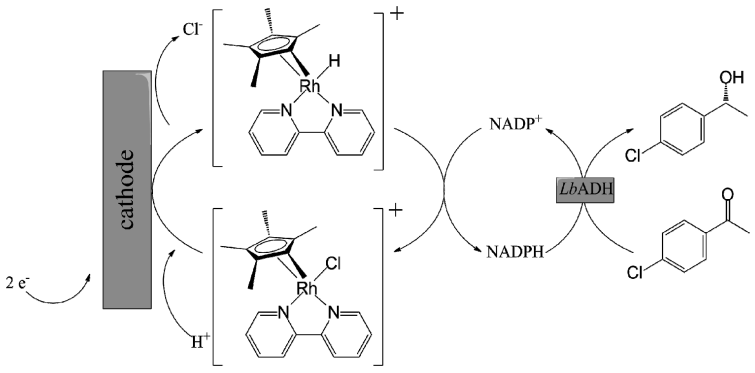
Dense propane can also be applied as non-reactive phase for *Lb*ADH catalysed transformations.[85] Both an aqueous/dense propane-biphasic system or a monophasic dense propane system with *Lb*ADH -lyophilisate immobilised on glass beads were investigated.[85] For the synthesis of (*R*)-phenylethanol (11), using the biphasic system led to 90 % conversion of acetophenone whereas the reaction with immobilised *Lb*ADH gave only 45 % conversion. This was in contrast to deactivation investigations. At 30 bar propane, in aqueous solution *Lb*ADH was less stable ($t_{1/2}$ = 0.2 h, 35 °C) than a corresponding freeze-dried preparation ($t_{1/2} \approx$ 1 h, 34 °C).

Several multi-step one-pot syntheses including one step being catalysed by *Lb*ADH are reported.[69,86,87] Chiral allylic alcohols (15) were obtained in a two-step synthesis by converting the product, an allylic ketone, of a Pd-catalysed Heck-reaction of aryliodides with butenone by simply adding buffer, cofactor, 2-propanol and *Lb*ADH to the transition-metal containing reaction mixture.[69] High space-time-yields of >1.0 mol L^{-1} d^{-1} and yields between 20 and 80 % were realised with this method.[69] A similar approach to phenylethanol derivatives (11) was published later by the same group.[87] Here, the first step was a Pd-catalysed coupling of aryliodides with acetic anhydride, which led to the corresponding acetophenone derivatives. Hydrolysing excess acetic anhydride was achieved by heating the reaction mixture with aqueous buffer. The enzymatic step was started by adding *Lb*ADH, $NADP^+$ and 2-propanol. Two diketo-acetophenone derivatives were converted in a three-step one-pot synthesis to give hydroxy acids (11).[86] In this case, the regioselectivity of *Lb*ADH was utilised to reduce a ketone in the presence of an aldehyde, while the aldehyde was reduced by an aldoketo reductase from *E. coli* (*EC*AKR). The oxidation of the primary alcohol was then performed by a dihydrodiol dehydrogenase from *Pseudomonas fluroescens* to give the corresponding acid. Except *Lb*ADH, all enzymes were used as whole-cell catalysts in *E. coli*. All three steps were carried out in one reaction mixture with the biocatalysts added stepwise after completion of the preceeding step. The medium always contained $NADP^+$ and 2-propanol for cofactor regeneration, although, no intermediate workup had to be carried out. On a 250 mg-scale, (2*S*)-hydroxy(phenyl)ethanoic acid (mandelic acid) was obtained with an overall yield of 90 % and an *ee* of 99 % and 3-[(1*R*)-1-hydroxyethyl]benzoic acid with an overall yield of 90 % (*ee* = 99 %).

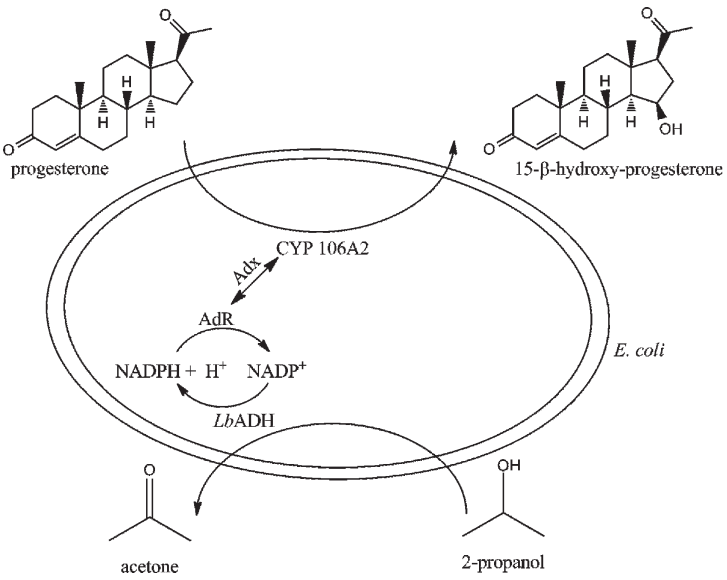
Electrochemical cofactor regeneration methods were not possible due to the lack of stability of *Lb*ADH in the presence of the redox mediator used, a rhodium bipyridin complex[88] (Fig. refechemie) and due to the adsorption of the enzyme on the porous carbon felt and subsequent deactivation.[75,89] After deactivation of the enzyme, the *ee* decreased dramatically due to the unselective direct reduction. Several approaches were tested to overcome these limitations. Adding a second protein, such as bovine serum albumin (BSA) kept the enzyme in solution and led to a TON of 74,000 for *Lb*ADH with an *ee* of >99.9 and a productivity of 120 mmol L^{-1} d^{-1} for (*R*)-phenylethanol (11).[89] By means of immobilisation, a direct contact of *Lb*ADH with the carbon felt

was avoided, thus a TON*Lb*ADH of 21,000 with a productivity of 74 mmol L^{-1} d^{-1} for (*R*)-phenylethanol (11) was possible.[89] The *ee* dropped slightly to >98.0 due to the direct reduction in compartments of the reactor where no enzyme was present.[89] Using a two-phase system with MTBE as second phase for the same substrate, acetophenone (11), the space-time-yield dropped to 25 mmol L^{-1} d^{-1} with the conversion not exceeding 60 %. These disadvantages may be overcome by the facilitated downstream-procedure in this case.[89] The spacial separation of *Lb*ADH immobilised on Sepabeads® from the polymer enlarged electrochemical mediator (Rhbpy) led to enhanced catalyst stability, so that electroenzymatic synthesis was possible with a linear product formation rate for (*R*)-4-chloro--phenylethanol (11) of 0.42 mmol L^{-1} h^{-1} and a space time yield of 10 mmol L^{-1} h^{-1}. One main drawback of this approach was the low TON of 3 for the cofactor.[75,90] The relatively complicated configuration for electroenzymatic synthesis as compared to a simple substrate coupled cofactor regeneration approach may be considered as another drawback.

Finally, the high stability and activity of *Lb*ADH was also used for simple cofactor regeneration in the hydroxylation of steroids to the corresponding 15*β*-hydroxy products.[91] For the hydroxylation step, the soluble P450 monooxygenase, CYP106A2 from *Bacillus megaterium* ATCC 13 368 was used. This enzyme requires an electron transfer partner, in this case bovine adrenodoxin (Adx), which was coexpressed together with the CYP106A2 in *Escherichia coli*. Adx acts as electron transfer agent from NADPH to CYP106A2, the cofactor regeneration was carried out by *Lb*ADH using 2-propanol as reducing agent (Fig. 8). Growing and resting cells of *E. coli* were tested as well as crude cell extract (CCE). Resting cells gave better conversion and less side products when compared to growing cells. Because steroids are not



F i g . 7 – *Reaction scheme for the reduction of acetophenone to R-phenylethanol with electrochemical cofactor-regeneration*[89,90]



F i g . 8 – *Reaction scheme for the hydroxylation of steroids with monooxygenase CYP106A2*[91]

actively transported through the cell membrane, CCE was tested and showed higher activity, but also a higher amount of side products compared to resting whole cells. After optimization, a space time yield of up to 18.3 mmol L^{-1} d^{-1} was achieved with CCE.

Another approach using *Lb*ADH for cofactor regeneration is the concurrent production of two enantio-enriched compounds.[92] The main reaction here was the oxidative kinetic racemic resolution of a ketone to the corresponding ester or sulfide oxidation to the corresponding sulfoxide by two Baeyer-Villiger monooxygenases (BVMO). BVMO need NADPH as redox equivalents with $NADP^+$ as coupled product. The *in situ* regeneration was done by the oxidation of an alcohol by *Lb*ADH or ADH from *Thermoanaerobacter species* (ADH-T). In this case, the kinetic racemic resolution of a long-chain alcohol (2-octanol, 2-undecanone, 2-hydroxy-6-methyl-hept-5-en (1)) was used for *Lb*ADH-catalysed cofactor-regeneration. Only for high conversion, high *ee* is possible for the non-converted substrates. *ee* for the alcohols was between 27 and 99 % depending on subtrates and enzyme combination. The authors do not comment on solubility restrictions of the long chain alcohols.

In a dynamic kinetic resolution, the spontaneous racemisation of *tert*-butyl-4-methyl-3,5-dioxo-hexanoate (7) was exploited to reduce the 4*S*,5*R*-alcohol[64] with *ee* = 99.2 % and 94 % diastereomeric excess.[64,65] The access to two other of the four possible diastereoisomers by using other biocatalysts is also described.[93]

Conclusion

*Lb*ADH is a versatile and robust catalyst. Furthermore, the tolerance of organic solvent/IL etc. as additives, as well as biphasic media, and gaseous dense reaction conditions is outstanding for an oxidoreductase. The substrate scope is broad and high regio- and stereospecifity can be obtained. Especially, the 2-keto motif has a high probability to be converted with high selectivity and activity. Here, the tolerance of organic cosolvents and biphasic systems allow compensation for water as the reaction medium. *Lb*ADH is used as whole-cell catalyst in *E. coli* as well as isolated enzyme, soluble or immobilised (Fig. 9). *A priori*, no choice for one of the regeneration methods can be substantiated. When isolated enzymes are used, the ratio *Lb*ADH / regeneration enzyme can be chosen independently. In whole-cell processes, intracellular cofactor concentrations are mostly sufficient, but expression levels of the enzymes are rather difficult to influence. This is especially an issue if cofactor regeneration is done in an enzyme-coupled approach, so that two enzymes are coexpressed in one organism. Different experiments have shown, that half-life of *Lb*ADH strongly depends on the presence of cofactor, magnesium, additives or organic cosolvents, pH and the nature of the buffer, ion strength, temperature, water activity, immobilisation, as well as non-miscible phases, and varies from single hours to more than 1000 h. Therefore, if stability is investigated, care has to be taken to ensure that process conditions are covered by experiments; otherwise, strong deviations from storage stability to process stability occur.

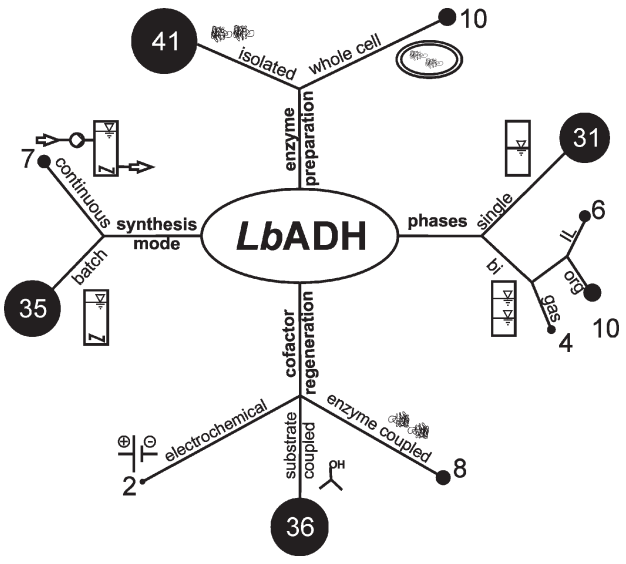


Fig. 9 – *Overview of the applications of LbADH with number of citations (the diameter of the circles is proportional to the number of citations)*

Abbreviations

BSA – bovine serum albumin
BTA – bis[(trifluoromethyl)sulfonyl]amide
BVMO – Bayer Villiger monooxygenase
CALB – B lipase from *Candida antarctica*
CCE – crude cell extract
CIP – Cahn-Ingold-Prelog
DCM – dry cell mass
*EC*AKR – aldoketo reductase from *Escherichia coli*
E. coli – *Escherichia coli*
ee – enantiomeric excess
ESI – electronic supporting information
FDH – formiate dehydrogenase
GDF – glucose facilitator from *Zymomonas mobilis*
GDH – glucose dehydrogenase
IE – isolated enzyme
IL – ionic liquid
*Lb*ADH – alcohol dehydrogenase from *Lactobacillus brevis*
*Lk*ADH – alcohol dehydrogenase from *Lactobacillus kefir*
MDH – malate dehydrogenase
MTBE – methyl-*tert*-butylether
NAD^+ – nicotinamide adenine dinucleotide (oxidised form)
NADH – nicotinamide adenine dinucleotide (reduced form)
$NADP^+$ – nicotinamide adenine dinucleotide phosphate (oxidised form)
NADPH – nicotinamide adenine dinucleotide phosphate (reduced form)
Rhbpy – pentamethylcyclopentadienyl-(2,2'-bipyridyl)-rhodium
scF – supercritical Fluid
SEM – scanning electron microscopy
TON – turnover number
WC – whole cell
WCM – wet cell mass
yADH – alcohol dehydrogenase from yeast

Supporting information for: Alcohol Dehydrogenase from Lactobacillus brevis: A Versatile Robust Catalyst for Enantioselective Transformations

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The first table provides a comprehensive guide to the substrate scope. The second table comprises the relevant literature and gives an overview.

Substrates converted by *Lb*ADH

Subst. No.	Residues			Citation
1	$R^1 = CH_3$	$R^2 = CH_3$		Zehentgruber *et al.* [95]
	$R^1 = CH_3$	$R^2 = C_6H_{15}$		Bräutigam *et al.* [2], Eckstein *et al.* [13,14,15], Kohlmann *et al.* [41], Rioz-Martinez *et al.* [66]
	$R^1 = CH_3$	$R^2 = C_2H_5$		Schroer *et al.* [71], Schumacher *et al.* [78], van den Wittenboer *et al.* [84]
	$R^1 = CH_3$	$R^1 = H$		Kwiecién *et al.* [43] *
	$R^1 = CH_3$	$R^2 = C_3H_7$		Eckstein *et al.* [15]
	$R^1 = CH_3$	$R^2 = C_4H_9$		Eckstein *et al.* [15]
	$R^1 = CH_3$	$R^2 = C_5H_{11}$		Eckstein *et al.* [15]
	$R^1 = CH_3$	$R^2 = C_7H_{15}$		Eckstein *et al.* [15], Kohlmann *et al.* [41]
	$R^1 = CH_3$	$R^2 = C_9H_{19}$		Rioz-Martinez *et al.* [66]
	$R^1 = C_2H_5$	$R^2 = C_5H_{11}$		Kohlmann *et al.* [41]
	$R^1 = CH_3$	$R^2 = C_8H_{17}$		Kohlmann *et al.* [41]
2	$R^1 = CH_3$	$R^2 = C_2H_4C_6H_5$		Hummel & Riebel [30], Hummel [31]
	$R^1 = CH_3$	$R^2 =$ Naphtyl		Hummel & Riebel [30], Hummel [31]
	$R^1 = CH_3$	$R^2 = CH_2C_6H_5$		Hummel & Riebel [30], Schroer *et al.* [72]
	$R^1 = CH_3$	$R^2 = C_2H_4CHC(CH_3)_2$		Rioz-Martinez *et al.* [66]
3	R1 = CH_2Phenyl	$R^2 = C_2H_5$		Hummel & Riebel [30]
	$R^1 = CH_3$	$R^2 = C_2H_5$		Hummel & Riebel [30], Hummel [31]
	$R^1 = CH_3$	$R^2 = CH_3$		Hummel & Riebel [30], Hummel [31]
4	$R^1 = CH_3$	$R^2 = CH_3$		Daußmann *et al.* [7,8], Ernst *et al.* [17], Hummel & Riebel [30], Ng & Jaenicke [55], Schroer *et al.* [71,73]
	$R^1 = CH_3$	$R^2 = CH_2C_6H_5$		Hummel & Riebel [30]
	$R^1 = CH_3$	$R^2 = C_2H_5$		Hummel & Riebel [30], Jeromin [34], Ng & Jaenicke [55]
	$R^1 = CH_2Cl$	$R^2 = C_2H_5$		Bräutigam *et al.* [1], Hummel & Riebel [30], Ng & Jaenicke [55]
	$R^1 = CH_3$	$R^2 = C_4H_9$		Hummel & Riebel [30]
	$R^1 = C_6H_5$	$R^2 = C_2H_5$		Hummel & Riebel [30]
	$R^1 =$ iso-C_3H_7	$R^2 = C_2H_5$		Hummel & Riebel [30], Ng & Jaenicke [55]
	$R^1 = CF_3$	$R^2 = C_2H_5$		Hummel & Riebel [30]
	$R^1 = C_2H_5$	$R^2 = C_2H_5$		Hummel & Riebel [30], Hummel [31]
	$R^1 = C_2H_5$	$R^2 = CH_3$		Hummel & Riebel [30], Hummel [31], Ng & Jaenicke [55]
5	$R^1 = CH_3$	$R^2 = C_2H_5$		Hummel & Riebel [30]
6	$R^1 = CH_3$	$R^2 = C_2H_5$		Hummel & Riebel [30]
	$R^1 = CH_3$	$R^2 = H$		Hummel & Riebel [30]
7	$R^1 = Cl$	$R^2 = H$	$R^3 =$ t-Bu	Müller *et al.* [50], Villela-Filho *et al.* [85], Wolberg *et al.* [91,92,93]
	$R^1 = H$	$R^2 = H$	$R^3 =$ t-Bu	Drochner & Müller [11], Müller *et al.* [50], Wolberg *et al.* [91,92]
	$R^1 = CH_3$	$R^2 = H$	$R^3 =$ t-Bu	Müller *et al.* [50], Wolberg *et al.* [92]
	$R^1 = C_2H_5$	$R^2 = H$	$R^3 =$ t-Bu	Wolberg *et al.* [92]
	R1 = (E)-PhCH=CH	$R^2 = H$	$R^3 =$ t-Bu	Wolberg *et al.* [92]
	$R^1 = H$	$R^2 = H$	$R^3 = CH_3$; CH_2CH_3; nPr; allyl; nHex; Bn; iPr;	Wolberg *et al.* [92]
	$R^1 = F$	$R^2 = H$	$R^3 =$ t-Bu	Wolberg *et al.* [92]
	$R^1 = OCH_3$	$R^2 = H$	$R^3 = CH_3$	Wolberg *et al.* [92]
	$R^1 = OCH_3$	$R^2 = H$	$R^3 =$ t-Bu	Wolberg *et al.* [92]
	$R^1 =$ BnO	$R^2 = H$	$R^3 =$ t-Bu	Wolberg *et al.* [92]
	$R^1 = H$	$R^2 = CH_3$	$R^3 =$ t-Bu	Ji *et al.* [36], Lüdeke *et al.* [44], Müller *et al.* [50], Wolberg *et al.* [92]
8	$R^1 = CH_3$	$R^2 = C_2H_5$		Kurina-Sanz *et al.* [42]
	$R^1 = CH_3$	$R^2 = C_3H_7$		Kurina-Sanz *et al.* [42]
	$R^1 = CH_3$	$R^2 = C_4H_9$		Kurina-Sanz *et al.* [42]
9	$R^1 = CH_3$	$R^2 =$ tert-C_4H_9		Hummel & Riebel [30]
	$R^1 = CH_3$	$R_2 = C_5H_{11}$		Hummel & Riebel [30]
	$R^1 = CH_3$	$R_2 = CH_3$		Hummel & Riebel [30], Hummel [31]
	$R^1 = C_2H_5$	$R_2 = C_3H_7$		Hummel & Riebel [30]
	$R^1 = CH_3$	$R^2 = CH_2CH(CH_3)_2$		Kurina-Sanz *et al.* [42]
	$R^1 = CH_3$	$R^2 = C_5H_{11}$		Kurina-Sanz *et al.* [42]
10	$R^1 = CH_3$	$R^2 = C_6H_5$		Kurina-Sanz *et al.* [42]
	$R^1 = CH_3$	$R^2 = CH_3$		Schroer & Lütz [70], Schroer *et al.* [72]
11	$R^1 = CH_3$			Cacchi *et al.* [3], Dreyer & Kragl [10], Eckstein *et al.* [15,16], Ferloni *et al.* [18], Hildebrand & Lütz [26,27], Hummel & Riebel [30], Hummel [31], Jeromin [34], Machielsen *et al.* [46], Ng & Jaenicke [55], Niefind *et al.* [56], Thorey *et al.* [81], Trivedi *et al.* [82,83], Villela-Filho *et al.* [85]; (Dimoula *et al.* [9], Schlieben *et al.* [68])*
	$R^1 = CH_3$	$R^2 = Cl$		Bräutigam *et al.* [1], Hummel & Riebel [30], Hummel [31]
	$R^1 = CH_3$	$R^3 = Cl$		Hummel & Riebel [30], Hummel [31]
	$R^1 = CH_3$	$R^4 = Cl$		Bräutigam *et al.* [1,2], Hildebrand & Lütz [28], Hummel & Riebel [30], Hummel [31], Weuster-Botz [87]
	$R^1 = CH_3$	$R^4 = F$		Cacchi *et al.* [3]
	$R^1 = CH_3$	$R^4 = C_2H_5$		Eckstein *et al.* [15], Hummel & Riebel [30], Hummel [31]
	$R^1 = CH_3$	$R^4 = CH_3$		Cacchi *et al.* [3]

Subst. No.	Residues		Citation
	$R^1 = CH_3$	$R^3 = CF_3$	Cacchi *et al.* [3]
	$R^1 = CH_3$	$R^4 = CO_2Et$	Cacchi *et al.* [3]
	$R^1 = CH_3$	$R^4 = NO_2$	Eckstein *et al.* [15]
	$R^1 = C_2H_5$		Hummel & Riebel [30], Hummel [31]
	$R^1 = H$		Hummel & Riebel [30], Hummel [31]
	$R^1 = CH_3$	$R^3 = CH_2O$	Gennaro *et al.* [21]
	$R^1 = CH_2OH$		Gennaro *et al.* [21]
			Kurina-Sanz *et al.* [42]
		4-Acetylpyridine	Jeromin [34]
12	$R^1 = CH_3$		Hummel & Riebel [30], Hummel [31]
	$R^1 = COOC_2H_5$		Hummel & Riebel [30]
	$R^1 = CH_3COOEt$		Hummel & Riebel [30]
13	$R^1 = H$	$R^2 = CH_3$	Hummel & Riebel [30], Hummel [31]
	$R^1 = CH_3$	$R^2 = C_6H_5$	Kihumbu *et al.* [39]
14	$R^1 = CH_2Cl$	$R^2 = C_6H_5$	Müller *et al.* [50], Schubert *et al.* [76]
	$R^1 = CH_2Cl$	$R^2 =$ TBS	Müller *et al.* [50], Schubert *et al.* [76]
	$R^1 = CH_2Cl$	$R^2 =$ TMS	Müller *et al.* [50], Schubert *et al.* [76]
	$R^1 = CH_2Br$	$R^2 =$ TMS	Müller *et al.* [50], Schubert *et al.* [76]
	$R^1 = CH_3$	$R^2 = C_6H_5$	Müller *et al.* [50], Schubert *et al.* [75,76]
	$R^1 = CH_3$	$R^2 =$ 4-MeO-C_6H_4	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 =$ 4-F-C_6H_4	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 =$ 4-Cl-C_6H_4	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 =$ 4-Br-C_6H_4	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 =$ 3-Br-C_6H_4	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 =$ 2-Br-C_6H_4	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 =$ 2-Pyridinyl	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 =$ 3-(Methyl)-2-thienyl	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 = H$	Müller *et al.* [50], Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 = SiMe_3$	Müller *et al.* [50], Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 = SiEt_3$	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 = SiMe_2$t-Bu	Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 = SiMe_2C_6H_5$	Müller *et al.* [50], Schubert *et al.* [75]
	$R^1 = C_2H_5$	$R^2 = H$	Müller *et al.* [50], Schubert *et al.* [75]
	$R^1 = C_3H_7$	$R^2 = H$	Müller *et al.* [50], Schubert *et al.* [75]
	$R^1 = C_5H_{11}$	$R^2 = H$	Müller *et al.* [50], Schubert *et al.* [75]
	$R^1 = CH_3$	$R^2 =$ Phenyl	Müller *et al.* [50], Schubert *et al.* [75]
15	$R^2 = NO_2$	$R^3 = CH_3$	Sgalla *et al.* [79]
	$R^3 = Cl$		Sgalla *et al.* [79]
	$R^2 = OCH_3$		Sgalla *et al.* [79]
	all R = H		Sgalla *et al.* [79]
	$R^2 = CH_3$		Sgalla *et al.* [79]
	$R^2 = CF_3$		Sgalla *et al.* [79]
	$R^3 = COCH_3$		Sgalla *et al.* [79]
	$R^3 = COOEt$		Sgalla *et al.* [79]
	$R^3 = NHCOCH_3$		Sgalla *et al.* [79]

Literature survey for *in vitro* catalysis with *Lb*ADH as derived for comparison (WC: whole cell; IE: isolated enzyme)

Ref.	Mode	Substrate Class No. (see: 4)	Cofactor Regeneration	$t_{\frac{1}{2}}$ / h	TON_{ADH} [1]	$TON_{NAD(P)(H)}$ / -	X / %	*ee* / %	STY / mmol L^{-1} d^{-1}
Bräutigam *et al.* [1]	WC, H_2O/IL, batch	4, 11	2-propanol	—	3 mmol/g_{cdw}	—	60-99	96; > 99.5 (biphasic)	2900 – 8640
Bräutigam *et al.* [2]	WC, H_2O/IL, fed-batch	1, 11	FDH (formate)		—	—	95 (2-Octanone), 96 (4-Cl-AP)	99.7 (2-Octanone), > 99.6 (4-Cl-AP)	1382 – 4608
Cacchi *et al.* [3]	IE, one-phase, batch (two-step one pot)	11	2-propanol	—	52000	125	76-92	98	110
Daußmann *et al.* [7]	IE, one-phase, rep. batch	4	2-propanol		—	74,000	94	> 99.8	786
Daußmann *et al.* [8]	IE, one-phase, rep. batch	4	2-propanol	loss of 10 % activity per cycle	—	74,000	94	> 99.8	—
Dimoula *et al.* [9]	immo. enzyme, gas/solid, ads. Phenomena	11	2-propanol	—	—	—	—	—	—
Dreyer & Kragl [10]	IE, aqueous/IL	11	2-propanol		—	—	—	—	—
Drochner & Müller [11]	IE, one-phase, batch	7	2-propanol		—	—	78	99.4	—
Eckstein *et al.* [14]	IE; H_2O/IL, H_2O/MTBE, batch	1	2-propanol		—	100	61, 88	> 99	14.4
Eckstein *et al.* [13]	IE, H_2O/MTBE, batch	1	2-propanol; GDH (glucose)		—	> 1000 with GDH rep batch	98 (2-propanol), 100 GDH	> 99.5	19.6 (2-propanol) 20 (GDH)
Eckstein *et al.* [15]	IE; one-phase and H_2O/MTBE; batch	1, 11	2-propanol; GDH; PTDH (phosphite); FDH; Hydrogenase (hydrogen); electrochemical	—	98000	7920	77.8 - 99.5	> 99.5	154
Eckstein *et al.* [16]	IE, H_2O/org. solv., batch	11	2-propanol	—	—	200 – 800	27.1 – 98	—	—
Ferloni *et al.* [18]	immo. enzyme, gas/solid, continuous	11	2-propanol	with excess sucrose: >100h(25°C), 40 (40°C), 9(50°C), 1(60°C) ; without sucrose: 1 (40°C)	∞ @ 25°C, 730,000 @ 40°C,260,000 @ 50°C, 86,000 @ 60°C	—	80	> 99.9	120
Gennaro *et al.* [21]	IE, one-phase, 3-step one-pot, batch	11	2-propanol	—	0.012 mmol U^{-1} / 0,013 mmol U^{-1}	78 – 85	88 – 99.7	> 95	7.1 – 7.8
Hildebrand & Lütz [26]	immo. enzyme, one-phase, continuous	11	2-propanol	20 @ 30°C (in solution); > 1200 @ 30°C (immo. enzyme)	2,500,000 (25,000)	20	60 – 98	> 99.5	250 – 1200
Hildebrand & Lütz [27]	IE and immo. enzyme; one phase and H_2O/org. solv.	11	electrochemical	see [85]	74,000	64	65 – 98	98 – 99.9	25 – 117
Hildebrand & Lütz [28]	immo. enzyme, one-phase, batch	11	electrochemical	immo. enzyme: > 1300, < 5 h (with [Rh(bpy)])	0,36 mmol $g_{immo.enzyme}{}^{-1}$	3	90	97.3	10
Hummel & Riebel [30]	IE, one-phase, activity tests	2, 3, 4, 5, 6, 11, 12	—	—	—	—	—	—	—
Hummel [31]	IE, one-phase, activity tests	2, 3, 4, 9, 11, 12	—	—	—	—	—	—	—
Jeromin [34]	immo. enzyme, one-phase, rep. batch	4, 11	2-propanol	stable for > 4 cycles (18°C)	0.016 mmol U^{-1}	900	74 – 100	> 99	50
Ji *et al.* [36]	IE, one-phase, batch	7	2-propanol	—	—	13.2	66	99.2 syn:anti 97:3	13.8
Ernst *et al.* [17]	WCs, one-phase, batch	4	FDH	—	200 μmol (g_{cdw} min)$^{-1}$	—	> 99	> 99	2060
Kihumbu *et al.* [39]	IE, one-phase, batch	13	FDH	—	0.65 mmol U^{-1}	11	90 (1*S*, 2*S*), 86 (1*S*, 2*R*)	*de* 99 (1*S*, 2*S*),98 (1*S*, 2*R*)	26
Kohlmann *et al.* [41]	IE, one-phase, batch	1	GDH	49 (buffer), 158 (IL/buffer)	840	800	>99	> 99	100-300
Kurina-Sanz *et al.* [42]	IE, one-phase, batch	8, 9, 10	2-propanol		—	50 – 100	25 – 97	> 99, *de* > 99	¡ 100
Kwiecién *et al.* [43]	structure investigations	1	—	—	—	—	—	—	—
Lüdeke *et al.* [44]	*Lb*ADH only as reference (see: [36])	7	2-propanol	—	—	—	—	—	—

Ref.	Mode	Substrate Class No. (see: 4)	Cofactor Regeneration	$t_{\frac{1}{2}}$ / h	TON_{ADH} [1]	$TON_{NAD(P)(H)}$ / -	X / %	*ee* / %	STY / mmol L^{-1} d^{-1}
Machielsen *et al.* [46]	IE, changing cofactor preference by molecular biology	11	—	—	—	—	—	—	—
Müller *et al.* [50]	Review, see: [31,32,36,37,64,74–76,90,92,93]	7, 14	—	–	—	–	—	—	—
Müller *et al.* [51]	IE, H_2O/MTBE, continuous	1, 11	2-propanol	– 26,000 15,400 10 – 60	0.32 – 0.99	200			
Ng & Jaenicke [55]	immo. WC, one-phase, rep. batch	4	2-propanol	—	258 mmol $(g_{wcw}\ d)^{-1}$	—	98 batch, 81 continuous	> 99	4600
Niefind *et al.* [57]	Crystal structure	—	—	—	—	—	—	—	—
[56]	Crystal structure and Mg^{2+} dependency	11	—	—	—	—	—	—	—
Schlieben *et al.* [68]	IE, changing cofactor preference by molecular biology	11	—	—	—	—	—	—	—
Schroer *et al.* [72]	WC, one-phase and H_2O/IL H_2O/MTBE, batch	2, 10	2-propanol	relative stability: 1 (no acetone-removal); 0.25 (with stripping), 0.82 (with pervaporation), 0.63 (with [BMIM][$(CF_3SO_2)_2N$])	21.8/390 mmol $g_{wcw}{}^{-1}$((R)-1-phenyl-2-propanol/(2R5R)-hexanediol)	—	24 – 95	—	15.2 – 325
Schroer *et al.* [71]	WC, one-phase, continuous	1, 4	2-propanol; FDH ; GDH	—	330 – 508 mmol $g_{wcw}{}^{-1}$	—	52 – 78	> 99 (no *ee* for Butanol)	max.: 6000 (3700)
Schroer *et al.* [73]	IE and WC, one-phase, batch	4	2-propanol	—	6 – 19 mmol g^{-1}_{cww}	5000 (IE), 10,000 (WC)	80 – 99	—	168 – 1000 IE/ 4000
Schroer & Lütz [70]	WC, one-phase, continuous	10	2-propanol	—	152 mmol g^{-1}_{wcw}	—	77	*ee*, *de* > 99	1458
Schubert *et al.* [75]	IE, one-phase, batch	14	2-propanol	—	0.058 mmol U^{-1}	2000	> 99	> 99	33
Schubert *et al.* [76]	IE, one-phase, batch	14	2-propanol	—	0.01 g U^{-1}	20,000	99	99	5.4
Schumacher *et al.* [78]	IE, one-phase, batch	1	2-propanol	—	0.009 – 0.24 mmol U^{-1}	100	60 – 80	33 – 43	72 – 3240
Sgalla *et al.* [79]	IE, one-phase, two-step one-pot, batch	15	2-propanol	—	0.016 mmol U^{-1}	180	21 – 80	> 99	1040
Thorey *et al.* [81]	immo. and IE, solid/dense propane and H_2O/dense propane	11	2-propanol	1.5 (aq. buffer) @ 36°C, 6.5 (H_2O/dense propane) @ 29°C, dense propane, 0.2 (H_2O/dense propane) @ 35°C	180 (solid/dense propane); 300 (H_2O/dense propane)	50 (solid/propane), 80 (H_2O/propane)	45 (solid/dense propane), 90 (H_2O/dense propane)	> 99.9	13.5 – 45
Trivedi *et al.* [82]	immo. enzyme, solid/gas, improvement of immobilisation procedure	11	—	—	—	—	—	—	—
Trivedi *et al.* [83]	immo. enzyme, gas/solid, cont.	11	2-propanol	990 (immob. enzyme), 4 (aq.)	4,200,000	3,360,000	90	–	260
Rioz-Martinez *et al.* [66]	IE, one-phase, batch	1, 2	2-octa-, 2-undecanol; 2-hydroxy-6-methyl-hept-5-en	—	1.25–1.88 µmol U^{-1}	50 – 75	41 – 53 (kinetic resolution)	66 – 99	5 – 7.5
van den Wittenboer *et al.* [84]	IE, H_2O/MTBE, continuous	1	2-propanol	—	31	350	70	91	210
Villela-Filho *et al.* [85]	IE, H_2O/MTBE, rep. batch	4, 11	2-propanol	1 (H_2O/DCM): (H_2O/MTBE: 1400(4°C); 480(20°C)	0.0025 mmol U^{-1}	—	95 (acetophenone), 98 ((*S*)-6-chloro-5-hydroxy-3-oxohexanoate)	> 99 (acetophenone), ¿ 99.5 ((*S*)-6-chloro-5-hydroxy-3-oxohexanoate)	—
Weuster-Botz [87]	WC, H_2O/IL, batch	4	FDH (Formate → CO_2)	—	3 mmol g^{-1}_{cdw}	—	97 – 99	99.7	2900
Wolberg *et al.* [93]	IE, one-phase, batch	7	2-propanol	—	0.035 mmol U^{-1}	300	> 90	> 99.5	23
Wolberg *et al.* [92]	IE, one-phase, batch	7	2-propanol	—	4,3 – 23 µmol U^{-1}	15 – 72	61 – 77	98.1 – 99.5	3.0 – 24.7
Wolberg *et al.* [91]	IE; one-phase fed-batch; H_2O/MTBE batch	7	2-propanol	—	200,000	96 (bisphasic 14,000)	84 (biphasic 70)	> 99.5 *S*	28
Zehentgruber *et al.* [95]	WC and IE, one-phase, batch and rep. batch	1	2-propanol	110(25 °C), 19(30°C)	—	4.3 – 102	54 – 96	—	0.8 – 18

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