Catalysis Science & Technology

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/catalysis

The selective addition of water

Verena Resch[a,b] and Ulf Hanefeld*[a]

[a]*Gebouw voor Scheikunde, Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, Julianalaan 136, 2628BL Delft, Nederland.*

[b]*Organische und Bioorganische Chemie, Institut für Chemie, Karl-Franzens-Universität Graz, Heinrichstrasse 28, 8010 Graz, Österreich.*

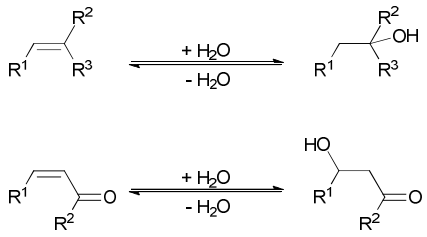
Abstract: Water is omnipresent and essential. Yet at the same time it is a rather unreactive molecule. The direct addition of water to C=C double bonds is therefore a challenge not answered convincingly. In this perspective we critically evaluate the selectivity and the applicability of the different catalytic approaches for water addition reactions, homogeneous, heterogeneous and bio-catalytic. Here we would like to discuss how to speed water addition up and even make it selective.

1. Introduction

Water is often seen as something that disturbs a reaction. Indeed, it is commonly used to "quench a reaction". Yet at the same time it is actually rather unreactive, being both a poor nucleophile and electrophile. Consequently the selective addition of water to carbon-carbon double bonds is known to be a chemically very challenging reaction. This, even though it is taught in every undergraduate course.[1-4] How then to take up this gauntlet and bring undergraduate chemistry to real life? How to realise short and efficient routes for the synthesis of alcohols by straightforward water addition to double bonds?

Even though water – in terms of sustainability and abundance – is an attractive reagent, it is rarely applied for the addition to double bonds in chemical processes. Indeed, only a few methods for the hydration of alkenes are reported,[5-9] of which only one is applied as a direct process for the synthesis of ethanol and similar alcohols.[10]

Difficulties lie mainly in the activation of water as a nucleophile. Compared to carbon or nitrogen nucleophiles, oxygen-containing nucleophiles, such as water or hydrogen peroxide, are known to be bad nucleophiles. In general the nucleophilicity can be increased when charged species of the nucleophile are employed. This is of course also true for carbon- and nitrogen-containing nucleophiles (nucleophilicity in decreasing order: CH_3^- > NH_2^- > OH^- > F^-). To improve the reactivity of water for the addition to carbon-carbon double bonds, a strong activation is necessary. In the textbook electrophilic addition reactions to carbon-carbon double bonds, the activation of the double bond is acid-induced (Scheme 1). Consequently, water is protonated and loses any nucleophilic character, the opposite of activation. Indeed, under these conditions almost any other nucleophile will add to the activated double bond; outcompeting water even if it is the solvent and thus present in excess. This is also represented in the few published procedures for performing the addition of water to double bonds; the presence of other nucleophiles is carefully avoided.[11]



Scheme 1 Water addition to isolated C=C bonds, i.e. an electrophilic addition, follows the rule of Markovnikov. A nucleophilic addition to a polarised double bond, a Michael addition, is observed for α,β-unsaturated carbonyl compounds.

The nucleophilic addition to carbon-carbon double bonds proceeds more readily in polarised, electron-deficient double bonds. These are the conditions of the Michael reaction (Scheme 1). But even here water addition is the exception and many successful Michael reactions with water as

inert solvent have been described as part of efforts to make chemistry more environmentally benign.[12-14]

The use of water as a benign and unreactive solvent has a second reason besides its poor nucleophilicity. In many addition reactions to carbon-carbon bonds the equilibrium of the water addition reaction is unfavourable, impeding it (see also Table 2). Both for electron-rich and isolated as well as conjugated, electron-poor double bonds the equilibrium can be on the side of the starting material; even if the reaction is performed in water. Thus poor nucleophilicity slows down a reaction with an unfavourable equilibrium, so that it is often not even noticed and other nucleophiles can be used in water. Here we would like to discuss how to speed water addition up and even make it selective.

2. Chemical catalysts

The textbook addition of water to carbon-carbon double bonds displays a very poor selectivity.[2-4] This is due to the fact that both the addition to electron-rich and to polarised double bonds (Scheme 1) is normally performed under acid catalysis. This does, however, induce a vast range of undesired side reactions such as isomerisations, polymerisations and rearrangements.

2.1. Acid catalysed addition of water to electron-rich C=C bonds

The large scale synthesis of “simple” alcohols is based on small alkenes normally derived from fossil fuels. One of the first bulk petrochemical processes from the 1920s was the hydration of propene.[15, 16] This is, however, an indirect process, in which first propene is treated with 60% sulfuric acid. In a second step the formed sulfate is steam-treated in a stripper and subsequently the isopropanol is removed at the top while the acid is collected at the bottom and recycled (Figure 1). The process has to be run carefully to avoid high temperatures that would cause ether formation. This indirect hydration process is actually a prime example how difficult it is to add

water to an electron-rich C=C bond. A process with concentrated sulfuric acid is not commonly used anymore.

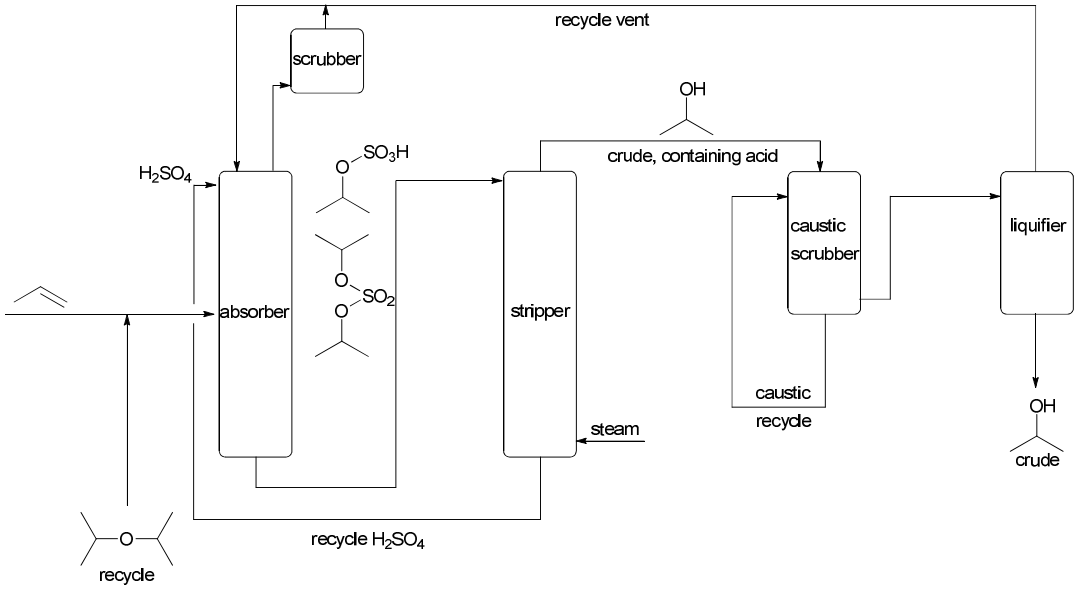


Figure 1 Indirect hydration of propene. This process demonstrates many of the problems that water addition reactions have: low reactivity (therefore sulfuric acid reacts first leading to multiple steps), side product formation (in particular ether).

In addition to the indirect process a direct process was developed, utilising heterogeneous acids. This came on stream commercially in 1951.[16] Processes based on vapour-liquid phase reactions with either sulfonated polystyrene ion-exchangers or tungsten oxide catalysts utilise high pressure. This calls for expensive equipment but has the advantage that the reaction equilibrium is forced towards the product side, as two molecules combine to form one product. With both catalysts high conversions per pass through the reactor were achieved (> 60%). A vapour-phase hydration at high pressure also utilising tungsten oxide, now immobilised on silica, yields even 95%. When low pressure and a phosphoric acid on silica are used conversions are only 10% per pass and large recycling streams have to be handled. Nonetheless, this has also been commercialised. Detailed investigations of the equilibrium and the kinetics under various reaction conditions are available.[15]

For ethanol, fermentation was replaced by the indirect hydration via the sulfuric acid method in 1930 but already in 1948 this started to be superseded by the direct hydration.[10] Although countless different acidic materials have been suggested as catalysts, many phosphoric acid supported celite (a natural silicate, the skeletons of diatoms), montmorillonite or similar carriers are used. The reaction is plagued by two side reactions, ether formation and polymerisation. At low temperatures ether synthesis becomes dominant, at high pressures polymerisation. Therefore an equimolar feed at 250-300 ºC is used with 5-8 MPa. Under these conditions, the equilibrium is then on the ethylene side with conversions below 25%. This causes large recycle streams (Figure 2), again demonstrating how difficult water addition reactions are.

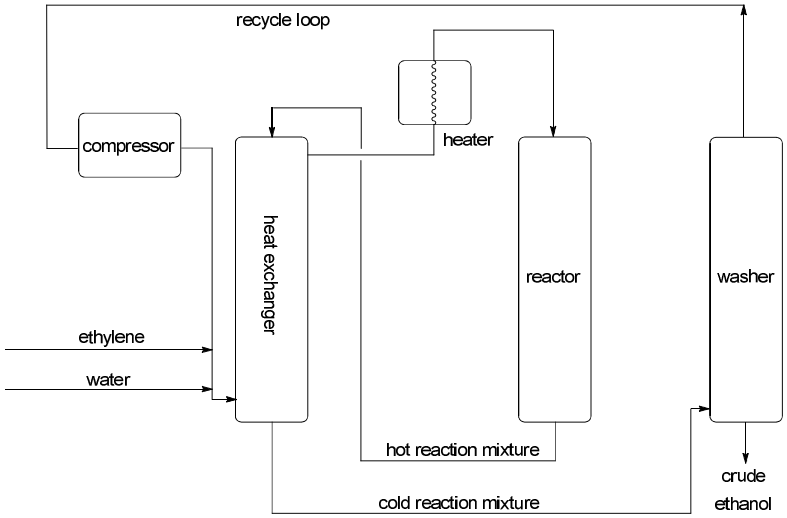
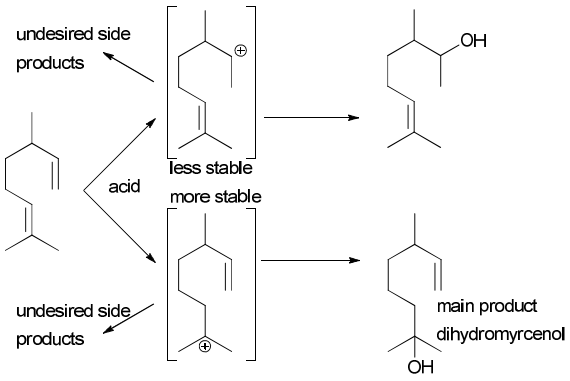


Figure 2 Direct hydration of ethene. This process demonstrates many of the problems that water addition reactions have: side product formation, here ether and polymer. Therefore the process cannot be run at optimal conditions for ethanol formation and a large recycle stream has to be taken care of.

The water addition to isobutene is industrially less important since the production of *tert*-butanol is often coupled with propene oxide production, starting from isobutane.[17] However, both the indirect hydration and the direct hydration of isobutene are employed.[18, 19] In particular the indirect hydration is utilised with the technical C4 feeds containing isobutene and *n*-butene. Again detailed studies on the kinetics and the equilibrium have been performed, revealing a situation less favourable than in the propene case.[20]

While the "simple" alcohols can be produced from an alkene that cannot isomerise and where the rule of Markovnikov ensures that only one regioisomer can be formed, problems become even more pronounced when terpenes are the starting materials.[21] Only two terpenes have been investigated more thoroughly for selective water addition reactions. Dihydromyrcene can be converted into dihydromycrenol with relatively high selectivity due to the difference in stability of the intermediate carbenium ions (Scheme 2). Biphasic systems with zeolites as catalysts as well as triflic acid in ionic liquids have been studied for this. [5, 22, 23] Similar studies for the structurally more demanding α–pinene gave product mixtures. The alcohols dominated but no single alcohol could be obtained as the only product.[24-27]



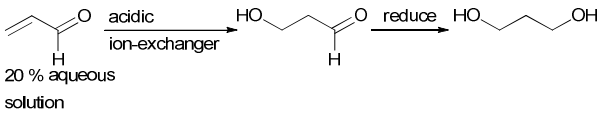
Scheme 2 Dihydromyrcene can be converted into dihydromyrcenol with good selectivity.

2.2. Chemo-catalytic addition of water to electron-deficient C=C bonds

The Michael addition of water can either be acid or base catalysed, activating either the α,β-unsaturated carbonyl compound or the nucleophile, water. However, it needs to be emphasised that almost every other nucleophile reacts more readily in Michael additions, making the water addition an exception. One recent example for a base catalysed addition of water was the use of amines to catalyse the addition of water to α,β-unsaturated carbonyl compounds.[6] In this study, proteinogenic α-amino acids were tested as catalysts to convert for example cyclohex-2-enone to 3-

hydroxycyclohexanone. The best results were obtained using L-lysine as the catalyst. However, also this reaction is limited by its equilibrium, which allows a maximum conversion of approx. 25%. Nevertheless, the conditions - in comparison to some of the examples mentioned above - are very mild and α-amino acids represent non-toxic and sustainable catalysts. No stereo-induction was observed although chiral α-amino acids were used as catalysts. Other recent approaches based on mimicking hydratases will be discussed in section 3.3.

The only process that was run on an industrial scale is the addition of water to acrolein,[28] yielding 3-hydroxypropanal. Starting with approx. 20% acrolein in water it was possible to reach greater 50% conversion with a selectivity of greater 80% for the product. Addition of acids to the acidic ion exchange catalyst (pH ~ 4) suppressed the polymerisation of acrolein that commonly decreased catalyst activity.[29, 30] The 3-hydroxypropanal was exclusively used to produce 1,3-propanediol (Scheme 3).[31] However, this process has been replaced with a biological route to this diol. Today it is produced via fermentation, starting either from glycerol or sugar. A key step in the bio-process is a dehydratase catalysed elimination of water from glycerol.[32-35]



Scheme 3 Michael addition of water to acrolein. The 3-hydroxypropanal is then reduced to 1,3-propanediol.

3. Enzymes as catalysts

In contrast to chemical catalysts, nature is very well capable of providing the right activation to use water as a nucleophile. Enzymes are able to use water as a substrate and also provide the right environment for asymmetric transformations allowing the synthesis of enantiomerically pure alcohols.[36] Given the small size of water, the asymmetric addition is even more remarkable. The active site geometry and potential co-factors involved in enzyme catalysed water addition reactions are essential for the reaction to proceed. Furthermore, their ability to bind both the

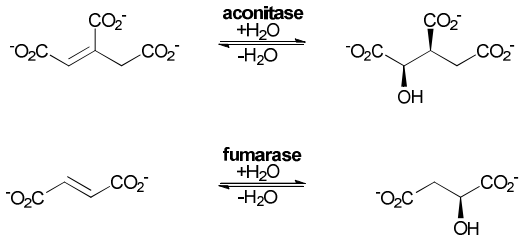
nucleophile and the electrophile – which leads to the stabilisation of the transition state – enhances the reaction. Basically an intramolecular reaction takes place.[37, 38]

Enzymes that catalyse the addition of water to carbon-carbon double bonds are called hydratases or hydro-lyases (E.C. 4.2.1.-). Living organisms harbour a vast variety of hydratases, which are involved in the primary metabolism such as the citric acid cycle. Apart from enzymes from the primary metabolism, some are also employed in the energy storage and release system of living organisms, were they are for example in charge of degrading fatty acids. The hydratases involved in metabolic pathways display high selectivities. In the primary metabolism perfect selectivities are indispensable to life. However, from a chemist's point of view, enantioselectivity is highly desired but in contrast to that, the substrate acceptance should be as broad as possible. This is unfortunately not always the case for the hydratases. Even though this limits the application, there are several examples that prove their potential, and application of hydratases on industrial scale is well established.[39]

3.1. Mechanistic aspects of enzymatic water addition

As for the chemical catalysts, the addition of water catalysed by enzymes can be grouped into two different types, depending on the substrate. The double bond can be either an isolated (electron-rich) double bond or conjugated to a carbonyl functionality (polarised and electron-poor), representing a Michael-type addition (Scheme 1).[40]

Hydratases have different features that allow the activation of water. In some cases the activation is performed with the help of a metal ion, which is located in the active site, in others the reaction is catalysed without cofactors. This of course also leads to a different mechanism. Aconitase (an iron-sulfur cluster containing enzyme) and fumarase C (which requires no cofactor) serve as two distinguished examples. Both enzymes perform the addition of water to similar substrates (see Scheme 4).



Scheme 4 Aconitase and fumarase catalyse the hydration of similar substrates but the cofactor chemistry and mechanistic aspects differ.

Class I fumarases (fum A and B) harbour an iron-sulfur cluster that is involved in catalysis. A second class of fumarases (class II, fum C) performs the same reaction without the help of any cofactor. The iron-sulfur cluster acts as a Lewis acid and is involved in the activation of the water molecule.[41] Aconitase is also an iron-sulfur cluster containing enzyme. Since detailed mechanistic studies on aconitase exist,[42] we will take aconitase as an example to illustrate the different activation mechanisms of water in comparison with fumarase C, a cofactor-independent fumarase.

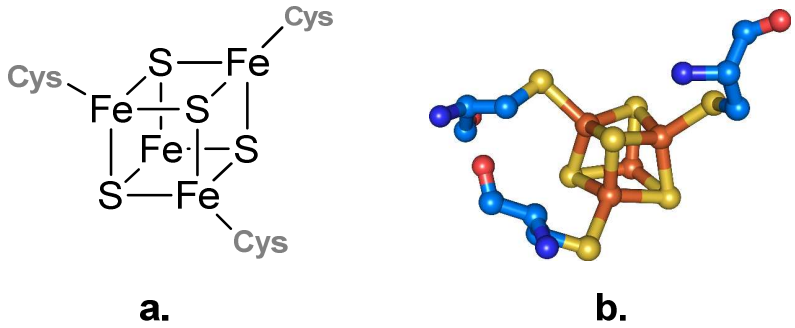
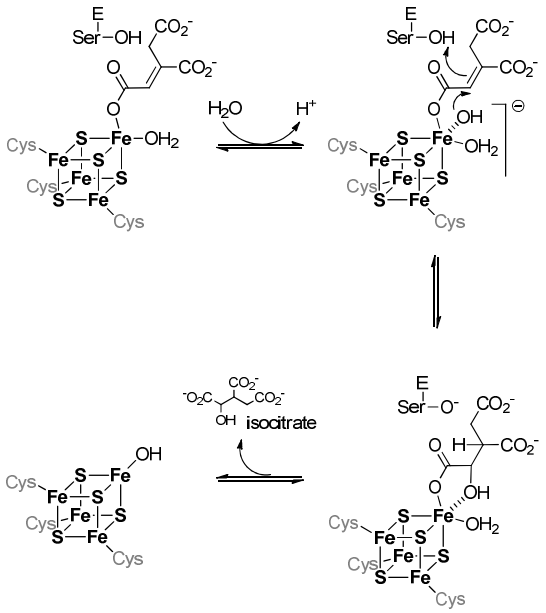


Figure 3 a. Schematic representation of an iron-sulphur cluster with three irons bound to cysteine residues. b. Iron-sulphur cluster as it is found in the crystal structure of aconitase from *Bos taurus*.[42]

The iron-sulfur cluster of aconitase consisting of 4 iron and 4 sulfur atoms [4Fe-4S] forming a cube-like structure that is bound to the protein backbone by three cysteine residues (see Figure 3). This allows one iron to remain without a binding partner and it can therefore act as a Lewis acid. It contributes to the reaction in two ways: first it helps to orientate the substrate in the

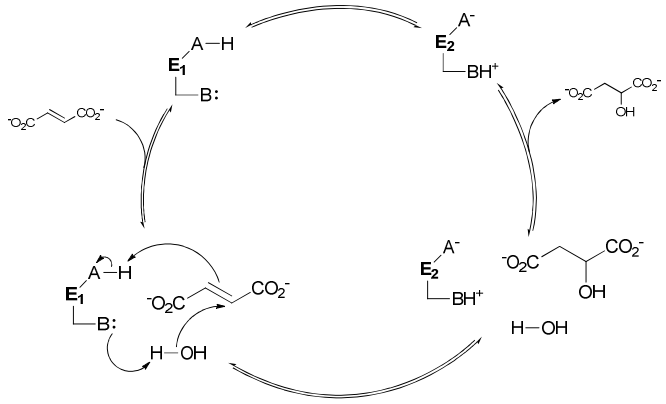
active site by forming a coordinative bond to the hydroxyl group of the carboxylate and its second function is to bind the water which serves as the second substrate. The binding of both substrates allows a close interaction and an intramolecular reaction is possible. The formed product – which is isocitrate in the case of aconitase – is released from the active site and the iron-sulfur cluster can be employed in another catalytic cycle (see Scheme 5).



Scheme 5 Hydration-part of the catalytic cycle of the iron-sulfur cluster containing aconitase.

In the case of the metal-independent fumarase, the mechanism for the water addition differs strongly due to the absence of a cofactor. Here the proton transfer is performed by two acid-base •-amino acid residues. The mechanism involves two states that are defined by the ionisation state of these two residues. They can be either in the form of a protonated acid and a deprotonated base (state E_1) or as deprotonated acid and protonated base (state E_2). In the E_1 state, the enzyme is able to bind fumarate as the substrate and the water addition to malate is performed. For the dehydration of malate, the enzyme needs to be in the E_2 state. In general the basic residue is involved in the deprotonation of the water molecule that is added to the double bond. The primary function of the acidic residue is to donate a proton to the substrate (see Scheme 6).[43] The two very

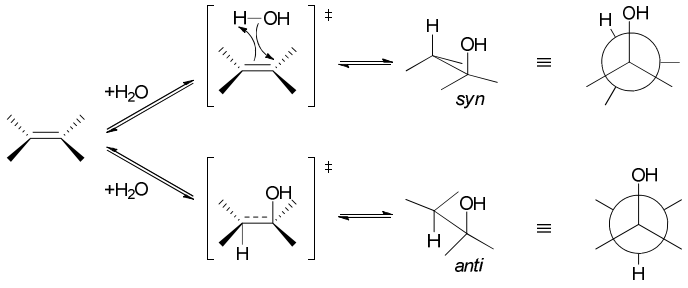
different catalytic mechanisms for aconitase and fumarase show how diverse the mechanisms of activation for both water and the substrate can be.



Scheme 6 Catalytic mechanism of metal-independent fumarase. The two acid/base residues (A and B) are essential. Depending on their ionisation state, either the E_1 form or E_2 form is present.

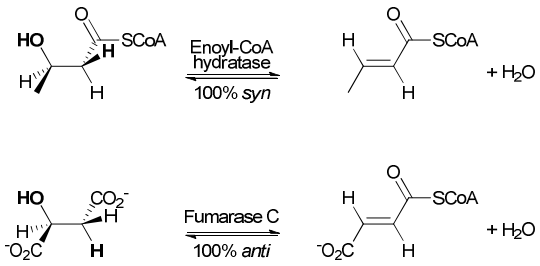
Most hydration reactions are equilibrium reactions and hydratases are also able to perform both the addition and elimination of water. Depending on the substrate, the equilibrium can either lie on the substrate or product side (for equilibrium yields of different industrially employed hydratases see Table 2).

In general, both the addition and elimination of water can occur either in *syn*- or *anti*-fashion (see Scheme 7). Depending on their mechanism, chemically (acid/base) catalysed addition and elimination reactions can show selectivity towards the *anti*-product or no selectivity is observed. In the case of an E2 mechanism (concerted) *anti*-stereoselectivity is again observed. If the reaction proceeds via an E1 mechanism (stepwise), no selectivity is observed. In contrast to chemical methods, enzymes are also able to perform the *syn*-addition/elimination. Depending on the enzyme, biocatalytic hydration and elimination reactions can show either *syn*- or *anti*-preference.[44]



Scheme 7 Depending on the enzyme, either *syn*- or *anti*-addition and elimination reactions are observed.

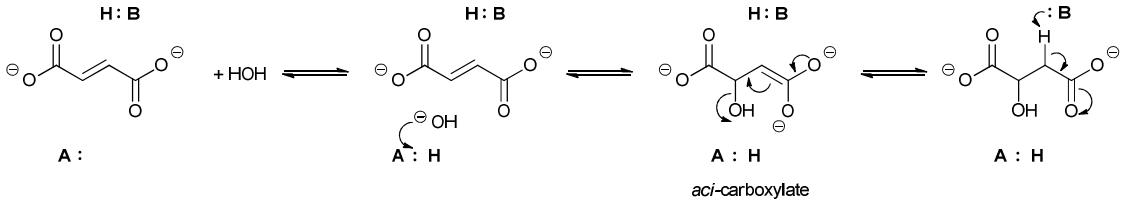
Studies showed that the preference depends for example on the position of the abstracted proton. If the proton is in α-position to the carboxylate group, *anti*-selectivity is observed. Abstracted protons that are in the α-position to the carbonyl group of a thioester lead to *syn*-selectivity.[41] Enzymes that catalyse the *anti*-addition/elimination belong to the aspartase/fumarase superfamily, which are for example fumarase, aconitase or enolase. Enzymes belonging to the enoyl-CoA-hydratase superfamily like for example enoyl-CoA hydratase can catalyse the *syn*-addition/elimination (see Scheme 8).[45]



Scheme 8 Enoyl-CoA hydratase and fumarase C catalyse the addition and elimination of water with different stereo-preference. In the case of enoyl-CoA hydratase selectivity towards *syn*-addition and elimination is observed, while fumarase C shows *anti*-preference.

Theories to explain the difference in selectivity suggest taking the nature of the substrate and the structural features of the enzyme into account. It is assumed that the acidity of the proton attached to the α-carbon plays a crucial role.[46, 47] In terms of the enzyme structure, two important features were recognised as important. For enzymes belonging to the enoyl-CoA hydratase superfamily performing exclusively the *syn*-addition and elimination of water a conserved

oxyanion hole involved in the stabilisation of the enolate anion is key. It is assumed that the reaction follows an E1cB-elimination mechanism, where first the elimination of a proton takes place, leading to the formation of an enolate. In the final step, the hydroxide serves as a leaving group.[44] If the addition or elimination is taking place in an *anti*-fashion – like it is the case with enzymes from the aspartase/fumarase superfamily – the reaction follows a concerted E2 mechanism. Enzymes from this group share active site residues that allow the stabilisation of *aci*-carboxylate intermediates (Scheme 9).[44, 48]



Scheme 9 Mechanism of the acid-base catalysed *anti*-addition/elimination reaction of enzymes from the aspartase/fumarase superfamily involving the *aci*-carboxylate as an intermediate.

While the *syn*/*anti*-selectivity differs with the type of enzyme used for catalysis, the chemically catalysed reaction shows only a preference towards the *anti*-addition. Therefore, in chemically acid-catalysed reactions regio- but not stereoselectivity can be observed. In contrast to these findings, both chemical as well as biocatalytic methods for the addition of water to electron-rich double bonds follow the rule of Markovnikov.

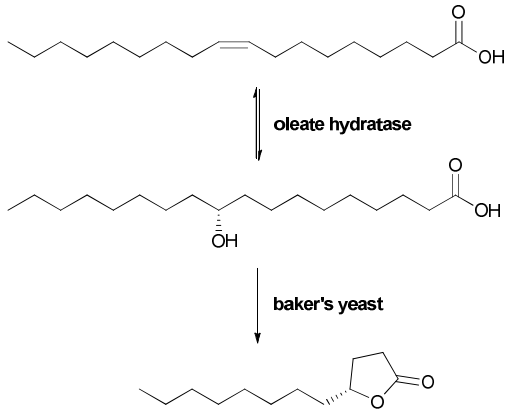
3.2. Enzyme catalysed addition of water to electron-rich C=C bonds

The addition of water to electron-rich double bonds is catalysed by a variety of different hydratases like oleate hydratase, carotenoid hydratases, linalool dehydratase-isomerase, kievitone hydratase, phaseollidin hydratase, limonene hydratase or acetylene hydratase, to name just a few.[1] The focus of this review lies on hydratases that are employed either in industrial processes or on laboratory scale, therefore we would like to focus on oleate hydratase and limonene hydratase in particular.

Oleate hydratase

Historically, discovery and first investigations of oleate hydratase (E.C. 4.2.1.53) date back to the 1970s, when its activity towards the hydration of oleic acid was first described.[49-51] Already back then the substrate specificity was investigated and different hydroxystearic acids were tested.[52] However, it took quite some time until detailed information about the enzyme became available. Recent investigations on an oleate hydratase from *Elizabethkingia meningoseptica* (formerly *Pseudomonas sp*. Strain 3266) showed that the enzyme is a monomer with a molecular mass of 73 kDa, which binds a catalytically non-essential calcium ion.[53] Another study describes an oleate hydratase from *Macrococcus caseolyticus* cloned and overexpressed in *E. coli*. This oleate hydratase is described as a dimeric enzyme with a molecular mass of 136 kDa containing an FAD-cofactor. The enzyme acts only on *cis*-9-double bonds to yield 10-hydroxyoctadecanoic acid and *cis*-12-double bonds giving rise to 10,13-dihydroxyfatty acids.[54] Very recently the crystal structure of an oleate hydratase from *Lactobacillus acidophilus* was described, suggesting its dependence on FAD. This assumption is supported by the presence of an FAD-binding pocket. Furthermore, an oleate hydratase from *Lysinibacillus fusiformis* showed activity towards several different substrates, being – next to oleic acid – palmitoleic acid, γ-linolenic acid, linoleic acid, myristoleic acid, and α-linolenic acid (substrates in decreasing order of activity).[55]

In general, many hydratases from different organisms are described. However, in most of the cases detailed characterisation is still missing. Oleate hydratases are nonetheless successfully applied in larger-scale biotransformations. One example is an oleate hydratase from a bacterial strain used in the production of γ-dodecalactone, which is known as an essential flavour compound in whiskey. In this process, (*R*)-10-hydroxystearic acid is produced in a fermentative approach, and is then further converted to γ-dodecalactone by baker's yeast giving an *ee* of 87% (see Scheme 10).[56, 57]



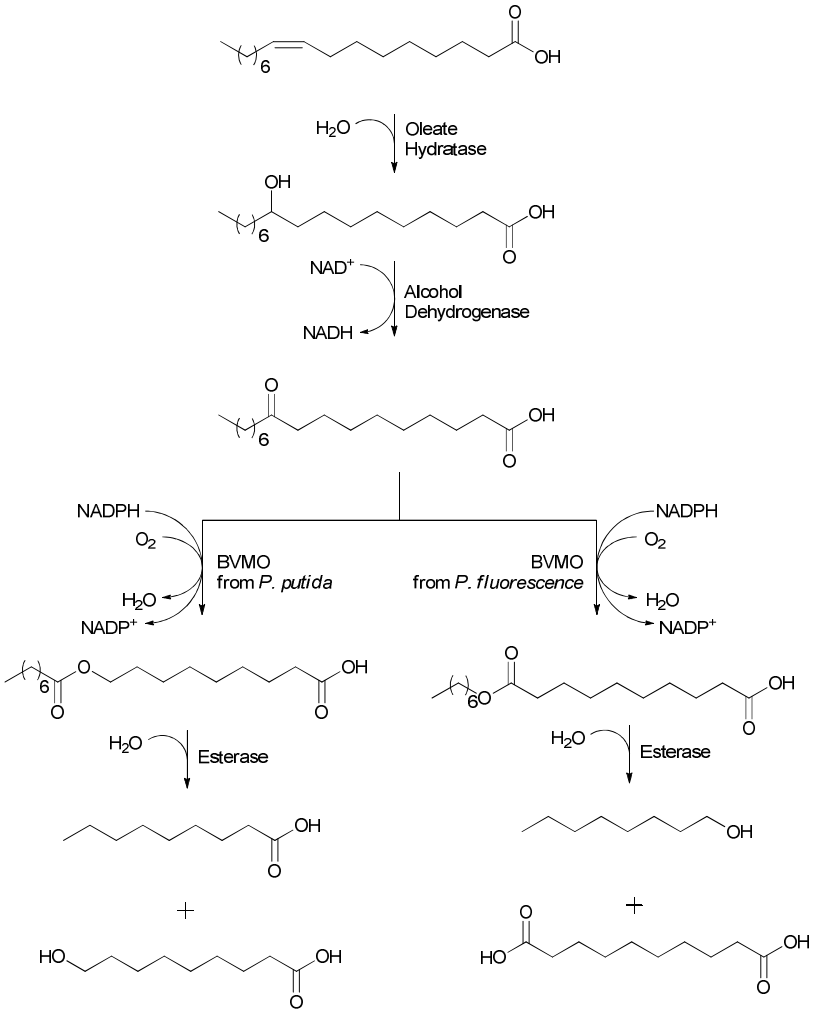
Scheme 10 Oleate hydratase in combination with baker's yeast for the enantioselective synthesis of the whiskey flavour compound γ-dodecalactone.

Oleate hydratases from different sources are also employed for the large-scale production of 10-hydroxystearic acid starting from oleic acid (Table 1). For example an oleate hydratase from *Stenotrophomonas nitritireducens* was employed in this bioprocess and a productivity of 7.9 g/L/h of 10-hydroxystearic acid was achieved.[58] In another process the use of oleate hydratase from *Stenotrophomonas maltophilia* (overexpressed in *E. coli*) is reported furnishing 10-hydroxystearic acid in a yield of 98% (w/w) which corresponds to a volumetric productivity of 12.3 g/L/h or 49 g/L after 4 h.[59] A third bioprocess again uses heterologously expressed oleate hydratase from *Stenotrophomonas maltophilia*. Using a whole-cell approach, a productivity of 8.2 g/L/h (46 g/L) of 10-hydroxystearic acid was reached. Furthermore, the isolation of 10-hydroxystearic acid gave rise to a yield of 70.9% and after recrystallisation a purity of 99.7% was achieved.[60]

Table 1 Comparison of large-scale approaches for the production of 10-hydroxystearic acid using either homologously or heterologously expressed oleate hydratase.

Entry	Organism	Productivity (g/L/h)	Yield (%)	Ref
1	*Stenotrophomonas nitritireducens*	7.9	100	[58]
2	*Stenotrophomonas maltophilia* (overexpressed in *E. coli*)	12.3	98	[59]
3	*Stenotrophomonas maltophilia* (overexpressed in *E. coli*)	8.2	86	[60]

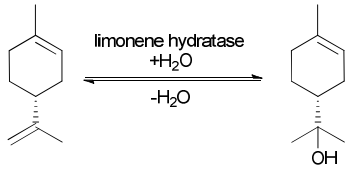
Oleate hydratase showed also great potential as an enzyme embedded in cascade reactions. A multistep enzyme-catalysed reaction sequence shows the combination of oleate hydratase, alcohol dehydrogenase, two different Baeyer-Villiger monooxygenases and an esterase. The enzymes were combined for the synthesis of long-chain α,ω-dicarboxylic and ω-hydroxycarboxylic acids using renewable fatty acids and plant oils.[61] The reaction started with oleic acid, which was converted into either *n*-nonanoic acid and ω-hydroxynonanoic acid or *n*-octanol and 1,10-decanedioic acid. In the sequence oleate hydratase catalysed the addition of water, which was followed by the oxidation of the hydroxyl group by the alcohol dehydrogenase. The Baeyer-Villiger monooxygenase-catalysed oxidation leads to the formation of an ester, which is further hydrolysed by the esterase (see Scheme 11). Very recently also the conversion of the hydroxy-acid by an alcohol dehydrogenase into an aldehyde and subsequently transaminase catalysed into the amino acid was described. Thus monomers for Nylon production are accessible from unsaturated fatty acids.[62]



Scheme 11 A multi-enzymatic cascade employing an oleate hydratase, an alcohol dehydrogenase, two different Baeyer-Villiger monooxygenases and an esterase giving rise to α,ω-dicarboxylic and ω-hydroxycarboxylic acids.

Limonene Hydratase

In nature, limonene hydratase is involved in the biodegradation of the monoterpene limonene. One part of this pathway involves the conversion of limonene to α-terpineol, which is the corresponding hydration product. Limonene represents an easily accessible compound since it is produced in large amounts as a side product in food industry.



Scheme 12 Addition of water to (*R*)-(+)-limonene catalysed by limonene hydratase to form (*R*)-(+)-α-terpineol.

Especially during processing citrus fruits, limonene is produced as a waste product and represents a perfect precursor for a variety of different flavour and fragrance compounds like for example menthol or carvone.[63] (*R*)-(+)-α-Terpineol for example is a common fragrance in perfume industry, since it is known for its strong lilac-like smell. In contrast to this, its (*S*)-enantiomer displays a strong conifer-like odour. Since the olfactory properties strongly depend on the enantiomer, the enantioselective addition of water to the double bond is highly important for the production of the pure fragrance. All studies on the enantioselective addition of water showed that limonene hydratase is very specific and only (*R*)-(+)-limonene was converted to (*R*)-(+)-α-terpineol (see Scheme12).

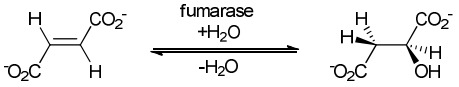
Limonene hydratases are often found in fungi (especially in those that grow on rotting citrus peel) like *Fusarium oxysporum* 152B[64-66], *Pleurotus sapidus*[67], *Aspergillus niger* (ATCC 16404, ATCC 9642 and ATCC 1004 strains)[68] and *Penicillium spp*.[69-71] but also bacterial sources like for example *Pseudomonas gladioli*[63, 72] *Escherichia coli*[73], and *Sphingobium spp*.[74] are known to harbour limonene hydratases. Due to (*R*)-(+)-limonene as a cheap starting material, also the chemical industry turned its interest to limonene hydratase. Several small-scale processes are running, giving access to 0.1 to 15.5 g of (*R*)-(+)-α-terpineol per litre of fermentation medium.[65, 68, 71, 75, 76] The most profitable process uses resting cells of *Sphingobium* sp. allowing the production of 130 g of (*R*)-(+)-α-terpineol per litre of medium within 96 h.[74]

3.3. Enzyme catalysed addition of water to electron-deficient C=C bonds

The addition of water to electron-deficient double bonds opens up a completely different class of substrates. Many enzymes that can perform this addition are known and well investigated. The most famous one is fumarase, but also malease, citraconase, aconitase, urocanase, enzymes with a hydratase-tautomerase bi-functionality, enoyl-CoA-hydratase, carnitine dehydratase, hydroxycinnamoyl-CoA hydratase lyase, Michael hydratase,[77] phenolic acid decarboxylases[78] or an artificial hydratase are described.[1, 79]

Fumarase

Fumarase is an enzyme that is industrially used in the large-scale production of (*S*)-malate (Scheme 13). It plays a very important role in primary metabolism, where its function is to catalyse the addition of water to fumarate to stereoselectively form (*S*)-malate, a reaction that is part of the citric acid cycle.[80]



Scheme 13 Fumarase catalyses the addition of water to fumarate forming (*S*)-malate.

Due to their important role in the primary metabolism, fumarases are ubiquitous in nature. Three different types of fumarases (fum A, fum B and fum C) are found in *E. coli* and are categorised in two different classes. Class I fumarases are dependent on Fe^{2+} and sensitive to heat. *E. coli* fumarase A and B are grouped in this class.[81, 82] In contrast to this, class II fumarases – fum C belongs to this group – are independent of Fe^{2+}; these enzymes are also not sensitive towards elevated temperatures and maintain activity at 50 ºC.[83-86] Investigations on the structure of fum C also allowed a detailed insight into the mechanism behind the hydration reaction (see Scheme 6).[43, 87-89] Due to their high stability only class II fumarases are used for industrial applications.

Malate is a very important compound in food industry. It is the second-most widely used acidulant holding approx. 10% of this market and represents an alternative to citric acid.[90] It is

furthermore discussed as a potential monomer for biodegradable polymers. In the traditional process for the production of (*S*)-malate, apple juice was used as a source for isolation of the compound. Since apple juice only contains 0.4–0.7% of (*S*)-malate, this method was soon proven to be inefficient. Chemical hydration processes for the production of malate often require harsh conditions. For example, in the largest chemical process maleic anhydride is converted to racemic malate by hydration. To hydrate maleic anhydride the reaction needs to be carried out at 180 °C and 1 MPa.[91]

Already in the late 1970s, fermentative processes using fumarase were invented to replace the traditional isolation method. Whole-cell approaches using immobilised cells of *Brevibacterium ammoniagene*[92], *Brevibacterium flavum*[93, 94] (representing two industrially established processes) or *Saccharomyces cerevisiae*[90] to develop continuous production systems were investigated. Using *Brevibacterium flavum* cells immobilised on κ-carrageenan gel gave rise to a conversion of 80% – which represents approx. the equilibrium conversion – and a production capacity of 468 t/a.[95, 96] In 1984 an enzyme-membrane-reactor-based production system was started by the former Degussa company.[97] This system allowed to recycle the enzyme while still performing homogenous enzyme catalysis. The same system was not only applied for the production of (*S*)-malate but also for natural α-amino acids.

Suspended whole cells from *Corynebacterium glutamicum* were used by AMINO GmbH in 1988 in a process that allowed the production of approx. 2000 t/a.[95, 98, 99] The main limitation in the water addition to fumarate is the equilibrium that governs the reaction. Even though the product side is favoured, full conversion cannot be achieved. To overcome this limitation, a precipitation strategy was used in some industrial processes. Calcium carbonate is used in an elegant way to precipitate both the fumarate and the malate and the reaction takes place in a slurry of the salts and the biocatalyst where only approx. 1% of the calcium salt is in solution (see Figure 4).[95]

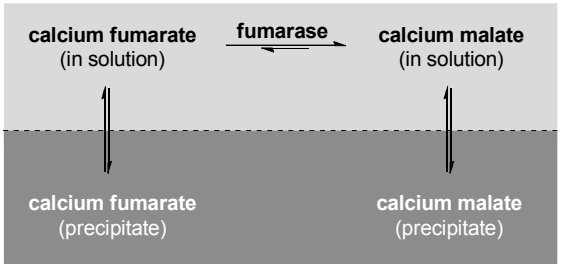


Figure 4 The calcium carbonate method allows to shift the equilibrium of the reaction by precipitating both fumarate and malate. The reaction mixture is a slurry of the salts and the biocatalyst.

The industrial production of (*S*)-malate by for example AMINO GmbH comprises the following steps: The reaction mixture contains imidazole buffer, fumarase (whole cells of *Corynebacterium glutamicum*), fumarate and calcium carbonate to achieve the shift of equilibrium. The biocatalyst can be removed from the reaction mixture by a simple filtration step. The calcium salt is removed by the addition of sulphuric acid leading to the formation of insoluble calcium sulfate – removable by filtration – and the soluble free (*S*)-malate. After evaporation of the solvent, (*S*)-malate is purified by crystallisation and isolated after a final centrifugation step (see Figure 5).

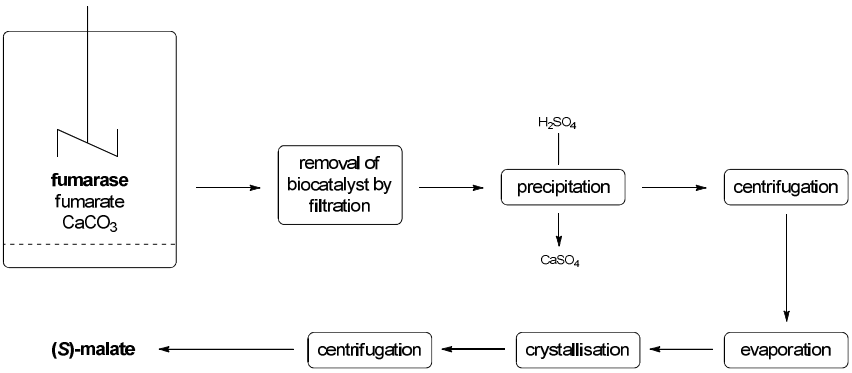
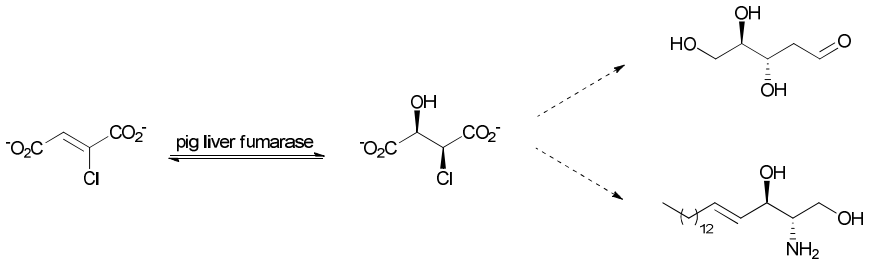


Figure 5 Flow scheme for the industrial process for the production of (*S*)-malate using whole cells of *Corynebacterium glutamicum*.

As an enzyme that is involved in the primary metabolism, fumarase shows a strict substrate specificity. What is desired and highly important for living organisms is a drawback when it comes to biocatalysis, where a broader substrate spectrum is appreciated. Experiments to elucidate the substrate scope were already carried out in 1968 using fumarase from pig heart.

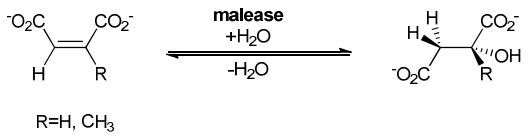
The enzyme showed activity towards the following substrates: fluorofumarate, fumarate, chlorofumarate, bromofumarate, acetylenedicarboxylate, iodofumarate, mesaconate (in decreasing order of activity). For nearly all tested substrates, the water addition took place in the *trans*-fashion. The *cis*-product was found for α-fluoromalate, where also a spontaneous decomposition to oxaloacetate was observed.[100] Later tests on the substrate specificity showed that also chloro-, fluoro- and difluoro-fumarate are accepted as substrates. Chlorofumarate was for example converted to L-*threo*-chloromalate, which was chemically transformed further to *trans*-D-*erythro*-sphingosine and 2-deoxy-D-ribose (see Scheme14).[101]



Scheme 14 Pig liver fumarase was used for the conversion of chloro-fumarate to L-*threo*-chloromalate, which was further converted to *trans*-D-*erythro*-sphingosine and 2-deoxy-D-ribose.

Malease

The use of fumarase allows the production of enantiomerically pure (*S*)-malate. Nature also provides a catalyst for the selective synthesis of the opposite enantiomer, (*R*)-malate. The enzyme described for this is malease, which is capable of performing the water addition to maleate (see Scheme 15). Like in the case of fumarase, malease is cofactor-independent and displays good stability. In contrast to fumarase, malease is not involved in the primary metabolism and is therefore also less abundant in nature. However it is found in both bacterial and mammalian sources.[39, 102-106]



Scheme 15 Malease catalyses the addition of water to maleic acid or citraconic acid.

The substrate spectrum of malease is – as is the case for fumarase – rather narrow and maleic acid and citraconic acid are accepted best, but small changes in the functional group pattern of these substrates are allowed.[104-106] For example chloromaleate and bromomaleate are hydrated to give the α-substituted malates (2*S*,3*S*)-3-chloromalate and (2*S*,3*S*)-3-bromomalate.[107]

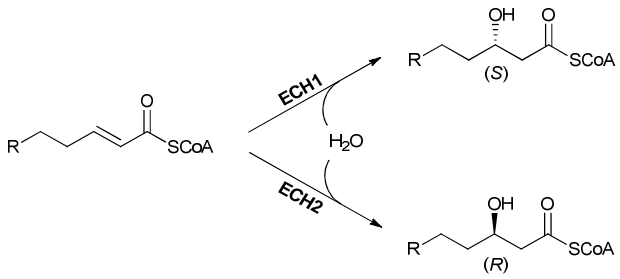
In industry, *Pseudomonas pseudoalcaligenes* containing malease is used for the large-scale production of malic acid. Starting this production process from maleic anhydride – which undergoes spontaneous hydrolysis under aqueous conditions – allows a more cost-efficient process.[95] In more recent studies, the use of permeabilised *Pseudomonas pseudoalcaligenes* cells in a continuous process is reported.[108]

Enoyl-CoA-hydratase

Enoyl-CoA-hydratase is an enzyme involved in the degradation pathway of fatty acids. It catalyses the addition of water to fatty acids. Depending on the fatty acid (linear or branched) different enoyl-CoA-hydratases are employed in the degradation. Not only the substrate specificity differs, also the enantioselectivity can be different. Depending on the enoyl-CoA-hydratase used, the water addition can be either (*S*)- or (*R*)-selective (see Scheme 16).[109-111] It also needs to be mentioned that in contrast to chemical methods, where only the *anti*-addition of water is observed, enoyl-CoA-hydratases catalyse the *syn*-addition.

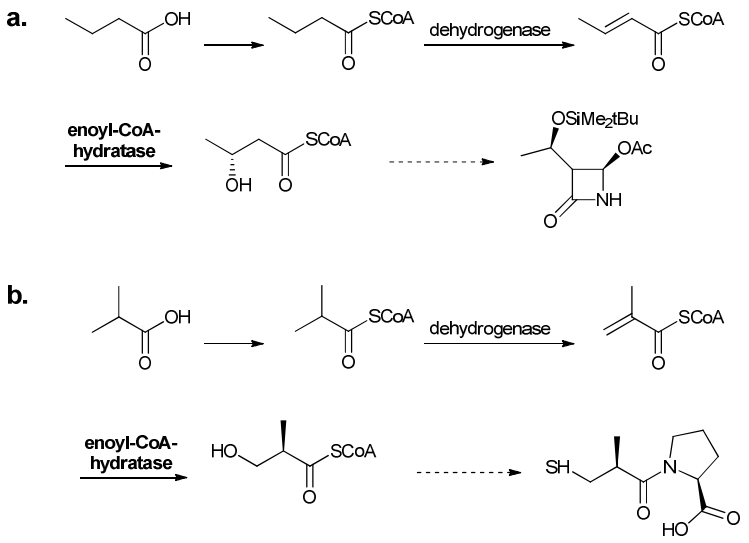
One example for an (*S*)-selective enoyl-CoA-hydratase is ECH1. This enzyme is also known as crotonase, and is able to perform the addition of water to substrates with a chain length between 4 and 20 carbon atoms.[45, 109] This rather broad substrate spectrum is achieved by a flexible loop in the active site, which allows to increase the hydrophobic binding pocked to bind larger substrates.[112] The water addition to a *trans*-enoyl-CoA-thioester takes place in a *syn*-fashion and (*S*)-3-hydroxyacyl-CoA is formed.[45, 112-115] ECH1 from bovine liver can be heterologously expressed in *E. coli*, which allows the production of larger amounts of enzyme.

The (*R*)-selective enoyl-CoA-hydratase ECH2 also catalysed the *syn*-addition with opposite enantioselectivity. *Trans*-2-enoyl CoA thioesters are accepted as substrates and (*R*)-3-hydroxyacyl-CoA is formed as the product.[116] The substrate specificity of ECH2 depends on the source. For example, ECH2s from bacterial sources show a preference towards short-chain substrates while ECH2s from eukaryotic sources prefer long-chain substrates.[114] The difference in enantioselectivity between ECH1 and ECH2 can easily be explained by the geometry of the active site. The active sites of the ECH1 and ECH2 behave like mirror images of each other.[115]



Scheme 16 The addition of water catalysed by ECH1 and ECH2 proceeds in a *syn*-fashion but displays a different enantioselectivity depending on the enzyme used.

In industry, enoyl-CoA-hydratase is employed for the production of D-β-hydroxyisobutyric acid from isobutyric acid. In a whole-cell process run by Kanegafuchi Japan, cells from *Candida rugosa* IFO 0750 M are used.[117] This process consists of three steps starting from butyric acid, which is converted into the corresponding CoA-thioester. This thioester is then enzymatically dehydrated to form the α,β-unsaturated compound, which serves as the substrate for the hydratase. In additional steps the hydrated thioester can then be converted to (*R*)-3-hydroxybutanoic acid (> 98% *ee*). The efficiency of this process is represented by a space-time-yield of 5–10 g/L/d.[95] (*R*)-3-Hydroxybutanoic acid represents an important building block for the synthesis of a carbapenem intermediate (see Scheme 17a).[95] This process can also be employed for the production of (*R*)-3-hydroxy-2-methylpropanoic acid, which represents a precursor for captopril (see Scheme 17b). For this process a space-time-yield of 5–10 g/L/d and a yield of 98% is reported.[95, 118]



Scheme 17 Industrial processes employing enoyl-CoA-hydratase from *Candida rugosa* IFO 0750 M. **a.** Route starting from isobutyric acid for the synthesis of the D-β-hydroxyisobutyric acid, which serves as a precursor for a carbapenem intermediate. **b.** Route starting from isobutyric acid for the synthesis of (*R*)-3-hydroxy-2-methylpropanoic acid, a building block in the captopril synthesis.

Recently also the concept of designing new enzymes with new activities has become more popular. Two examples with pyridine-based ligands of Cu catalysts were described. In one case DNA was utilised as chiral backbone, in the other case the complex was embedded into a homodimeric protein. Remarkably the DNA based catalyst catalysed a *syn*-addition of water.[119] When alcohol was present in the reaction mixture this was used as nucleophile, rather than water, again demonstrating the difficulty in utilising water.[120] In the other example the design of an artificial metallo-enzyme with hydratase activity was achieved by employing the homodimeric protein LmrR – a transcriptional regulator – as the second coordination sphere for a Cu(II) phenanthroline complex that is responsible for the activation of water.[79] Even though this method is not applicable on large scale yet, it represents a different approach for the design of novel enzymes. Again other nucleophiles were preferred over water.[121]

4. Conclusion and Outlook

Water is and remains a poor electrophile and nucleophile that is difficult to activate. Its direct application as a reagent in addition reactions to C=C double bonds therefore remains challenging. Chemical catalysts to date are not very successful in the activation of water (section 2), yet there is hope. Building on the knowledge of biological systems the first artificial hydratases have been developed opening up new avenues for chemo-catalysis (section 3.3).[79,119] In stark contrast to the chemical approaches hydratases are the backbone of life. They may serve as models for better chemical catalysts but of course they are catalysts in their own right. Indeed they have proven their value in several industrial processes (section 3).

In general, the asymmetric addition of water to double bonds represents a very efficient and green method for the production of secondary and tertiary alcohols.[122] Water represents an abundant and safe nucleophile, being both the solvent and the substrate, and hydratases are the perfect tools to perform this reaction. Selected hydratases like fumarase, malease or enoyl-CoA-hydratase have already proven to be versatile tools for the biocatalytic synthesis of fine chemicals. They are employed on industrial scale in well-established processes still running today on large annual production scales (see Table 2).

Table 2 Comparison of hydratases employed in industry.[95] [a] no numbers available, [b] equilibrium yield for (3*R*)-3-hydroxybutanoyl-CoA, which serves as the precursor for β-hydroxy-*n*-butyric acid.

Product	Enzyme	Organism	Company	yield [%]	equilibrium yield [%]	Annual production [t]
(*S*)-malate	fumarase	*Corynebacterium glutamicum* (suspended whole cells)	AMINO GmbH	85	82[123, 124]	2000
(*S*)-malate	fumarase	*Brevibacterium flavum* (immobilised whole cells)	Tanabe Seiyaku Co., Ltd.	> 70	82[123, 124]	468
(*R*)-malate	malease	*Pseudomonas pseudoalcaligenes* (immobilised whole cells)	DSM	> 99	100[105]	-[a]
β-hydroxy-*n*-butyric acid	enoyl-CoA hydratase	*Candida rugosa* IFO 0750 M (suspended whole cells)	Kanegafuchi Chemical Industries Co., Ltd	-[a]	85[b,125]	-[a]
β-hydroxy-isobutyric acid	enoyl-CoA hydratase	*Candida rugosa* IFO 0750 M (suspended whole cells)	Kanegafuchi Chemical Industries Co., Ltd	98	-[a]	-[a]

These hydratases are employed for very specific reactions and due to their narrow substrate spectrum, a broad applicability is not possible. However, in recent years methods for the discovery of novel enzymes as well as the techniques to engineer known biocatalysts have significantly improved. Structural investigations allow a deeper insight in the mechanisms behind hydratase-catalysed reactions and catalytically essential residues can be identified. This might allow the expansion of the range of hydratases. Furthermore, promiscuous hydratase activity for different decarboxylases was discovered. In this study seven phenolic decarboxylases from different sources were used to hydrate five different hydroxystyrenes as substrates.[78]

Promisingly there are also organisms with reported hydratase activity that were not yet investigated in detail. For example the use of resting cells from *Rhodococcus rhodochrous* for the hydration of the lactones 3-methyl-2-butenolide and 3-ethyl-2-butenolide to give the corresponding (*R*)-3-hydroxy-3-alkylbutanolides is reported, but detailed investigations on the enzyme performing this interesting reaction are still missing.[77,126,127] A hydratase activity from *Alicycliphilus denitrificans* was however recently shown to be an artefact. This was due to a coupled assay used, indicating that great care has to be taken when identifying these enzyme activities.[128, 129]

Summarising, the addition of water is a demanding task. Chemically only the most simple molecules with a limited number of functional groups can be converted reasonably well. But even in these cases activity and in particular selectivity are missing. Hydratases on the other hand represent efficient and highly selective catalysts for the addition of water, a reaction that is still underrepresented and difficult to achieve in organic chemistry. Using water as the nucleophile and substrate at the same time represents not only an elegant but also a very green route to the production of a variety of different alcohols. The current limitations arising from the substrate scope of the known enzymes can be challenged by modern protein engineering techniques and new enzyme discovery to broaden the toolbox of hydratases available for industrial applications.[130]

Acknowledgement

An “Erwin-Schroedinger” Fellowship (J3292) is generously provided by the Austrian Science Fund (FWF) for V.R. The authors thank all their colleagues and group members for the many fruitful discussions and the joint work that helped in forming the ideas formulated in this perspective.

References

1. J. Jin and U. Hanefeld, *Chem. Commun. (Cambridge)*, 2011, **47**, 2502-2510.
2. J. E. McMurry, *Organic Chemistry*, Cengage Learning, 8th edn., 2012.
3. K. Schwetlick, *Organikum*, Wiley, 23 edn., 2009.
4. J. Clayden, N. Greeves and S. Warren, *Organic Chemistry*, Oxford University Press, 2nd edn., 2012.
5. P. N. Davey, M. J. Earle, J. T. Hamill, S. P. Katdare, D. W. Rooney and K. R. Seddon, *Green Chem.*, 2010, **12**, 628-631.
6. V. Resch, C. Seidler, B.-S. Chen, I. Degeling and U. Hanefeld, *Eur. J. Org. Chem.*, 2013, 7697-7704.
7. S. Wang, Z. Zhang, C. Chi, G. Wu, J. Ren, Z. Wang, M. Huang and Y. Jiang, *React. Funct. Polym.*, 2008, **68**, 424-430.
8. X. Wang, D. Sui, M. Huang and Y. Jiang, *Polym. Adv. Technol.*, 2006, **17**, 163-167.
9. L. Xue, B. Jia, L. Tang, X. F. Ji, M. Y. Huang and Y. Y. Jiang, *Polym. Adv. Technol.*, 2004, **15**, 346-349.
10. N. Kosaric, Z. Duvnjak, A. Farkas, H. Sahm, S. Bringer-Meyer, O. Goebel and D. Mayer, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2012, DOI: 10.1002/14356007.a09_587.pub2.
11. V. J. Nowlan and T. T. Tidwell, *Acc. Chem. Res.*, 1977, **10**, 252-258.
12. Y. Li, C. Wang, G. Jia, S. Lu and C. Li, *Tetrahedron*, 2013, **69**, 6585-6590.
13. H. M. Meshram, N. Satish Kumar, J. B. Nanubolu, L. Chandrasekhara Rao and N. Nageswara Rao, *Tetrahedron Lett.*, 2013, **54**, 5941-5944.
14. C. Pan and Z. Wang, *Coord. Chem. Rev.*, 2008, **252**, 736-750.
15. L. Petrus, R. W. De Roo, E. J. Stamhuis and G. E. H. Joosten, *Chem. Eng. Sci.*, 1984, **39**, 433-446.
16. A. J. Papa, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2012, DOI: 10.1002/14356007.a22_173.pub2.
17. D. A. O'Sullivan, *Chem. Eng. News*, 1985, **63**, 10-11.
18. H.-D. Hahn, G. Dämbkes, N. Rupprich, H. Bahl and G. D. Frey, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, 2013, DOI: 10.1002/14356007.a04_463.pub3.
19. Z. Lei, Y. Yang, Q. Li and B. Chen, *Catal. Today*, 2009, **147**, S352-S356.
20. L. Petrus, R. W. De Roo, E. J. Stamhuis and G. E. H. Joosten, *Chem. Eng. Sci.*, 1986, **41**, 217-226.
21. J. Monteiro and C. Veloso, *Top. Catal.*, 2004, **27**, 169-180.
22. P. Botella, A. Corma, J. M. L. Nieto, S. Valencia, M. E. Lucas and M. Sergio, *Appl. Catal. A: Gen.*, 2000, **203**, 251-258.
23. Y. Liu, Z. Zhou, G. Yang, Y. Wu and Z. Zhang, *Int. J. Chem. Reactor Eng.*, 2010, **8**, 1542-6580.
24. N. Comelli, M. C. Avila, C. Volzone and M. Ponzi, *Cent. Eur. J. Chem.*, 2013, **11**, 689-697.
25. A. M. Chibiryaev, A. Yermakova and I. V. Kozhevnikov, *J. Supercrit. Fluids*, 2010, **51**, 295-305.
26. M. C. Ávila, N. A. Comelli, E. Rodríguez-Castellón, A. Jiménez-López, R. Carrizo Flores, E. N. Ponzi and M. I. Ponzi, *J. Mol. Catal. A: Chem.*, 2010, **322**, 106-112.
27. T. Mochida, R. Ohnishi, N. Horita, Y. Kamiya and T. Okuhara, *Microporous Mesoporous Mater.*, 2007, **101**, 176-183.
28. D. Arntz, A. Fischer, M. Höpp, S. Jacobi, J. Sauer, T. Ohara, T. Sato, N. Shimizu and H. Schwind, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2012, DOI: 10.1002/14356007.a01_149.pub2.
29. C. Kohlpaintner, M. Schulte, J. Falbe, P. Lappe, J. Weber and G. D. Frey, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, 2013, DOI: 10.1002/14356007.a01_321.pub3.
30. T. Haas, T. Hahm, R. Vanheertum, W. Hofen and L. Deusser, WO 01/09073 A1, 2001.
31. C. J. Sullivan, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, 2012, DOI: 10.1002/14356007.a22_163.
32. E. Celinska, *Biotechnol. Adv.*, 2010, **28**, 519-530.

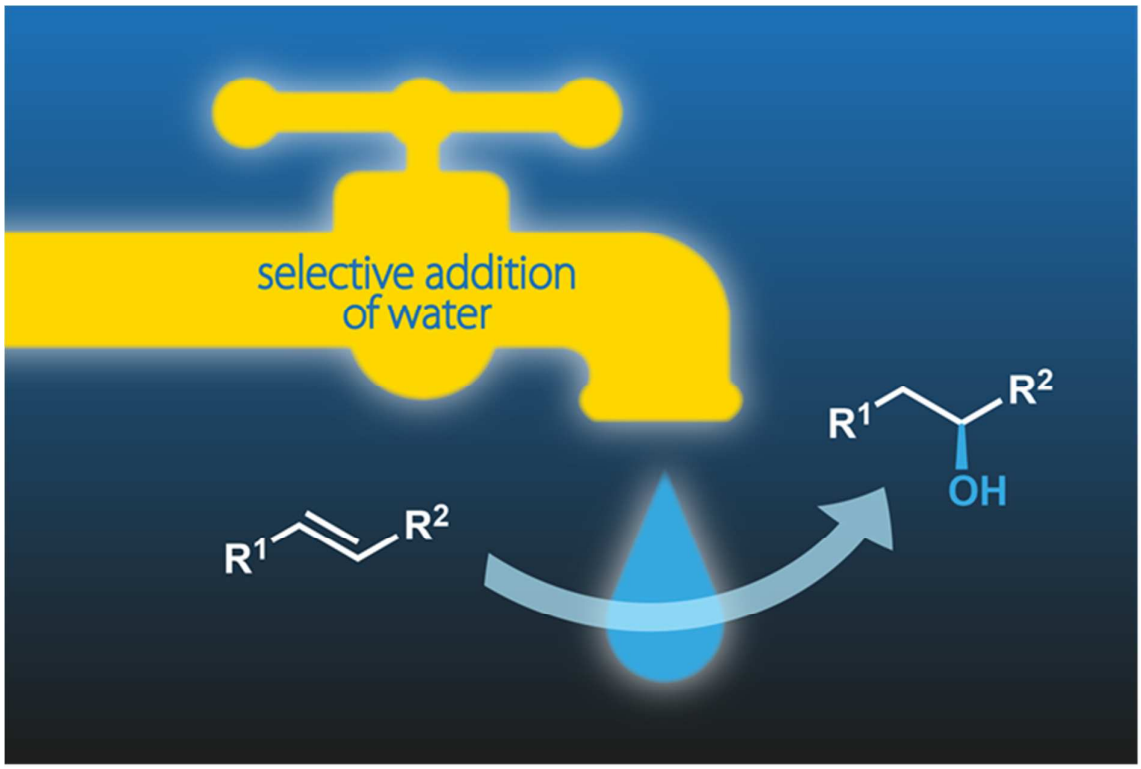
33. D. M. Adkesson, A. W. Alsop, T. T. Ames, L. A. Chu, J. M. Disney, B. C. Dravis, P. Fitzgibbon, J. M. Gaddy, F. G. Gallagher, W. F. Lehnhardt, J. C. Jefferson, M. L. Luyben, M. Seapan, R. E. Trotter, G. M. Wenndt, E. K. Yu, WO 2004/101479 A2, 2004.
34. V. E. T. Maervoet, M. De Mey, J. Beauprez, S. De Maeseneire and W. K. Soetaert, *Org. Process Res. Dev.*, 2010, **15**, 189-202.
35. A. J. Mattam, J. M. Clomburg, R. Gonzalez and S. S. Yazdani, *Biotechnol. Lett.*, 2013, **35**, 831-842.
36. H. Gröger, *Angew. Chem. Int. Ed. Engl.*, 2014, **53**, 3067-3069.
37. V. E. Anderson, M. W. Ruszczycky and M. E. Harris, *Chem. Rev.*, 2006, **106**, 3236-3251.
38. X. Zhang and K. N. Houk, *Acc. Chem. Res.*, 2005, **38**, 379-385.
39. M. J. van der Werf, W. J. J. van den Tweel, J. Kamphuis, S. Hartmans and J. A. M. de Bont, *Trends Biotechnol.*, 1994, **12**, 95-103.
40. T. Tokoroyama, *Eur. J. Org. Chem.*, 2010, 2009-2016.
41. Mohrig, K. Moerke, D. Cloutier, B. Lane, E. Person and T. Onasch, *Science*, 1995, **269**, 527-529.
42. H. Lauble, M. C. Kennedy, H. Beinert and C. D. Stout, *J. Mol. Biol.*, 1994, **237**, 437-451.
43. I. A. Rose and T. M. Weaver, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 3393-3397.
44. J. R. Mohrig, *Acc. Chem. Res.*, 2013, **46**, 1407-1416.
45. H. M. Holden, M. M. Benning, T. Haller and J. A. Gerlt, *Acc. Chem. Res.*, 2001, **34**, 145-157.
46. K. R. Hanson and I. A. Rose, *Acc. Chem. Res.*, 1975, **8**, 1-10.
47. J. A. Gerlt and P. G. Gassman, *J. Am. Chem. Soc.*, 1992, **114**, 5928-5934.
48. V. Puthan Veetil, G. Fibriansah, H. Raj, A.-M. W. H. Thunnissen and G. J. Poelarends, *Biochemistry*, 2012, **51**, 4237-4243.
49. E. N. Davis, L. L. Wallen, J. C. Goodwin, W. K. Rohwedder and R. A. Rhodes, *Lipids*, 1969, **4**, 356-362.
50. W. G. Niehaus, A. Kisic, A. Torkelson, D. J. Bednarczyk and G. J. Schroepfer, *J. Biol. Chem.*, 1970, **245**, 3790-3797.
51. L. L. Wallen, R. G. Benedict and R. W. Jackson, *Arch. Biochem. Biophys.*, 1962, **99**, 249-253.
52. A. Kisic, Y. Miura and G. J. Schroepfer, Jr., *Lipids*, 1971, **6**, 541-545.
53. L. E. Bevers, M. W. H. Pinkse, P. D. E. M. Verhaert and W. R. Hagen, *J. Bacteriol.*, 2009, **191**, 5010-5012.
54. Y.-C. Joo, K.-W. Jeong, S.-J. Yeom, Y.-S. Kim, Y. Kim and D.-K. Oh, *Biochimie*, 2012, **94**, 907-915.
55. B.-N. Kim, Y.-C. Joo, Y.-S. Kim, K.-R. Kim and D.-K. Oh, *Appl. Microbiol. Biotechnol.*, 2012, **95**, 929-937.
56. S. Gocho, N. Tabogami, M. Inagaki, C. Kawabata and T. Komai, *Biosci., Biotechnol., Biochem.*, 1995, **59**, 1571-1572.
57. A. Wanikawa, K. Hosoi, I. Takise and T. Kato, *J. Inst. Brew.*, 2000, **106**, 39-44.
58. B.-N. Kim, S.-J. Yeom and D.-K. Oh, *Biotechnol. Lett.*, 2011, **33**, 993-997.
59. Y.-C. Joo, E.-S. Seo, Y.-S. Kim, K.-R. Kim, J.-B. Park and D.-K. Oh, *J. Biotechnol.*, 2012, **158**, 17-23.
60. E.-Y. Jeon, J.-H. Lee, K.-M. Yang, Y.-C. Joo, D.-K. Oh and J.-B. Park, *Process Biochem.*, 2012, **47**, 941-947.
61. J.-W. Song, E.-Y. Jeon, D.-H. Song, H.-Y. Jang, U. T. Bornscheuer, D.-K. Oh and J.-B. Park, *Angew. Chem., Int. Ed. Engl.*, 2013, **52**, 2534-2537.
62. J.-W. Song, J.-H. Lee, U. T. Bornscheuer and J.-B. Park, *Adv. Synth. Catal.*, 2014, **356**, 1782-1788.
63. K. R. Cadwallader, R. J. Braddock, M. E. Parish and D. P. Higgins, *J. Food Sci.*, 1989, **54**, 1241-1245.
64. M. R. Marostica and G. M. Pastore, *Food Chem.*, 2006, **101**, 345-350.
65. J. Bicas, F. Barros, R. Wagner, H. Godoy and G. Pastore, *J. Ind. Microbiol. Biotechnol.*, 2008, **35**, 1061-1070.
66. J. L. Bicas, C. P. de Quadros, I. A. Néri-Numa and G. M. Pastore, *Food Chem.*, 2010, **120**, 452-456.
67. J. Onken and R. G. Berger, *J. Biotechnol.*, 1999, **69**, 163-168.

68. I. Rottava, G. Toniazzo, P. F. Cortina, E. Martello, C. E. Grando, L. A. Lerin, H. Treichel, A. J. Mossi, D. de Oliveira, R. L. Cansian, O. A. C. Antunes and E. G. Oestreicher, *LWT - Food Sci. Technol.*, 2010, **43**, 1128-1131.
69. A. Adams, J. C. R. Demyttenaere and K. N. De, *Food Chem.*, 2002, *80*, 525-534.
70. A. Z. M. Badee, S. A. Helmy and N. F. S. Morsy, *Food Chem.*, 2011, **126**, 849-854.
71. Q. Tan and D. F. Day, *Appl. Microbiol. Biotechnol.*, 1998, **49**, 96-101.
72. K. R. Cadwallader, R. J. Braddock and M. E. Parish, *J. Food Sci.*, 1992, **57**, 241-244, 248.
73. N. Savithiry, T. Cheong and P. Oriel, in *Biotechnology for Fuels and Chemicals*, eds. B. Davison, C. Wyman and M. Finkelstein, Humana Press, 1997, vol. **63-65**, ch. 20, pp. 213-220.
74. J. L. Bicas, P. Fontanille, G. M. Pastore and C. Larroche, *Process Biochem.*, 2010, **45**, 481-486.
75. M. Pescheck, M. Mirata, B. Brauer, U. Krings, R. Berger and J. Schrader, *J. Ind. Microbiol. Biotechnol.*, 2009, **36**, 827-836.
76. S. G. A. Prieto, V. J. A. Perea and L. C. C. Ortiz, *Vitae*, 2011, **18**, 163-172.
77. H. Holland and J.-X. Gu, *Biotechnol. Lett.*, 1998, *20*, 1125-1126.
78. C. Wuensch, J. Gross, G. Steinkellner, K. Gruber, S. M. Glueck and K. Faber, *Angew. Chem., Int. Ed. Engl.*, 2013, **52**, 2293-2297.
79. J. Bos, A. Garcia-Herraiz and G. Roelfes, *Chem. Sci.*, 2013, **4**, 3578-3582.
80. C. A. Lamartiniere, H. D. Braymer and A. D. Larson, *Arch. Biochem. Biophys.*, 1970, **141**, 293-302.
81. D. H. Flint, *Arch. Biochem. Biophys.*, 1994, **311**, 509-516.
82. Y. Ueda, N. Yumoto, M. Tokushige, K. Fukui and H. Ohya-Nishiguchi, *J. Biochem. (Tokyo)*, 1991, **109**, 728-733.
83. S. A. Woods, S. D. Schwartzbach and J. R. Guest, *Biochim. Biophys. Acta*, 1988, **954**, 14-26.
84. D. H. Flint, M. H. Emptage and J. R. Guest, *Biochemistry*, 1992, **31**, 10331-10337.
85. T. Weaver, M. Lees, V. Zaitsev, I. Zaitseva, E. Duke, P. Lindley, S. McSweeny, A. Svensson, J. Keruchenko, I. Keruchenko, K. Gladilin and L. Banaszak, *J. Mol. Biol.*, 1998, **280**, 431-442.
86. J. Yang, Y. Wang, E. M. Woolridge, V. Arora, G. A. Petsko, J. W. Kozarich and D. Ringe, *Biochemistry*, 2004, **43**, 10424-10434.
87. T. Weaver, *Acta Crystallogr., Sect. D*, 2005, **61**, 1395-1401.
88. T. Weaver and L. Banaszak, *Biochemistry*, 1996, **35**, 13955-13965.
89. T. Weaver, M. Lees and L. Banaszak, *Protein Sci.*, 1997, **6**, 834-842.
90. E. Bressler, O. Pines, I. Goldberg and S. Braun, *Biotechnol. Prog.*, 2002, **18**, 445-450.
91. K. Lohbeck, H. Haferkorn, W. Fuhrmann and N. Fedtke, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, 2000, DOI: 10.1002/14356007.a16_053.
92. K. Yamamoto, T. Tosa, K. Yamashita and I. Chibata, *Eur. J. Appl. Microbiol. Biotechnol.*, 1976, **3**, 169-183.
93. I. Takata, K. Yamamoto, T. Tosa and I. Chibata, *Enzyme Microb. Technol.*, 1980, **2**, 30-36.
94. I. Chibata, T. Tosa and I. Takata, *Trends Biotechnol.*, 1983, **1**, 9-11.
95. A. Liese, K. Seelbach and C. Wandrey, *Industrial Biotransformations; Second, Completely Revised and Extended Edition*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2006.
96. T. Tosa and T. Shibatani, *Ann. N. Y. Acad. Sci.*, 1995, **750**, 364-375.
97. W. Leuchtenberger, M. Karrenbauer and U. L. F. Plöcker, *Ann. N. Y. Acad. Sci.*, 1984, **434**, 078-086.
98. H. J. Danneel and R. Geiger, DE 4430010 C1, 1996.
99. H. J. Danneel and R. Geiger, DE 4424664 C1, 1995.
100. J. W. Teipel, G. M. Hass and R. L. Hill, *J. Biol. Chem.*, 1968, **243**, 5684-5694.
101. M. A. Findeis and G. M. Whitesides, *J. Org. Chem.*, 1987, **52**, 2838-2848.
102. J.-L. Dreyer, *Eur. J. Biochem.*, 1985, **150**, 145-154.
103. B.-F. He, T. Nakajima-Kambe, T. Ozawa and T. Nakahara, *Process Biochem.*, 2000, **36**, 407-414.
104. M. J. van der Werf, W. J. van den Tweel and S. Hartmans, *Appl. Environ. Microbiol.*, 1992, **58**, 2854-2860.

105. M. J. van der Werf, W. J. J. van den Tweel and S. Hartmans, *Appl. Environ. Microbiol.*, 1993, **59**, 2823-2829.
106. M. J. van der Werf, W. J. J. van den Tweel and S. Hartmans, *Eur. J. Biochem.*, 1993, **217**, 1011-1017.
107. M. Ueda, H. Yamada and Y. Asano, *Appl. Microbiol. Biotechnol.*, 1994, **41**, 215-218.
108. M. J. F. Michielsen, C. Frielink, R. H. Wijffels, J. Tramper and H. H. Beeftink, *J. Biotechnol.*, 2000, **79**, 13-26.
109. J. K. Hiltunen and Y.-M. Qin, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids*, 2000, **1484**, 117-128.
110. G. Agnihotri and H.-w. Liu, *Bioorg. Med. Chem.*, 2003, **11**, 9-20.
111. P. Bhaumik, M. K. Koski, T. Glumoff, J. K. Hiltunen and R. K. Wierenga, *Curr. Opin. Struct. Biol.*, 2005, **15**, 621-628.
112. B. J. Bahnson, V. E. Anderson and G. A. Petsko, *Biochemistry*, 2002, **41**, 2621-2629.
113. A. F. Bell, Y. Feng, H. A. Hofstein, S. Parikh, J. Wu, M. J. Rudolph, C. Kisker, A. Whitty and P. J. Tonge, *Chem. Biol.*, 2002, **9**, 1247-1255.
114. M. K. Koski, A. M. Haapalainen, J. K. Hiltunen and T. Glumoff, *J. Biol. Chem.*, 2004, **279**, 24666-24672.
115. M. K. Koski, A. M. Haapalainen, J. K. Hiltunen and T. Glumoff, *J. Mol. Biol.*, 2005, **345**, 1157-1169.
116. T. Hisano, T. Tsuge, T. Fukui, T. Iwata, K. Miki and Y. Doi, *J. Biol. Chem.*, 2003, **278**, 617-624.
117. J. Hasegawa, M. Ogura, H. Kanema, N. Noda, H. Kawaharada and K. Watanabe, *J. Ferment. Technol.*, 1982, **60**, 501-508.
118. J. Crosby, *Tetrahedron*, 1991, **47**, 4789-4846.
119. A. J. Boersma, D. Coquière, D. Geerdink, F. Rosati, B. L. Feringa and G. Roelfes, *Nature Chem.*, 2010, **2**, 991-995.
120. R. P. Megens and G. Roelfes, *Chem. Commun. (Cambridge)*, 2012, *48*, 6366-6368.
121. J. Bos, F. Fusetti, A. J. Driessen and G. Roelfes, *Angew Chem Int Ed Engl*, 2012, **51**, 7472-7475.
122. R. Kourist and U. Bornscheuer, *Appl. Microbiol. Biotechnol.*, 2011, *91*, 505-517.
123. R. M. Bock and R. A. Alberty, *J. Am. Chem. Soc.*, 1953, **75**, 1921-1925.
124. H. A. Krebs, *Biochem. J.*, 1953, **54**, 78-82.
125. J. R. Stern and A. del Campillo, *J. Biol. Chem.*, 1956, **218**, 985-1002.
126. B.-S. Chen, L. G. Otten, V. Resch, G. Muyzer and U. Hanefeld, *Stand. Genomic Sci*, 2013, **9**, 175.
127. M. Müller, *ChemBioEng Rev*., 2014, **1**, 14–26.
128. J. Jin, A. J. Straathof, M. H. Pinkse and U. Hanefeld, *Appl. Microbiol. Biotechnol.*, 2011, **89**, 1831-1840.
129. V. Resch, J. Jin, B.-S. Chen and U. Hanefeld, *AMB express*, 2014, **4**, 30.
130. T. Davids, M. Schmidt, D. Böttcher and U. T. Bornscheuer, *Curr. Opin. Chem. Biol.*, 2013, **17**, 215-220.

Graphical abstract

Water is omnipresent and unreactive. How to speed water addition up and even make it selective is highlighted in this perspective.



Biographic sketch

Verena Resch was born in Austria in 1984. She studied biochemistry and molecular biology at the University of Graz and graduated as a MSc in 2008 under the supervision of Prof. Wolfgang Kroutil establishing multi-enzyme cascades. Staying in the same group she received her PhD in organic chemistry in 2011 working on the use of alkaloid pathway enzymes in organic synthesis. In 2012 she started as a post-doctoral fellow for two years with an Erwin-Schrödinger Fellowship from the Austrian Science Fund at the University of Technology in Delft in the group of Prof. Ulf Hanefeld working on hydratases and chemo-enzymatic cascades. She just returned to Graz, where she continues her work as Erwin-Schrödinger fellow.

Ulf Hanefeld was born in 1966 in Köln, Germany, and grew up in then (West) Berlin and London. In 1993 he received his PhD from the Georg-August-Universität zu Göttingen, having performed the research both in Göttingen (Prof. H. Laatsch) and Seattle (Prof. H. G. Floss). After postdoctoral years with Prof. C. W. Rees (Imperial College London), Prof. J. Staunton (Cambridge) and Prof. J. J. Heijnen and Dr. A. J. J. Straathof (TU Delft), he received a fellowship from the Royal Netherlands Academy of Arts and Sciences (KNAW). He rose through the ranks at the Technische Universiteit Delft and his research in Delft focuses on enzymes, their immobilisation and application in organic synthesis.