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Catalysis Science & Technology

REVIEW

From sequential chemoenzymatic synthesis to integrated hybrid catalysis: Taking the best of both worlds to open up the scope of possibilities for a sustainable future.

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Enzymatically catalyzed reactions are highly efficient but higher scale extrapolation is limited to a certain class of stable and easily recyclable enzymes. Heterogeneously catalyzed reactions are often limited by the multiphasic nature of catalytic systems and/or variations in the distribution of active sites. For these reasons, the development of highly selective and efficient hybrid catalysts is extremely needed and is now well admitted that disruptive innovation is highly desirable in process engineering, (bio)catalyst design and materials science. An optimal integration of all types of catalysis is the key for efficient global implementation of sustainable processes. This review gives an overview of all the pathways that integrate chemical and biological catalysis, beginning with combined chemoenzymatic processes in cascade to progressively open the field of hybrid catalysis in a one-pot concomitant execution. We have emphasized on the factors that have to be considered to understand their catalytic synergy. A better understanding of hybrid catalytic systems is an essential tool not only in the choice of the best route of their implementation but also in the synthesis of a wide range of fine chemicals.

1. Introduction

After more than a decade of intense research on biomass valorisation devoted to switch from classical refineries to biorefineries, one must acknowledge that many hard challenges are still being faced, especially when non-edible cellulosic biomass is considered as a feedstock. Transposition of mature concepts used in oil refining such as the use of solid oxides [1], promoted or not by transition metals, have shown to face activity and stability issues when it comes to convert highly oxygenated compounds. Currently, cellulose [2] itself as well as aldoses or alditols [3] contained in / or producible from cellulose, are in addition preferentially converted in aqueous media for solubility, reactivity and processability reasons. This paradigm adds a challenge of the use and recycling of highly water-tolerant catalysts, [4] which is not addressed to the widely used silicic [5] or aluminic [6] carriers (or mixed oxides) in refining and petrochemistry for gaseous or liquid hydrocarbons conversion. Innovative solutions have thus been developed to circumvent their hydrothermal instability, for instance by coating of mesoporous oxides [7] or over-coating of metal-promoted oxides [8] with carbon. Selectivity is also a major issue for efficient conversion of cellulose or sugars to high-value chemicals through chemical catalysis. Direct conversion of cellulose to ethylene glycol is one of the typical examples to illustrate this (Figure 1).

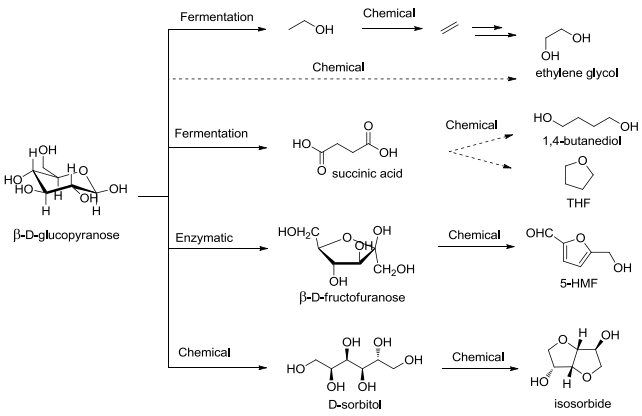

Figure 1. Examples of valorisation products from glucose *via* chemocatalytic and/or biocatalytic pathways mentioned in the introduction.

Despite the advantages of a one-step conversion compared to the multi-step industrial process, coproduction of ethylene glycol, propylene glycol and *1,2*-butanediol face major separation challenges even with the most selective catalysts composed of homogeneous $H_2WO_4$and heterogeneous Ni/C [9]. As a result, industrial processes making use of chemical catalysis to valorise

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biosourced/oxygenate substrates are implemented as downstream steps, letting the biocatalysis as an entry point of the biorefinery with enzymatic saccharification and/or fermentation step [10-11]. Less functionalized substrates like alcohols, diols, carboxylic acids or pyrones can thus be obtained and chemocatalysis may then take over for further transformation to olefins, [12] diolefins or unsaturated carboxylic acids, [13] for instance. Then, glucose can be hydrogenated to sorbitol [14] and subsequently dehydrated to isosorbide [15]. On the other hand, biocatalysis, whether enzymatic or fermentative, has gained increasing interest in this context, being particularly fitted to selectively transform biosourced products into desirable chemicals and fuels in water.

Thanks to the boom of metabolic and genetic engineering, researchers try to push back the frontiers of operating conditions of biological catalysts in terms of organic solvent use, temperature and acidity range or substrates scopes [16]. A couple of industrial processes are already under operation with first generation sugars and are on the verge to be commercialized with lignocellulosic-derived feedstocks. After adequate pretreatment steps, cellulolytic cocktails are indeed able to hydrolyze cellulose to glucose [17] at such a level of selectivity at low temperatures that chemical catalysis cannot compete yet, even if research efforts are clearly oriented toward this direction [18]. In the field of biofuels, glucose can be fermented to bioethanol by simultaneous saccharification and fermentation. Concerning specialty chemicals, besides many molecules still under development, one may highlight the case of succinic acid [19]. This chemical has been historically obtained by a relatively eco-inefficient process of *n*-butane oxidation followed by hydrogenation/hydration of maleic anhydride [20]. It is now industrially operated by BioAmber in Canada [21] by fermentation of first-generation sugars. If second generation glucose becomes available in a close future, these first generation processes could switch to cellulosic sugars. A major enzymatic process would benefit from these change: glucose isomerization to fructose is, as a matter of fact, the largest enzymatic process and runs for high fructose corn syrup (HFCS) production catalysed by xylose isomerase (EC 5.3.1.5). This isomerization is limited by thermodynamic equilibrium at an almost equimolar mixture of glucose and fructose. This equilibrium hampers further valorisation of fructose to the high potential *5*-hydroxymethylfurfural (HMF) and requires advanced sugar separation. Despite high specificities and selectivities combined with enhanced productivities, enzymatic and fermentation steps development are limited by high dilution, high production costs and complex recycling. For enzymatic transformations specifically, higher scale extrapolation is moreover limited to a certain class of stable and easily recyclable enzymes, immobilized xylose isomerase for HFCS production being the most representative example. Nevertheless, the extremely large family of oxidoreductases, of which the use would make sense for a rich scope of reactions of highly oxygenated substrates to modify their chemical functionalities, suffer from major drawbacks. Oxidases like those from the EC 1.1.3 sub-subclass release hydrogen peroxide (H_2O_2) while oxidizing the desired substrate, which is deleterious for the enzyme at low concentration levels. Combination of oxidase with catalase (EC 1.11.1.6) is thus compulsory to maintain the oxidase activity, hydrogen peroxide being rapidly decomposed to H_2O and O_2 by the catalase. Such complex and expensive enzymatic combinations are also encountered in the case of cofactor-dependent redox active enzymes. High costs of cofactors like the famous nicotinamide adenine dinucleotide NAD(P) make their regeneration mandatory, so that the oxidation of a substrate has to be accompanied by a compensative reduction of another substrate, which can largely complicate products separation if other organic products are formed. These multiple enzymatic systems are unimaginable at commercial scales. New paradigms have to be developed to circumvent these drawbacks, which limit the spread of biocatalytic process concretization. These introductive examples show us how disruptive innovation are needed concomitantly in process engineering for reaction and separation, in (bio)catalyst design and materials science [22]. New conceptual approaches are required to address the challenges of sustainable fuels and chemicals as stated by the European Cluster on Catalysis in their Science and Technology Roadmap on Catalysis for Europe [23]. It also appears clearly that second generation biorefineries and biochemistry facilities, in opposition to oil refineries and petrochemistry facilities predominantly rely on heterogeneous catalysis, further requiring all types of catalysis: homogeneous, heterogeneous and bio- catalysis. It is now becoming more and more admitted that better integrated and more synergistic combinations between the two worlds of catalysis could produce improvements in terms of performance, selectivity and eco-efficiency. An optimal integration of all these types of catalysis will be the key for efficient global implementation of renewable resources conversion towards a sustainable chemistry. This review paper thus proposes to give an overview of all the pathways that have been described so far to integrate chemical and biological catalysis, beginning with more or less integrated chemoenzymatic synthesis working in cascade to progressively open the field of hybrid catalysis where both catalysis work in tandem in a one-pot concomitant execution. The first part of the review will synthesize the numerous examples that have emerged as sequential one-pot multi-steps syntheses. Both catalyses can be operated under different conditions with changes in pH, temperature, addition of solvent between the steps, so that the integration extent is somewhat limited. Only costly separation steps and intermediates isolations are spared. This approach is a first attempt towards compatibility and cross inhibition understanding between catalysts and substrates/products, as the catalyst of the first step stays in the reaction middle for the next step, irrespective of the order in which the catalytic steps are conducted. For instance, undesired products resulting from the lack of selectivity of a first chemical catalysis step may inhibit enzymes or yeasts such as, for example, furfurals generated during pre-treatment of the biomass before enzymatic saccharification [24]. Inversely, peptidic residues of enzymes from a first biological step may inhibit the action of the chemical catalysts [25]. Some reviews have already drawn up reports of such successive chemoenzymatic one-pot multi-steps examples, and a constantly increasing number of successful examples appear in the literature. Yet, rare examples describe synergetic effects between both catalyses. This approach is valuable for process simplification, separation costs reduction upon understanding about compatibility issues not only between catalysts but also between reaction media. After an overview of typical examples of one-pot multi-steps chemoenzymatic synthesis, we specifically emphasize here what we consider as an even more disruptive approach with the so-called “hybrid catalysis” concept [26], where chemical catalysts and biological catalysts concomitantly work in tandem.

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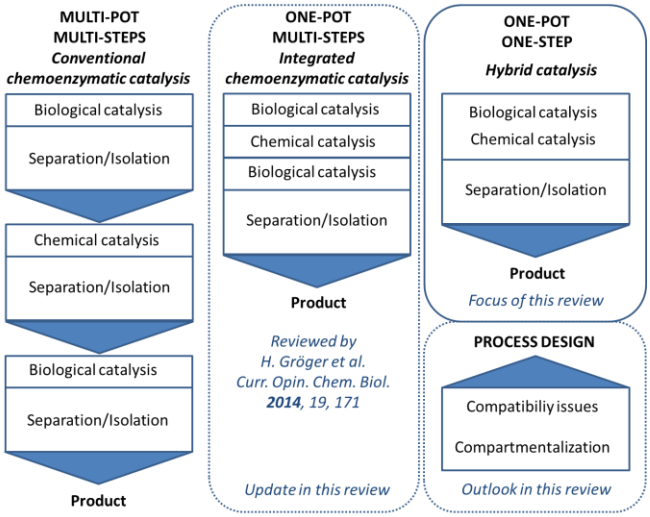

Figure 2. Different levels of integration of biological and chemical catalysis

It corresponds to the third column in Figure 2 added to the two first columns presented by Gröger *et al.* [27] in a founding paper we will come back on further. Such simultaneous operations are of particular interest to solve the aforementioned drawbacks of enzymatic catalysts, by, *e.g.*, displacing thermodynamic equilibrium for isomerases, eliminating or valorising H_2O_2 for oxidases and regenerating cofactors for oxidoreductases, while beneficially getting rid from the use of any additional enzymatic systems. As compatibility between catalysts and/or substrates is a major issue for catalysts integration in tandem reactions, compartmentalization and new reactor designs are of major interest in this area. The necessity of compartmentalization has already been introduced with different concepts like multiphasic systems, phase segregation with membranes, catalysts immobilization or nanoreactors [28-29]. These different aspects are broadly tackled in this review all along the examples when necessary and will deserve a specific reviewing and perspectives in a near future.

2. Chemoenzymatic one-pot multi-steps syntheses.

2.1. History

The numerous reviews, concepts and perspective articles illustrate the amazingly growing interest for the field of chemoenzymatic transformations. It is important to get back to these historical contributions foreshadowing the birth of this new catalysis area, which our contribution aims to update and expand. The potential of combining biocatalysts and chemical catalysts was first highlighted in 2007, a golden year for biomass valorisation [30], by B. Shanks [31] after the seminal work the same year of Zhang *et al.* [32], Woodley [33] and Dumesic *et al.* [34] for selective synthesis of respectively HMF and *2,5*-dimethylfuran (2,5-DMF) by chemical pathway using fermentation sugars as feedstock. The authors propose to rethink other existing chemical trees to create new sequences, like the transformation of succinic acid obtained by fermentation to *1,4*-butanediol or tetrahydrofuran by chemical catalysis (see Figure 1). Some years later, these transformations are currently under finalization by BioAmber to expand their chemicals library [35]. No specific integration between the different steps was investigated. New examples were provided in 2011 by A. Marr *et al.* [36] and the concept of compartmentalization was evoked for the first time for moderately compatible catalysts. Some main domains of hybrid catalysis applications were identified and briefly described: dynamic kinetic resolution (DKR), cofactor regeneration and biomass valorisation. A recent book chapter by the same authors supplement the previous work on DKR [37]. As a result, the present review will not emphasize this part of chemoenzymatic catalysis. These fields were enriched by J. F. Hartwig *et al.* [38] in 2013 in a perspective paper dealing with one-pot multistep chemoenzymatic reactions focusing on asymmetric synthesis allowed by enantiospecific and enantioselective biocatalysts. DKR, well-known for decades without being named as a hybrid system, associates, for instance, lipases and organometallic complexes for deracemization reactions in organic solvents. The very first one-pot one-step transformations were also described with the first attempts to circumvent compatibility issues between catalysts. H. Gröger *et al.* [24] took over this work underlining the potential of aqueous phase transformations using metal catalysis and/or organocatalysis in combination with biocatalysis. A focus on heterogeneous catalysts is carried out by Dumesic *et al.* [39] summarizing the challenges and outlooks to fill the gap specifically in the case of saccharides valorisation. They also showed how advances in protein engineering open the diversity of microorganisms for an amazing variety of target molecules. Some specialty chemicals can for instance be difficult to synthesize from fossil resources whereas chemoenzymatic pathways from simple sugars affords them in a minimal number of steps, for instance cortalcerone *via* glucosone by a recombinant strain of *Escherichia Coli* [40]. Chemically catalysed dehydration of cortalcerone followed by isomerization produce furylglycolic acid, a new monomer for the synthesis of original biosourced polyesters. *In vitro* and *in vivo* chemoenzymatic cascade catalysis with applications in synthetic biology were reviewed by Rudroff *et al.* [41] to emphasize the mimicking of biological networks to develop multistep one-pot reactions advantageously combining the individual assets of each type of catalysis. Complex syntheses of glycans or glycoproteins are also taking advantage of orthogonal interdisciplinary cooperation of chemistry and biology to yield new strategies in the field of glycobiology [42]. After this short historical point, the next section will detail chemoenzymatic approaches sorted by the type of chemical catalysis involved, beginning with homogeneous and heterogeneous non-metallic catalysis, followed by homogeneous and heterogeneous metal-based catalysts, and finally with hybrid catalytic systems.

2.2. Non-metallic Homogeneous or Heterogeneous catalysis and biocatalysis.

The development of organic catalysis over the last twenty years and its progress in terms of enantioselectivity and activity, often bioinspired, are favourable for further coupling with biocatalysis [43]. The proof of concept was given by Gröger *et al.* [44] in 2009 with the sequencing of an aldolization reaction catalysed by a chiral proline derivative followed by reduction with an alcohol dehydrogenase (EC 1.1.1.1). Specific review on combination of organocatalysts and biocatalysts in sequential or one-pot

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procedures was further provided in 2017 by Bisogno *et al*. [45]. In 2013, Kudo *et al.* [46] described a Friedel-Crafts reaction using an immobilized peptide, an amino acid polymer, in a water/THF mixture followed by an α-oxyamination reaction catalysed by laccase (EC 1.10.3.2). It is not unusual to encounter enzymatic reactions capable of tolerating the presence of organic catalysts, like amino acid derivatives, themselves enzyme components. However, no laccase/peptide compatibility problem was pointed out, immobilization restricting the possibility of detrimental interactions between both catalytic systems. It is noteworthy that the first signs of compatibility between enzymes and organic solvents are observed therein. Several reviews have been then published on the subject, providing arguments against certain preconceived ideas whereby enzymatic catalysis could only take place in water [46]. In the context of biomass valorisation, conversion of hexoses to HMF is an extremely important topic [47]. It is thus of specific interest to carefully describe the different chemoenzymatic tentative to reach HMF from glucose. As well documented now in the literature, the best yields of HMF are obtained from fructose in aprotic polar solvents by chemical catalysis. Under equivalent conditions, glucose is less reactive and its dehydration less selective towards HMF. Reasoning similar to that of proposed by van Bekkum *et al.* [48] in 1980 to obtain mannitol from a glucose/fructose mixture is therefore possible. Indeed, it would appear that fructose would dehydrated faster than glucose to HMF and the thermodynamic equilibrium of glucose/fructose enzymatic isomerization limited to 50/50 would be favourably displaced. In addition, chemical isomerization, whether acid- or base-, homogeneously or heterogeneously catalysed, has still not reached the same performance level as enzymatic catalysis [48]. A chemoenzymatic or hybrid synthesis involving a glucose isomerase (EC 5.3.1.18) and a dehydrating catalyst therefore seems highly relevant. Conducting each reaction in separate reactors would only enabling reaching the thermodynamic limit of about 50 % isomerization per pass of glucose to fructose and would thus induce the necessity for glucose and enzyme recycling, as reported by Afonso *et al.* [49] in a tentative integration of enzymatic isomerization and chemical dehydration in wet tetraethylammonium bromide involving a large number separation and recycling steps. Dumesic *et al.* first attempted a hybrid catalysis experiment to convert glucose directly to HMF by mixing thermophilic glucose isomerase in aqueous medium with a dehydration acid heterogeneous catalyst [50]. The enzyme was specifically developed for the reaction since dehydration of sugars is favoured at temperatures higher than 100 °C. However, this concept is not efficient enough since the temperature is still too low for the dehydration. In addition, dehydration of fructose in aqueous medium is not thermodynamically favoured and HMF is highly sensitive to rehydration to levulinic acid. The authors therefore moved towards a one-pot multi-step chemoenzymatic approach with enzymatic isomerization before dehydration at higher temperature and addition of an organic solvent to separate the produced HMF from water. With yields of about 30 % HMF, the sequential method still remains more attractive. The direct approach is therefore not obvious. Note that the development of new enzymes by genetic engineering is generally tedious and very time-consuming for a potentially random result in terms of improved performance. In the studies conducted by Dumesic's team, the presence of residual glucose does not seem desirable since its reactivity with the dehydration catalyst is not zero, producing undesirable by-products, such as humins. Selective separation of glucose and fructose therefore seems essential. To limit the separation costs and the number of steps in the chemoenzymatic approach, a compartmentalization methodology has been considered since 2010. It consists in chemically shifting the glucose/fructose isomerization equilibrium using a method for selective functionalization or protection of fructose. These aspects will be briefly mentioned in the following paragraph. An attractive chemoenzymatic pathway to access HMF therefore consists in working in a two-phase medium in the presence of complexing agents such as aluminated or borated derivatives [51]. The latter are chosen for their preferential affinity with fructose compared with glucose for two reasons: shifting of the isomerization equilibrium towards fructose and transfer of the fructose complex or HMF produced to the organic phase. The first complexation example was described in the 70s in the presence of sodium tetraborate, shifting the enzymatic isomerization equilibrium towards fructose: a global yield of 88 % fructose and fructoborate complex was obtained instead of the usual 50 % limitation [52]. This method was implemented for the first enzymatic isomerization/chemical dehydration coupling described in 2010 by Qi *et al.* [53]. Dehydration nevertheless takes place in the aqueous phase, with HMF being transferred to a *1*-butanol organic phase. In view of the studies conducted by Draffin *et al.* [54] demonstrating that lipophilic arylboronic acids could complex and transport fructose across a water/organic solvent interface, the isomerization/dehydration concept was improved by conducting enzymatic isomerization in aqueous phase (Figure 3).

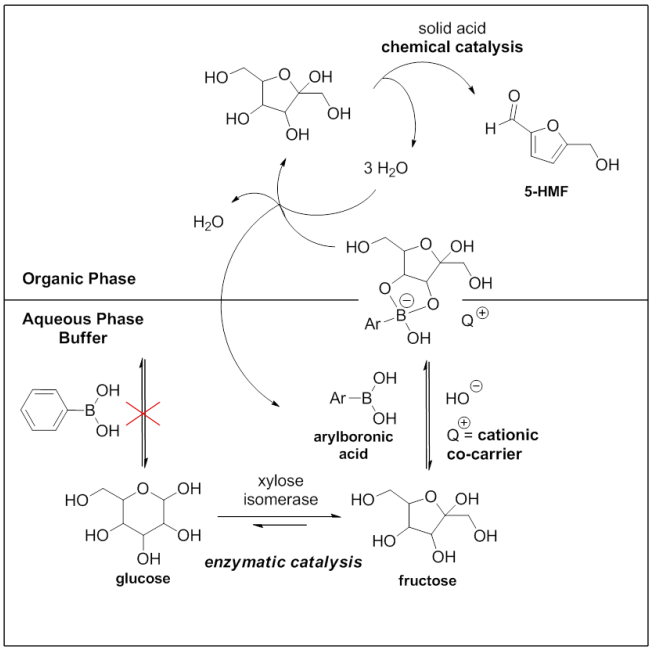

Figure 3. Concept of isomerization/dehydration in a two-phase medium in the presence of boronic acid and a carrier. Adapted from Ref. {54].

Fructose was then selectively complexed with boronic acid, the as-formed boronic ester was transported to the organic phase and fructose was finally decomplexed/dehydrated to HMF in an organic phase using a single acid catalyst. An alternative pathway developed recently consist in carrying out fructoboronate ester

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hydrolysis at the interface with a second aqueous phase where fructose is released and subsequently dehydrated to HMF. This process can be either operated in a sequential [55] or continuous fashion. In this latter variation, enzymatic and chemical catalysis run simultaneously thanks to an efficient transportation of the complex between both aqueous phases through the organic phase (Figure 4). This feature is currently under optimization in a work of the present authors, and the results will be reported in due time.

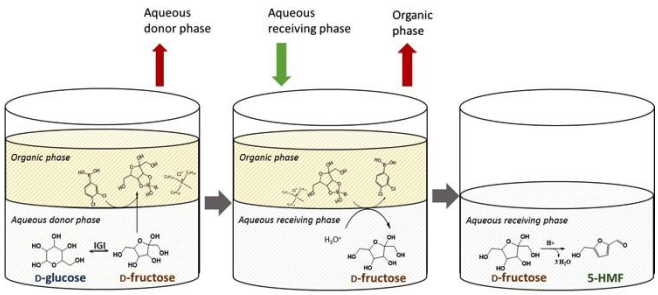

Figure 4. Triphasic chemoenzymatic implementation of HMF production from glucose. Adapted from Ref. [55].

A similar pathway with extraction was also described earlier by Alipour *et al.* [56]. The sequencing is more complex since it adds a step consisting in releasing the fructose/boronic acid complex in an ionic liquid before the dehydration step and a second extraction of HMF to an organic solvent. An excellent yield of 80 % yield of HMF could be obtained from glucose through this variant (Figure 5).

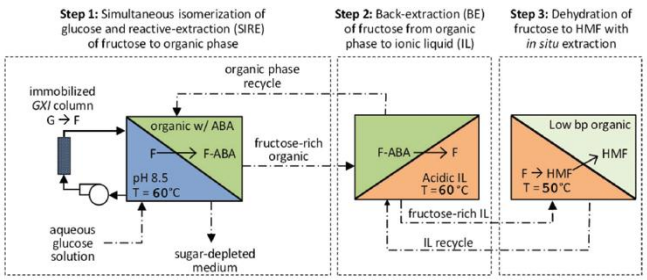

Figure 5. Diagram illustrating the chemoenzymatic synthesis of HMF from glucose with double transport of the fructose-carrier complex. Reproduced from Ref. [56] with permission from The Royal Society of Chemistry.

In view of recent publications, Palkovits *et al.* are also investigating this methodology, focusing only on the isomerization step, which let open possibilities for subsequent valorisation of fructose [57]. Furthermore, a proposal of cascade reactions using for all steps the same siliceous porous support functionalized differently for supporting the enzymatic catalyst consisting of saccharification of cellulose to glucose, then isomerization of glucose to fructose and subsequent dehydration of fructose to HMF in an organic solvent was put forward by Wu *et al.* [58]. While this method sounds definitely elegant, the final yield obtained through this pathway did not exceed 40 %. These studies all demonstrate that chemoenzymatic conversion of glucose to HMF, a concrete challenge in the field of biomass valorisation, is far from being obvious. It requires process innovation to minimize the number of steps while maximizing the yield: two-phase media, membranes, carriers, complexants, extractions, releases, etc. They nevertheless provide a wealth of information on the compatibilities between glucose isomerase and the catalysts, complexants, carriers and organic solvents. Among furanics, furfural and furfuryl alcohol are also two industrially relevant commodity chemicals [59], as well as building blocks [60]. Two-steps xylose dehydration (Figure 6) catalysed by an acidic heterogeneous catalysis SO_4/SnO_2-Montmorillonite (MMT) and enzymatic hydrogenation of the resulting furfural into furfuryl alcohol using recombinant *Escherichia Coli* CCZU-K14 afforded quantitative yields of alcohol [61]. Temperature and pH had however to be adapted between the chemical and the enzymatic steps.

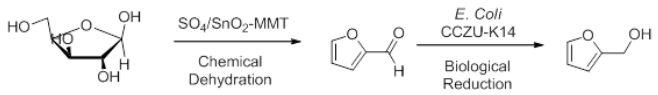

Figure 6. Chemoenzymatic one-pot two-steps synthesis of furfuryl alcohol from xylose. Adapted from Ref. [61].

Besides valorisation of biosourced substrates, other relevant examples involving non-metallic chemocatalysis and enzymatic catalysis have proven the attractiveness of one-pot two-steps approaches to enhance yields and selectivities to high added-value chemicals. Recently, Bornscheuer *et al.* [62] presented a one-pot chemoenzymatic reaction for the synthesis of (*1S*,*2R*)-*1*-(methoxycarbonyl)cyclohex-*4*-ene-*2*-carboxylic acid. They started with the esterification of anhydride in the presence of methanol and sulfuric acid (Figure 7). The as-obtained diester was then sequentially transformed into the desired product using a recombinant pig-liver esterase (ECS-PLE06). The one-pot process enabled obtaining higher yields (up to 84%) when compared to the classical two-step synthesis route (only 70%). Moreover, the authors found that the conventional acid-catalysed esterification was not sufficient to completely convert anhydride to esters. To overcome this issue, they performed esterification using thionyl chloride, which gave full conversion and excellent enantiomeric excess (higher than 99.5%).

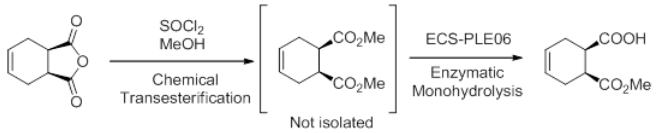

Figure 7. Chemoenzymatic one-pot two-steps transesterification and monohydrolysis. Reproduced from Ref. [62] with permission from The American Chemical Society.

Hailes *et al.* [63] reported a chemoenzymatic reaction cascade to provide access in one pot to chiral tetrahydroisoquinoline alkaloids (THIAs) from low-cost starting materials (Figure 8). A Pictet–Spenglerase norcoclaurine synthase-catalysed reaction was followed by a chemical cyclisation to give two new heterocyclic rings with excellent enantioselectivity and doubled yield compared to previously described conventional pathways.

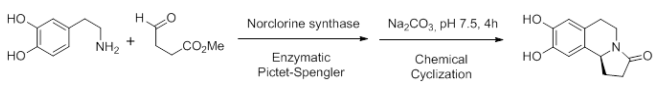

Figure 8. Chemoenzymatic one-pot two steps enantioselective synthesis of alkaloids. Adapted from Ref. [63] with permission from The Royal Society of Chemistry.

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A base-induced ring-closure has also been used to enantioselectively synthesize aromatic epoxides from prochiral α-haloketones (Figure 9) after an enzymatic reduction catalysed by a substrate-tolerant medium-chain dehydrogenase from *Kuraishia capsulate* CBS1993 [64].

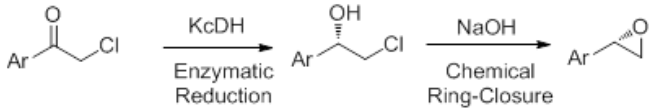

Figure 9. Chemoenzymatic one-pot two steps enantioselective synthesis of aromatic epoxides. Adapted from [64].

A one-pot, two-step procedure combining *1,3*-dipolar cycloaddition and an enantioselective reduction (Figure 10) mediated by *Daucus carota* (carrot root) has been described by Souza Oliveira *et al.* [65]. The synthesis was accomplished by first employing the biocatalyst followed by a "click" reaction in aqueous medium under very mild conditions to yield the corresponding chiral disubstituted *1,2,3*-triazoles.

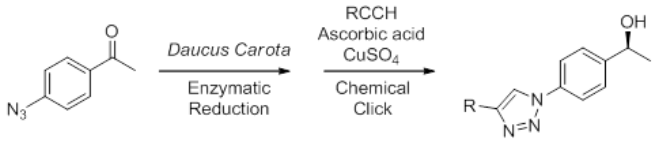

Figure 10. Chemoenzymatic one-pot two-steps enantioselective reduction and click-chemistry. Adapted from Ref. [65] with permission from Elsevier.

Another interesting example of a one-pot chemoenzymatic process was given by Liardo *et al*. [66]. They developed a hybrid organo-biocatalytic system for the asymmetric conversion of racemic alcohols into amines. In this one-pot oxidation-transamination process, AZADO (*2*-azaadamantane *N*–oxyl) displayed high catalytic activity for the oxidation of secondary alcohols and ω-transaminase high yields for the amination reaction. In all cases, the desired amines were obtained in high yields with excellent enantiomeric excess.

2.3. Homogeneous or Heterogeneous Metallic catalysis and biocatalysis.

It is interesting to observe that, during the last decade, considerable interest has been shown in chemoenzymatic syntheses involving organometallic catalysts composed of a metal and an organic ligand. It would be quite logical to expect reactions catalysed by metallic complexes derived from biocompatible metals frequently encountered in enzymes or metalloenzymes such as zinc, copper, iron and calcium. Surprisingly, the first studies in this field focused on traditional organic chemistry metals such as palladium, rhodium and ruthenium [67]. Examples of chemoenzymatic syntheses involving metalloenzymes and biocompatible metals have only been described more recently [68], enabling novel synthetic cascades [69] reported in the section dedicated to fully hybrid catalytic system (see section 3.3.).

The very first example described in 2006 involving organometallic catalysts consists in a chemical hydrogenation step of *N*-acetylaminoacrylate in aqueous phase catalysed by a rhodium complex followed by enantiospecific enzymatic hydrolysis by acylase (EC 3.5.1.14 ; CLEA AM) after adjusting the temperature and pH and adding a buffer solution (Figure 11). To carry out the reaction in hybrid catalysis in aqueous phase, the rhodium complex had to be immobilized on a porous silica support (1-AITUD-1), but the system led to poorer performance [70].

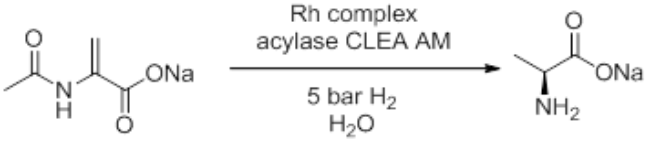

Figure 11. First chemoenzymatic synthesis involving an organometallic complex. Adapted from [70].

Compartmentalization by immobilization can thus be a way to implement successive chemoenzymatic pathway into hybrid catalysis, but can lead to severe loss in activity. Other sequences of organometallic catalysis followed by enzymatic or whole cell catalysis have been described over the last decade. For example, a one-pot chemoenzymatic method for the synthesis of a variety of new iminocyclitols, from readily available non-phosphorylated donor substrates, has been developed by Wong *et al.* [71] thanks to the discovery of a novel fructose-*6*-phosphate aldolase (EC 4.1.2.13), particularly tolerant to functional groups. The broad substrate tolerance afforded the synthesis of these new iminocyclitols in good yields and a concise fashion (Figure12).

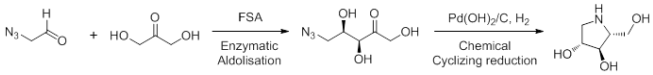

Figure 12. Chemoenzymatic one-pot two-steps synthesis of iminocyclitols. Adapted from [71].

H. Gröger and W. Hummel reported the Suzuki coupling between phenylboronic acid and brominated acetophenone followed by enantioselective reduction of the ketone by an enzyme such as alcohol dehydrogenase with no intermediate purification (Figure 13) [72]. The pH had to be adjusted and *iso*propanol added to guarantee the activity of the enzyme. The initial objective of the authors was to work in hybrid catalysis, but boronic acid proved to be a strong inhibitor of the enzyme. It had therefore to be fully converted prior addition of the enzymatic catalyst. Extensive research work on crossed inhibition must therefore be conducted rigorously before coupling the two worlds of catalysis into a hybrid system.

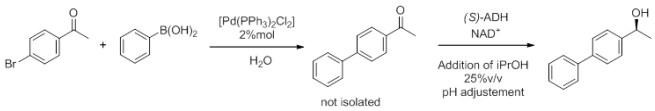

Figure 13. Successive chemoenzymatic syntheses without isolating the intermediate organic product. Adapted from [72].

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To avoid the use of sensitive phosphines, Cacchi *et al.* [73] prepared palladium nanoparticles stabilized primarily within the protein cavity of a highly thermostable Dps protein (DNA binding protein from starved cells) Pd_{np}/Te-Dps. This "pre-catalyst" was used in an analogous one-pot two-step Suzuki-Miyaura cross-coupling followed by an enantioselective enzyme catalysed reduction to form chiral biaryl alcohols. Similar cases of enzymatic hydrogenation of cetophenones have been conducted after Heck couplings steps catalysed by palladium complexes without isolating the intermediate product [74].
Wacker oxidations were also successfully sequenced with alcohol dehydrogenase (Figure 14) [75].

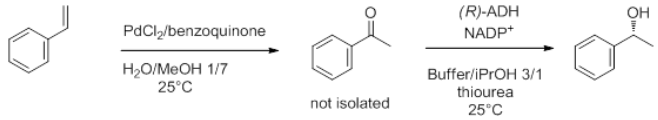

Figure 14. Sequential Wacker oxidation and stereoselective hydrogenation by alcohol dehydrogenase. Adapted from Ref. [75] with the permission from Wiley.

In the latter case, system efficiency has since then been optimized through compartmentalization with a polydimethylsiloxane membrane [28]. The example of Mihovilovic *et al.* [76] demonstrates that enzymes such as Bayer-Villiger monooxygenases are active to convert substituted cyclopentanones in the presence of an organic solvent resulting from the heterogeneous metallic hydrogenation step catalysed by Rh/C of the corresponding cyclopentenone, operated continuously (Figure 15). Use of an adjuvant such as Triton X-100 remains desirable to simplify transfer of the compounds between the two phases, but, surprisingly, the performance levels are satisfactory despite the presence of organic solvent, not miscible with water.

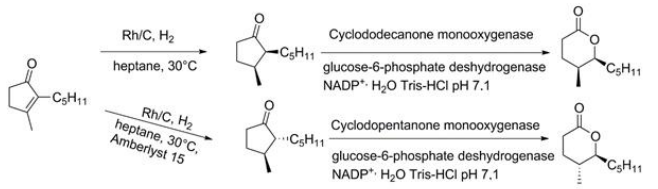

Figure 15. Chemoenzymatic synthesis of chiral lactones by succession of metallic and enzymatic catalyses. Adapted from [76].

As already suggested, compartmentalization thanks to multiphasic systems appears to be a valuable solution in many transformations concerned by catalysts incompatibility. Transfer of intermediates becomes a significant step towards efficiency. Sensitivity of biocatalysts with the envisioned organic solvent has to be verified considering the miscible part of organic solvent with water. In chemical organometallic catalysis, another major reaction has been coupled with biological catalysis: olefin metathesis. The first case in one-pot two-steps describes (Figure 16) a first step of ring-closing metathesis (RCM) of a bis-allylmalonate by a ruthenium complex and the second step is a monohydrolysis into monoester by a pig-liver esterase, as already depicted in a previous work [77].

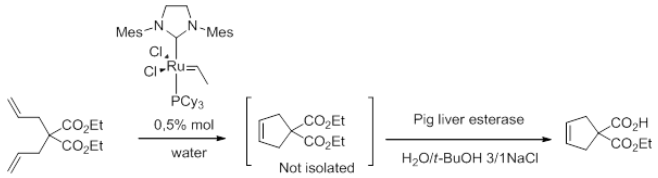

Figure 16. First example of metathesis in a chemoenzymatic synthesis. Adapted from [77].

In an intermediate step, the salinity was adjusted and a co-solvent added. Enzymatic activity was not disturbed by the presence of the residual ruthenium complex. The two steps were carried out successively.
A recent example involving metathesis shows even more clearly the potential of chemoenzymatic synthesis, at the boundary between chemoenzymatic integration and hybrid catalysis, as a multiphasic system is used. The reactions are conducted in a two-phase medium with true synergy between both steps. Hartwig *et al.* [78] described the production of epoxides from olefins or fatty acids, as shown in Figure 17.

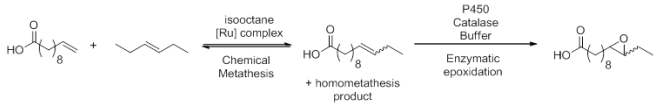

Figure 17. Biphasic tandem chemoenzymatic metathesis and epoxidation of olefins. Adapted from Ref. [78] with the permission from Wiley.

They used a second-generation Grubbs catalyst known for its metathesis activity, and the cytochrome P450 BM3 of *Bacillus megaterium* for selective epoxidation of one, and only one, unsaturated fatty acid, from a mixture. The team paid a special attention to the compatibility between both catalysts. The metathesis catalyst was selected for its stability under air atmosphere and its activity in protic solvents. The reaction took place in a two-phase *iso*octane/water medium: organic medium for metathesis and aqueous medium for epoxidation since the Grubbs catalyst is sensitive to the oxygenated medium. *Iso*octane is a suitable solvent for metathesis and is also biocompatible with cytochrome P450, even if miscibility with water is poor. In this case, there is a true synergy since the product cannot be obtained with such a high yield if the two steps are conducted successively, regiospecific oxidation shifting the metathesis thermodynamic equilibrium favourably to a single product.
Another interesting example involving metathesis in a one-pot cascade metathesis−aromatization sequence was given by Scalacci *et al*. [79]. They described for the first time the ability of the MAO-N enzyme, oxidase variant from *Aspergillus niger*, to catalyse oxidation-aromatization of *3*-pyrrolines into pyrroles in whole cell (Figure 18). As reported by the authors, excellent yields were obtained for –Cl, -CH_3 or alkyl substituents as compared to the non-substituted pyrroles due to the lower reactivity of electron-rich diallylanilines toward the ring-closing metathesis.

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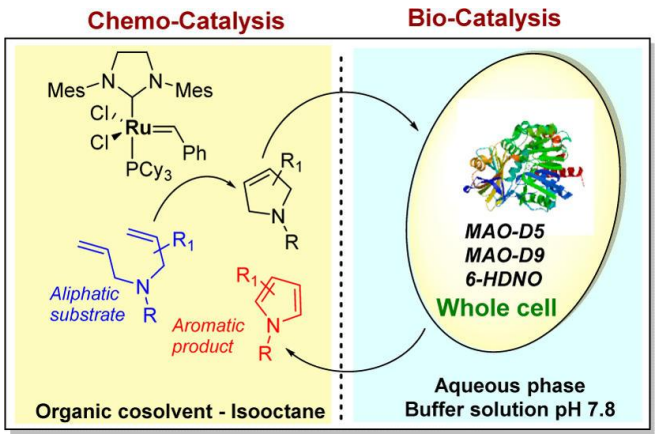

Figure 18. Biphasic tandem chemoenzymatic ring-closing metathesis and heterocycle oxidation. Adapted from Ref. [79] with the permission from American Chemical Society.

Like Hartwig's work [78], homogeneous ruthenium catalysis was carried out in a hydrocarbonated solvent, isooctane, while biocatalysis was confined in whole cell in an aqueous phase.

Among the rare examples of the use of biocompatible metals, Sonoike *et al.* report the one-pot chemoenzymatic synthesis of chiral *1,3*-diols in aqueous solvent at room temperature [80] by combining an enantioselective aldol reactions catalysed by Zn^{2+} complexes of *L*- and *D*-phenylalanyl-pendant [*12*]aneN$_4$ with an enantioselective reduction of the aldol products. The enzymatic step uses oxidoreductases with the regeneration of the NADH cofactor.

The integrated heterogeneous metal/enzymatic catalyst was applied also for the one pot relay synthesis of optically active and biologically relevant amines and amides from aldehydes and ketones or alcohols [81], which is exemplified with capsaicin (Figure 19). They developed a mild and efficient Pd catalysed process of synthesis of primary amines from aldehydes or ketones using HCO_2NH_4 as both, the amine source and the reducing agent. Pd was immobilized on aminopropyl-mesocellular foam (Pd^0-AmP-MCF) and both reactions occurred in toluene at 80 °C thanks to the lipophilicity of the identified lipase. The catalytic reactions were highly chemoselective to amines and glycine esters in excellent yields (up to 99% ee).

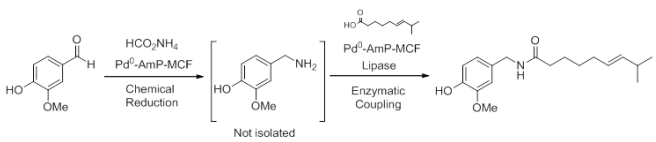

Figure 19. One-pot two-steps synthesis of capsaicin by chemical reduction and enzymatic peptidic coupling. Adapted from Ref. [81] with the permission from American Chemical Society.

Moreover, the integration with enzyme (lipase; EC 3.1.1.3) enabled direct one-pot amide formation. The role of Pd was double. First, it accelerated the transfer hydrogenation of the *in situ* generated imine intermediates (confirmed by HRMS analysis) and secondly it accelerated the decomposition of formate/formic acid, which is essential for the one-pot transformation because remaining HCO_2NH_4 inhibited the catalytic activity of the lipase.

One very interesting example of a successful one-pot process between two incompatible catalysts was recently published by Sand and Weberskirch [82]. They reported the combination of a lipase-based ester hydrolysis with a water-sensitive Cu/bipyridine-catalysed oxidation reaction in aqueous medium. To perform this reaction, they encapsulated the Cu complex in an amphiphilic diblock copolymer structure to avoid direct contact with the enzyme. The as-prepared nanocatalysts displayed no significant loss in activity and were active in five consecutive runs (80% substrate conversion). The one-pot tandem reaction conducted for the acetyl esters transformation to the corresponding aldehydes showed high yields of 73–93% for various tested aromatic and allylic acetic esters. Numerous chemoenzymatic reactions have therefore been developed in the recent years, with an increasing focus on integration to minimize the number of steps and maximize the performance levels. Incompatibilities between the catalysts or the conditions under which they exhibit optimum activity frequently prevent a true tandem implementation with cohabitation. Innovating process solutions have therefore been designed to still optimize the productivity of the systems. These examples give fruitful insights into the compatibility of different types of catalysts and participate to sweep away some prejudices about their activity and stability in the presence of unusual substrates, solvents or co-catalysts. Examples and perspectives dealing with a full integration towards hybrid catalysis are subsequently reported in the following section.

3. Introduction to hybrid catalysis

3.1. Concept and pioneering studies

One of the major challenges of hybrid catalysis therefore lies in the compatibility of the substrates with the catalysts, and in the implementation of the reactions at identical pH, temperature, atmosphere, solvent and kinetic ranges. The optimum operating conditions for the two types of catalysis may in fact sometimes be quite different. For example, Figure 20 provides an overview of the biocatalysis and chemical catalysis operating ranges in terms of temperature. Both worlds share a common broad range of temperatures, but the operating temperature in hybrid catalysis will be determined by the most thermosensitive catalysts, in other words the biocatalysts: enzymes, fungi, yeasts or bacteria, which exhibit optimum activity between ambient temperature and about 100°C or even over for the most thermophilic biocatalysts [83]. In this operating range, numerous chemical catalysts, in particular in homogeneous catalysis, also exhibit their optimum activity.

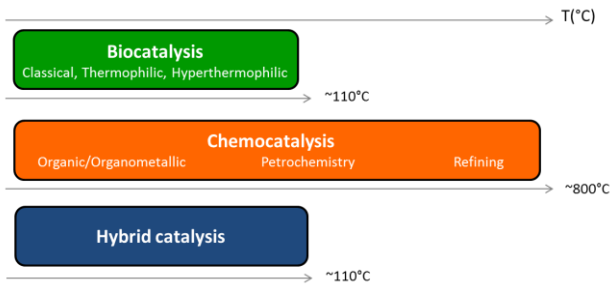

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Figure 20. Range of common temperatures for hybrid catalysis.

The big challenge therefore consists in the rationalized use of the advantages of each type of catalysis in order to access new conversions that cannot be achieved by each type of catalysis taken independently - or with lower yields. The objective is therefore to obtain a synergistic and/or complementary effect between the reactions. This effect lies in the realization of perfectly simultaneous operation of the biocatalyst and the chemical catalyst (whether homogeneous or heterogeneous): as mentioned above, this "hybrid catalysis" association between both types of catalysis requires total reaction compatibility: kinetics, solvent, temperature, impurities, etc. Apart from the numerous cases of dynamic kinetic resolution, there are still very few examples related to the development of hybrid catalysis.

In order to yield and efficient process involving two types of catalysis operating in tandem in the same reactor, each reaction must obviously be compatible with the other ones. We have seen in the previous section that isolation of catalysts through the implementation of two-phase media or the use of membranes could enable simultaneous development of the action of both types of catalysis. To move towards a hybrid solution, other methods, mostly quite ingenious, have been reported. It is quite amusing to observe that before experiencing a significant revival of interest in the recent years thanks to biomass conversion and the need for new catalysis paradigms, the concept of hybrid catalysis and the challenges it involves were perfectly described in the introduction of the pioneering studies published in 1980 by van Bekkum *et al.* [84]. The authors mentioned that the simultaneous action of biocatalysis and chemical catalysis is attractive for numerous catalytic processes in solution. For example, they envisioned an enzymatic conversion of a substrate into an intermediate product, which is simultaneously converted by the chemical catalyst into the desired product. They had already identified that the problems to be overcome for these catalytic combinations are the limited operating condition sequences, the selectivity of the chemical catalyst for the intermediate as well as mutual inhibition of the catalysts. The scene was set. They describe an apparently simple but finely balanced combination between an immobilized glucose isomerase catalysing the glucose-fructose isomerization and a Pt/C heterogeneous hydrogenation catalyst to produce *D*-mannitol from fructose. Previously, isomerization was carried out chemically, but, in that case, the kinetics was much slower than that of hydrogenation, inducing the presence of a large quantity of *D*-sorbitol in the medium obtained by hydrogenation of the initial non yet isomerized glucose [85]. The enzyme requires a minimum of salinity to stabilize the pH of the medium through a controlled quantity of $MgSO_4$ and $CaCO_3$, which must however not be too high due to possible inhibition of the platinum of the hydrogenation catalyst. The chemical catalyst was chosen for its glucose/fructose hydrogenation selectivity in favour of fructose and the hydrogenation kinetics slow enough to operate from a significant instantaneous concentration of fructose. Ruthenium was also a preferred metal, but suffered more from the presence of the enzyme. The enzyme extensively deactivated Raney nickel and the iridium-based catalysts were not sufficiently active. These studies represent in particular the first signs of what would lead to the development of Dynamic Kinetic Resolution (DKR) amongst the very first examples of hybrid catalysis. The objective is to achieve enantiomeric enrichment from a racemic mixture with joint action by a chemical catalyst and an enzyme, often a lipase or a protease. Lipases such as CAL (*Candida Antarctica* Lipase, A or B) are especially suitable since they are robust, organic solvent-tolerant and can withstand temperatures of up to 100 °C without being deactivated. The chemical catalysts used are mostly palladium-, rhodium-, iridium- and nickel-based organometallic catalysts chosen according to their activity and their compatibility with the enzyme. No general compatibility rule can be derived from the numerous examples and each combination must be experimentally tested to find the best synergy. DKR has been described and reviewed on many occasions [86], including a recent complete review by Leino *et al.* [87] and a book chapter by Marr *et al.* [36]. For these reasons, DKR will not be detailed further in this paper. Only selected examples of DKR that describe disruptive approaches for hybrid catalysis will be discussed. It is worth pointing out that recent developments have implemented enzymes that are more sensitive than lipases and have broadened the spectrum of accessible reactions [88].

After the pioneering studies on hybrid catalysis conducted by Kieboom and van Bekkum [89], it took 20 years before Kieboom then described a chemoenzymatic one-pot multi-steps sequence integrating the three main types of catalysis in the same reactor: homogeneous, heterogeneous and enzymatic [89]. Enzymatic oxidation of methylgalactoside, organocatalysed dehydration of oxidized product, then reduction on a metallic heterogeneous catalyst follows each other elegantly in this example (Figure 21). This one-pot/multi-step synthesis involves modifying the atmosphere and temperature between the steps.

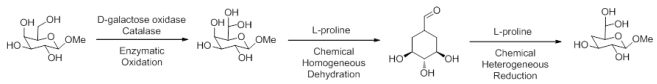

Figure 21. Example of chemoenzymatic cascade reaction involving homogeneous, heterogeneous and enzymatic catalysis. Adapted from [89].

Some examples clearly describe the compatibility problems due to coupling of both catalysis and the solutions designed to overcome these difficulties: sequential catalysis, immobilization of catalysts on supports, confinement or encapsulation, membranes, two-phase media, etc.

In the following part, we give an overview of the hybrid catalytic concept, its promises, realizations and perspectives.

3.2. Catalysis integration at the highest: two catalysts in one.

Turner *et al.*, already well established in the field of DKR, suggest using whole cells to isolate the enzymatic biocatalysts they contain [86]. This methodology has been applied to deracemize a cyclic secondary amine, a process difficult to achieve by DKR contrary to the case of primary amines. They combined *E. Coli* cells overexpressing a monoamine oxidase onto which palladium nanoparticles were deposited as non-selective hydrogenation catalyst (Figure 22). The enzyme only oxidizes the secondary amine of *S* stereochemistry in the initial racemic mixture of *1*-methyltetrahydro*iso*quinoline, while the hydrogenation catalyst returns an equimolar *R* and *S* mixture. The medium is therefore progressively enriched in the *R*-enantiomer, not oxidized by the enzyme. Air/hydrogen cycles are nevertheless necessary to

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operate the oxidase enzyme and the Pd-NP catalyst in turn. Both catalysts are therefore compatible due to sequestration of the enzyme in the whole cell, which produces it, but the catalysis operate in turn.

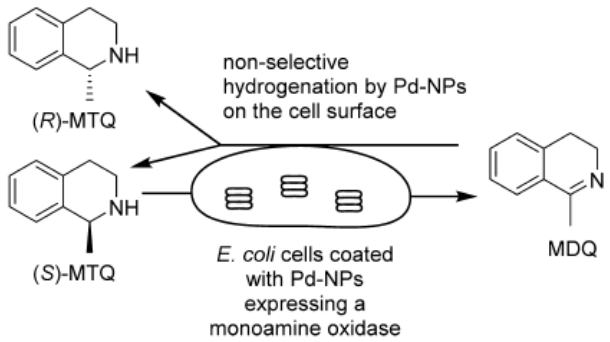

Figure 22. Deracemization of a chiral amine involving coated *E. coli* cells with Pd-NPs. Reproduced from Ref. [86] with permission from the American Chemical Society.

A hybrid Pd-lipase catalyst was also studied in the DKR of the α-methylbenzylamine. Itabaiana *et al.* [90] studied immobilization of lipase B from *Candida Antarctica* (CaLB) by covalent bond on functionalized silica containing palladium. The racemization and acetylation steps proceeded simultaneously catalysed by Pd and lipase. It was shown that the amount of the immobilized Pd did not affect the final conversion. Moreover, the highest enantiomeric excess values were obtained with 1 wt.% of Pd and the reaction proceeded without immobilized Pd nanoparticles (but using $Pd/BaSO_4$ as the racemization catalyst) has demonstrated lower enantiomeric excess, proving better activity and stability of the Pd-lipase hybrid material.

Integration of multiple catalytic systems with creation of new biocompatible enzymatic mimics was studied also by Xue *et al.* [91]. The authors synthetized graphene–haemin–glucose oxidase tandem catalyst for biomimetic generation of anthithrombotic species. They showed that haemin could be conjugated with graphene forming as very effective catalyst for L-arginine oxidation using hydrogen peroxide. Moreover, glucose oxidase (EC1.1.3.4) covalently linked onto graphene surface generated hydrogen peroxide through the oxidation of blood glucose. This permitted one-pot continuous generation of nitroxyl species from physiologically abundant glucose and *L*-arginine.

The use of microorganisms (bacteria, yeasts) or whole cells often leads to more disadvantages than advantages due to the nutrients required, which form another source of contamination [92]. In the literature therefore, hybrid catalysis, like the chemoenzymatic syntheses described above, focuses mostly on the use of enzymatic catalysts for the biocatalytic component.

The highly ambitious alternative consisting in implementing only one "catalyst" involving several orthogonal catalytic functions has also been reached by Palomo *et al.* [93]. They took up this challenge by developing "nanobiohybrid" catalysts synthesized as assemblies of enzymes and nanoparticles (Figure 23).

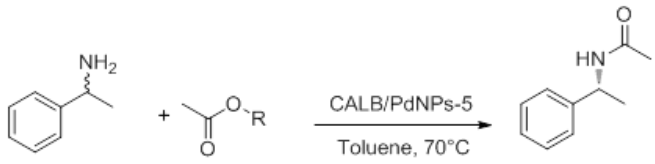

Figure 23. DKR of a racemic amine into enantiopure amide catalysed by a nanobiohybrid. Adapted from Ref. [93].

Apart from an interesting activity in pallado-catalysed C-C couplings (Suzuki, Heck), DKR reactions have been conducted with CALB-PdNPs catalysts with enzymatic transesterification and metal racemization.

Incorporation of metal catalysts in proteins is a major research direction for the synthesis of artificial metalloenzymes. Incorporating a biotinylated iridium complex in the framework of streptavidin made it biocompatible with other enzymes, which was not the case when used as free complex in the medium. Its coupling with mono-oxidases or mono-oxygenases generates active and selective original hybrid systems for various conversions described in the article (Figure 24) [94].

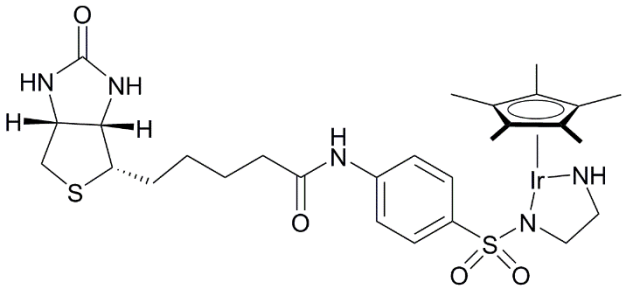

Figure 24. Biotinylated iridium complex for application in hybrid catalysis.

3.3. Sequestration towards compatibility

Adopting a supramolecular chemistry approach, Toste *et al.* [95] described the encapsulation of Au(I) and Ru(II) complexes in Ga_4L_6 tetrahedral clusters to combine them with esterases, lipases and alcohol dehydrogenases, extending the range of possible conversions beyond DKRs and other deracemization methods (Figure 25). The yields obtained in hybrid catalysis are the same as those obtained by successive steps, but avoiding the intermediate purification step presents significant improvement of transformation efficiency.

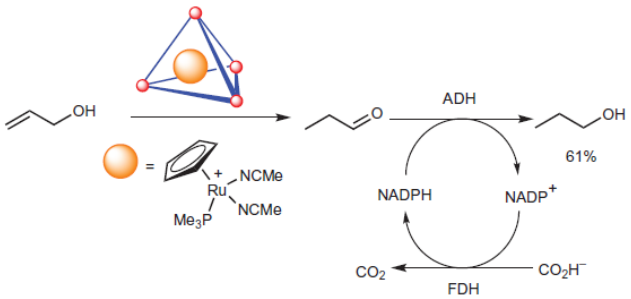

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Figure 25. Encapsulation of a ruthenium complex for compatibilisation with an alcohol dehydrogenase. Adapted from Ref. [95] with the permission from Springer Nature

3.4. Compatible catalysis working in synergy.

Very rarely, this sequestration is not required and one of the most spectacular examples to date is probably that developed by Kroutil *et al.* in 2010, which illustrates the combination of an iridium-based organometallic catalyst and an alcohol deshydrogenase [96]. In this article, no less than 7 organic products (substrates/products), two enzymes and an iridium complex cohabit in a buffered water/toluene medium, giving a highly elegant example of deracemization (Figure 26). A racemic mixture of chlorohydrins is oxidized into chloroketone by an iridacyclic cationic complex $[Ir]^+$. Another hindered chloroketone is used as hydrogen transfer agent. The chloroketone produced is then selectively reduced by alcohol dehydrogenase (ADH) operating with an NADH cofactor. The NADH is regenerated by a formate dehydrogenase (FDH; EC 1.2.1.2). The hindered chloroketone hydrogen donor was chosen to not be reduced by alcohol dehydrogenase and each catalytic system therefore operates perfectly orthogonally without interacting on the substrates of another catalytic system.

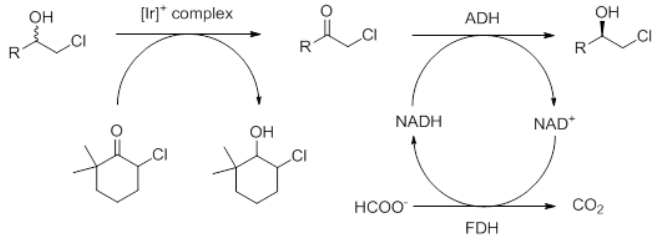

Figure 26. Hybrid catalysis multi-component complex system for racemization of halohydrins. Adapted from Ref. [96] with the permission from Royal Society of Chemistry.

More intriguingly, hybrid catalysis has also found interest in polymer synthesis. A first report by Heise *et al.* [97] paved the way for integrating chemical and biocatalytic polymerizations by synthetizing block copolymers in one-pot two-steps. Enzymatic ring-opening polymerization by Lipase B *Candida Antarctica* afforded poly(caprolactone) macroinitiator for subsequent nitroxide-mediated atom-transfer radical polymerization (ATRP). Stereospecific and stereoselective synthesis of polyesters or polystyrenes containing asymmetric centres of controlled conformations could then be obtained by this methodology [98]. Thanks to mild conditions and biocompatible metallic promoters like copper, ATRP is a methodology of choice to combine with biocatalysis for the efficient synthesis of functional polymers.

An outstanding example of a fully integrated hybrid catalytic system then appears for the synthesis of copolymers in supercritical CO_2 by combining the enzymatic ring-opening polymerization (ROP) of ε-caprolactone with this time a copper-mediated ATRP of methacrylates [99]. Copper-mediated ATRP has also been combined with a lipase-catalytic monomer transesterification (Figure 27), which proved to be compatible for an efficient approach to linear polymeric hydrocarbons branched with functional groups like esters or alcohols [100].

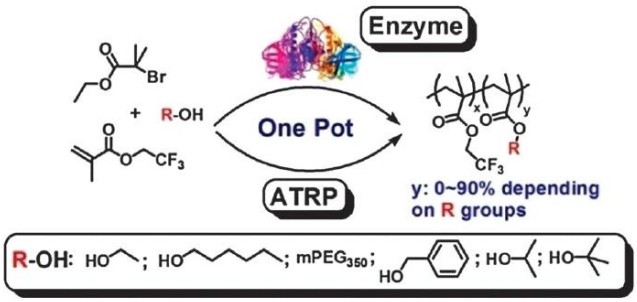

Figure 27. Hybrid catalytic system for polymer synthesis *via in situ* enzymatic monomer transformation. Adapted from Ref. [100] with the permission from Royal Society of Chemistry.

Another elegant use of hybrid catalysis is described for aldoximes preparation combining the oxidation of primary alcohols by acetic acid bacteria (Figure 28) with the subsequent condensation of the aldehyde with hydroxylamine [101]. Interception by hydroxylamine kinetically avoids the over-oxidation of the intermediate aldehyde to carboxylic acid, which nicely shows the advantages of combining chemical catalysis to enzymatic catalysis to orient selectivity and avoid undesired by-products formation.

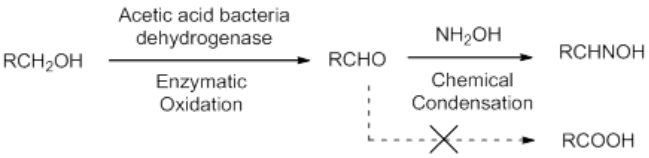

Figure 28. Hybrid catalytic system for simultaneous oxidation and condensation to aldoximes. Adapted from Ref. [101].

Until now, we have described only homogeneous systems as chemical catalysts. More rarely, heterogeneous catalysts are involved in hybrid systems. This type of association was reported by Kamwilaisak *et al*. [102] who used titanium oxide as a heterogeneous support and catalyst in the degradation of lignin by laccase at 50 °C and pH 5.0. They used also H_2O_2 to increase the catalytic performances of the laccase catalyst. However, it was shown that the lignin degradation increased using both processes separately rather than using both catalysts in one pot reaction. The results indicated that the combination of photocatalytic and biocatalytic reactions was more efficient using a two-stage process, with TiO_2 first and laccase second.

Unlike successive implementation, experimental and methodological tricks must be developed when working in hybrid catalysis to limit crossed interactions between the catalytic systems, in addition to the compatibility of reaction conditions. The successful examples reported above clearly demonstrate the increasing interest in coupling these two worlds of catalysis and entire research domains have emerged requiring combined skills in enzymatic catalysis and chemical catalysis.

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4. Hybrid catalysis to unlock enzymatic catalysis barriers.

It can be observed that the most promising enzymes to convert oxygenated products arising from biomass involve the large class oxidoreductases, oxidases (EC 1.1.3) and alcohol dehydrogenases being one of the most frequently encountered in chemoenzymatic transformations. The main drawbacks of those enzymes is almost systematically the need of a second enzymatic system, which creates two major barriers for the implementation of enzymatic catalysis at a larger scale:

- With oxidases, such as glucose oxidase, the aminoacid-oxidases and the laccases, the enzyme uses most often oxygen as oxidizing agent, converting it into hydrogen peroxide. Since hydrogen peroxide is toxic for the enzyme, a catalase is required to convert it back into water and oxygen;
- With alcohol dehydrogenases, whether used for dehydrogenation or hydrogenation (most examples described yet for chemoenzymatic synthesis involve an enantioselective hydrogenation), these enzymes depend on cofactors such as NAD(H) and NADP(H), which must be regenerated by a second redox enzymatic system. The use of an expensive cofactor, which must be regenerated often, prevents the deployment of this family of enzymes in large-scale processes.

Chemoenzymatic and hybrid syntheses reveal the two major barriers of the two main classes of enzymes capable of valorising biomass. These barriers hinder the development at larger scale due to the cost associated with parallel enzymatic systems based respectively on catalase and cofactor regeneration enzymes. These coupled systems also require substrates and co-substrates, which may create additional constraints when separating the products of interest and recycling the catalysts. These two main barriers can be exemplified in a complex immobilized multi-enzymatic transformation of glycerol to dihydroxyacetone published by Lopez-Gallego *et al.* [103] Glycerol deshydrogenase is accompanied by NAD^+ as cofactor, which needs to be regenerated from reduced form NADH. This regeneration is enzymatically carried out by NADH oxidase. This enzyme has the advantage of not using organic substrate, like formate deshydrogenase, but as an oxidase produces hydrogen peroxide. As a classical way to eliminate H_2O_2 to prevent enzyme inhibition and substrate unwanted further oxidation, a catalase is used to convert it into water and oxygen (Figure 29).

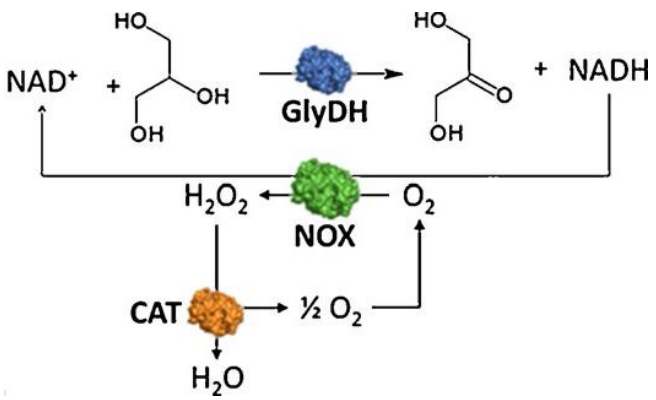

Figure 29. Immobilized multi-enzymatic system to dehydrogenate glycerol, regenerate NAD^+ and eliminate H_2O_2. Adapted from Ref. [103] with the permission from Wiley.

Immobilization of the three enzymes allows reduced products inhibition and shows again the advantages of compartmentalization toward efficient hybrid catalysis. Nevertheless, despite the attractiveness of selective biosourced glycerol valorisation, the production and use of three different enzymes can be considered as an insurmountable hurdle towards scaling-up. Hybrid catalysis can thus be advantageous with an objective of overcoming these barriers: how could chemical catalysis replace enzymatic coupled catalysis systems to simplify the systems and make them more eco-efficient In the light of all the chemoenzymatic synthesis and hybrid catalysis studies previously described, we will describe separately the two broad study pathways, namely *in situ* catalytic elimination or valorisation of hydrogen peroxide and regeneration of NAD/NADH type cofactors, which we consider the two fields for which enzymatic catalysis could take advantage in being coupled with chemical catalysis in a hybrid implementation.

4.1. *In situ* catalytic conversion of hydrogen peroxide

Oxidases are a sub-class of oxidoreductases, which catalyse oxidation reactions by using dioxygen as electron acceptor, as shown on Figure 30 [104].

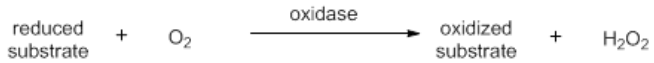

Figure 30. General reaction diagram catalysed by an oxidase.

The main families of H_2O_2-producing enzymes as well as their partner H_2O_2-decomposing enzymes are described in a book chapter by Adanyi *et al.* [105] and include glucose oxidase or galactose oxidase, which can be involved in the transformation of polysaccharide substrates. These enzymes operate without cofactor but release hydrogen peroxide. Hydrogen peroxide may be detrimental to enzyme activity, even at low concentrations, or may oxidize further substrates or products. In whole cell, enzymes of the catalase family breakdown hydrogen peroxide to maintain satisfactory cellular enzymatic activity. In contrast, the synthetic use of isolated oxidases requires a complementary cell-mimicking enzymatic system, which may incur a non-negligible additional cost for a process. Instead of a catalase, it could therefore be interesting to use an orthogonal chemical catalyst to eliminate hydrogen peroxide, or even better, generate a second valuable oxidized product. Figure 31 proposes a non-exhaustive list of products frequently used in fine chemistry deriving from an oxidation reaction that could use hydrogen peroxide. This approach could provide an efficient pathway for the biochemist to make use of oxidases. From the chemist's point of view, this pathway could reversely be interesting to generate hydrogen peroxide *in situ* for chemical oxidation reactions without having to store a harmful oxidizing agent on site [106-107].

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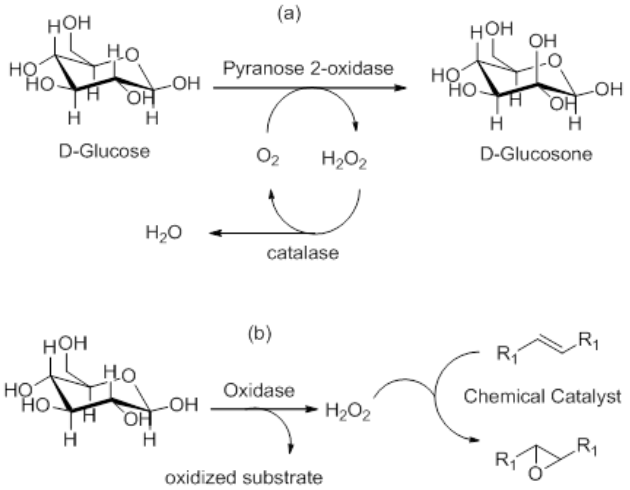

Figure 31. Concepts of (a) elimination and (b) valorisation of hydrogen peroxide within hybrid catalytic systems (for sake of clarity reactions are actually not equilibrated in this scheme).

In studies from 1991 and 2001, the potential of titanium silicalite zeolite (TS-1 (Titanium Silicalite-1) or Ti-β, Ti-MOR, Ti-MCM-22) as candidates for coupling with enzymatic oxidation had been mentioned but not exemplified [108]. Contrary to purely acidic zeolites, these particular categories of solids have been described to catalyse reactions under gentle temperature conditions, which are therefore compatible with enzymatic activity (< 100 °C). In 2010, Woodley, Grunwladt *et al.* in collaboration with the companies Haldor Topsøe and Novozymes first illustrated this concept by oxidizing D-Glucose to D-Gluconolactone with a glucose oxidase (GOx), further hydrolysed to targeted gluconic acid, while coproduced H_2O_2 was used by a TS-1 zeolite to convert allylic alcohol to glycidol (Figure 32) [109].

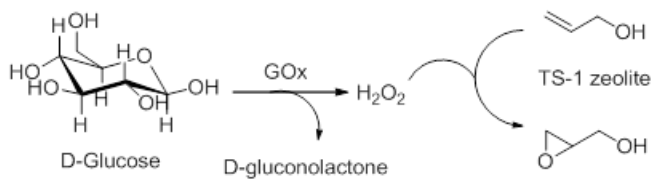

Figure 32. Example of hybrid catalysis to valorise H_2O_2 and produce D-gluconolactone and glycidol.

Then they generalized the use of the TS-1 and Sn-β for the valorisation of biosourced and petrosourced compounds *via* oxidation reactions to break down the hydrogen peroxide released by oxidases and in parallel oxidize substrates into valuable chemicals (Figure 33) [110].

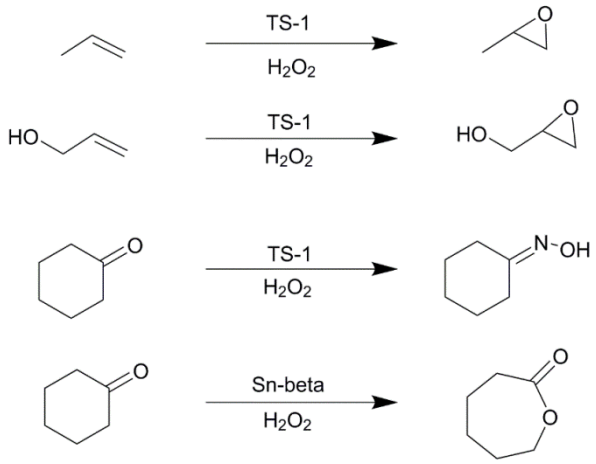

Figure 33. Selected examples of oxidation under conditions catalysed by zeolites in the presence of H_2O_2.

The main barrier with this approach, as with any example of hybrid catalysis, is harmonization of the reaction kinetics: if the chemical catalyst eliminates H_2O_2 too slowly, it will build up and the enzyme could be irreversibly deactivated. Extensive work on optimization of reaction conditions and design of catalysts is therefore required, with a case-by-case study for each enzyme/chemical catalyst combination and reaction of interest.

At the same time, spectacular progress has been made in directed evolution of new enzyme strains to make them more hydrogen peroxide tolerant. Takahashi *et al.*, for example, made a natural strain of a manganese peroxidase (EC 1.11.1.13) 9 times more stable than the original strain [111]. This progress can lower the kinetic constraints with the objective of allowing hybrid catalysis. Chemists and biochemists are therefore working together to progressively remove this drawback and allow deployment on a larger scale. The example of the Cetus process, developed in the 80's and abandoned since unprofitable, could possibly benefit from the development of hybrid catalysis to become again commercially attractive [112]. The process was based on a two-step quantitative chemoenzymatic conversion of glucose into fructose, avoiding a direct enzymatic isomerization by glucose isomerase limited by thermodynamics and separation cost of resulting glucose/fructose mixture. At the time, fructose was only used as a sweetener in the food industry (pure fructose or synthetic glucose/fructose mixtures such as HFCS not produced by traditional enzymatic isomerization), therefore with rather low added value. Selective production of fructose from glucose is now becoming increasingly important due to the growing interest in HMF and derivatives. The Cetus process proposed a first enzymatic process with selective and quantitative oxidation of *D*-glucose into *D*-glucosone. The reduction of *D*-glucosone catalysed by Pd/C also selectively produced *D*-fructose (Figure 34).

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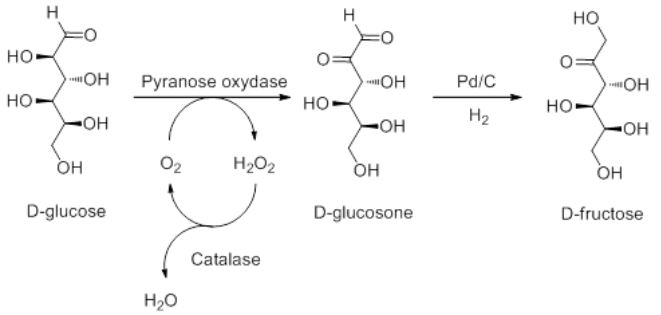

Figure 34. Cetus process as developed in 1980 by Cetus Corporation.

In view of the higher added value of fructose transformation to fine chemistry application *via* HMF compared to the sweetener use, this process would deserve to be deeply revisited. A recent study by Sun *et al.* [113] shows the attractiveness of reconsidering this process by replacing Pd/C by Ni-based catalyst in ethanol instead of water which allows enhancing the yields in fructose significantly.

In the original process, hydrogen peroxide was broken down by a catalase. To implement a hybrid process with H_2O_2 elimination and valorisation, a condition to make this process profitable would be to find a complementary target molecule that has a market share close to that of fructose and which is easy separable from fructose. If this feature imposes too many constraints, chemical catalysis could only play the role of catalase by breaking down hydrogen peroxide into oxygen and water. The oxygen could then be reused by the oxidase as oxidizing agent. To limit interactions and simplify separation, active redox heterogeneous catalysts could be useful inspired by Fenton-like chemistry like iron-containing zeolites [114] or manganese oxide (pyrolusite) [115].

The next section details the other major barrier identified in the field of enzymatic catalysis, for which hybrid catalysis could bring solutions to avoid multiple enzymatic systems: regeneration of oxidoreductase cofactors.

5. Regeneration of cofactors

Unlike fossil resources, lignocellulosic biomass has a high oxygen/carbon ratio with various chemical functions such as alcohols and aldehydes for sugars, or ethers and phenols for lignin. The enzymes of the oxidoreductase family seem to be suitable for selective conversion of these resources under gentle conditions. Amongst these biosourced products, the sugars and the alcohol-sugars are extremely important alongside glucose, fructose and sorbitol for the highest volumes. To selectively modify these oxygenated functions, for example to convert a polyol into a ketose or an aldose on a quite specific position, or selectively reduce a sugar into a given epimer, the alcohol dehydrogenases of the oxidoreductase family are more suited than chemical catalysis.

Oxidoreductases are the most represented class in the enzyme classification, reaching almost a quarter of the used enzymes [116]. Their associated cofactor is essential for their operation. Nicotinamides [NAD(P)H/NAD(P)$^+$] are the most commonly used cofactor couples (Figure 35). Thus, 80 % of the known oxidoreductases use the NAD/NADH couple as cofactor and 10 % their phosphorylated form NADP/NADPH [117].

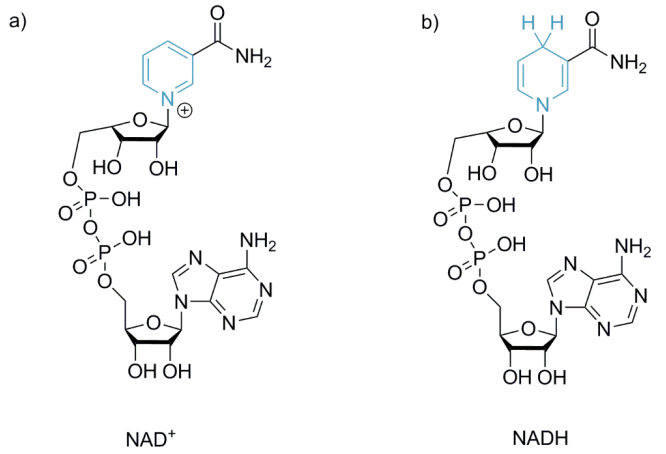

Figure 35. (a) NAD^+ and (b) NADH structures

The other cofactor families, such as the FAD/$FADH_2$, FMN/$FMNH_2$ and PQQ/$PQQH_2$ couples, are more confidential. These cofactors are converted by the enzyme stoichiometrically with respect to the substrate during the reaction. Their high price combined with the stoichiometric quantities required in the reaction considerably limit the economies of scale of these reactions without regenerating the cofactor. Various parameters must be taken into account to develop an efficient regeneration system, for example stability of the enzyme and its cofactor; operating conditions; reaction progress; compatibility of reaction rates; methods used to separate/purify/isolate the various substrates and products. Several regeneration methods are frequently described in the literature: enzymatic regeneration systems and chemical regeneration systems. We will first briefly describe the principle of enzymatic regeneration and the barriers encountered during its implementation. We will then see which relevant chemical solutions have been described to replace them and which perspectives for hybrid catalysis research could be proposed in this context.

5.1. Enzymatic regeneration

Two approaches can be considered during enzymatic regeneration of a cofactor. The first approach, known as "coupled-substrate regeneration", uses the production enzyme to regenerate the cofactor (Figure 36; S = Substrate, P = Product). Apart from the substrate to be converted, a second sacrificial substrate is added to conduct the two reactions: synthesis and regeneration.

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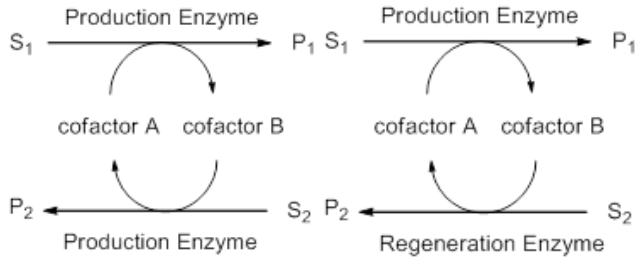

Figure 36. Enzymatic regeneration of cofactor.

The first case is rarely applied for several reasons: very few enzymes are active for two different substrates S_1 and S_2. Regeneration of the cofactor by converting S_2 into P_2 will occupy catalytic sites and slow down the production of P_1 from S_1. In order to be efficient, regeneration must also preferably be carried out in the presence of excess S2, which slows down the reaction of interest even more. The potential presence of 4 organic products (S_1, S_2, P_1, P_2) in this case makes the products difficult to separate.

The second approach, known as "coupled enzyme" regeneration, also uses a second substrate, but in the presence of a second enzyme (Figure 36; on the right). Extensive research is being conducted on this second approach. The most widely applied regeneration method and the only one used on industrial scale is that involving Formate dehydrogenase (FDH) and formate as a substrate. Regeneration of NADH is therefore accompanied by the production of carbon dioxide, as described on Figure 37. To avoid the tedious steps required to separate the substrates or regeneration organic products (S2 and P2 on Figure 36), it is better to choose substrate/inorganic couples, which are easy to separate. The FDH regeneration enzyme engages the $CO_2/HCOO^-$ couple, with CO_2 being evacuated from the system in the gas phase. Redox couples such as O_2/H_2O and H_2O/H_2 may also be ideal couples for simplified and less expensive implementations.

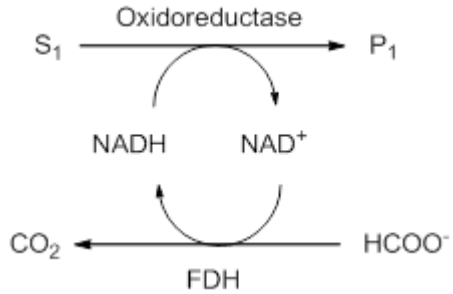

Figure 37. Enzymatic regeneration of the cofactor with the enzyme FDH (Formate Deshydrogenase).

Although the regeneration product can be easily evacuated from the reaction medium in the case of FDH, the major drawback of this method is due to the cost of producing the regeneration enzyme. Addition of formic acid/formate depending on the pH may also inhibit the production enzyme [118].

In the case of an oxidation reaction, therefore coupled with reduction of NAD^+ into NADH, enzymatic regeneration of NAD^+ is preferably carried out with the enzyme NADH-oxidase [119]. This enzyme reduces the dioxygen into hydrogen peroxide. The regeneration kinetics are very slow, however [120] and the formed hydrogen peroxide produces numerous detrimental effects on the enzymatic reactions [121]. Once again, we encounter the barrier mentioned in the previous section, with the same type of methodology to be implemented to eliminate hydrogen peroxide. Triple, highly complex oxidoreductase/NADH-oxidase/catalase combinations must therefore be set up. In this context, simple regeneration by hybrid catalysis with the enzyme and a chemical catalysis may be far more preferable. To overcome the disadvantages of cofactor enzymatic regeneration, numerous studies have been conducted on non-enzymatic methods, which will be now globally described by broad catalyst families.

5.2. Chemical regeneration

In the context of chemical regeneration of the cofactor, a molecule must selectively transfer the charges between a reducing or oxidizing agent and the cofactor. Various types of chemical catalysis have therefore been considered.

5.2.1. Regeneration by inorganic salts

Inorganic salts were tested for NADH regeneration, such as sodium dithionite $Na_2S_2O_4$ and sodium borohydride $NaBH_4$ [122], conventional reducing agents in organic chemistry. At high concentrations, these salts nevertheless deactivate the enzymes [123]. In addition, these salts are used stoichiometrically and with no treatment procedure. There are therefore consequences on the cost of implementation and the pollution generated. Although NADH regeneration has been reported, note that no solution with equivalent oxidizing salts has been proposed for NAD^+ regeneration.

5.2.2. Organic catalysis

According to the literature, the most efficient organic molecule found to date for NAD^+ regeneration is pyrroloquinoline quinone (PQQ or methoxanine) shown on Figure 38. It is also a cofactor in its own right of quinoprotein glucose dehydrogenase, for example.

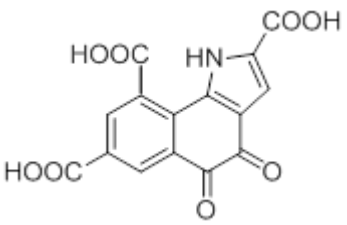

Figure 38. PQQ molecule used for NAD^+ regeneration

After transferring a hydride from NADH and a proton from the medium, PQQ is reduced to $PQQH_2$ with NAD^+ regeneration. $PQQH_2$ then reduces a mole of dioxygen from the medium to form hydrogen peroxide by regenerating PQQ. This method can therefore be used to introduce the cofactor in catalytic quantity with respect to the substrate (1:20), giving a yield of 75 %, which amounts to a turnover number (TON = number of moles

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converted per mole of catalyst) of 15.6 for NAD^+ [124]. Like NADH oxidase, the main disadvantage of this organic molecule is the production of hydrogen peroxide, which inhibits numerous enzymes.

However, other organic compounds were also active for the cofactor regeneration. In their work on NADH regeneration, Okamoto *et al.* [125] studied an artificial metalloenzyme based on streptavidin variants and a biotionlylated iridium complex. Five different nicotinamide mimics of $mNAD^+$ were tested as cofactors in the catalytic reduction of Michael Acceptors by ene reductase. It was shown *(1)* that the catalytic activity (in terms of enantioselectivity) strongly depends on the nature of the mNADH, and *(2)* that when increasing the reaction time from 4 to 7 h the total loading of Ir complex could be reduced from 0.1% to 0.05%. The best combination for the most efficient artificial cofactor regeneration was found to be composed of tionylated complex and S112A Variant of streptavidin.

5.2.3. Heterogeneous catalysis

Quite recently, the very first example of nicotinamide cofactor regeneration by a heterogeneous catalyst was reported by Yiu's team [126]. In this example, the catalyst used to regenerate NADH is platinum on alumina Pt/Al_2O_3. Under a pressure of 9 bar of hydrogen in aqueous phase, the catalyst exhibited a regeneration yield of 50 % in a 6 hours reaction at 37 °C. The regeneration system was then used *in situ* with conversion of propanal to propanol catalysed by an alcohol dehydrogenase derived from the strain *Saccharomyces cerevisiae.* The regeneration yields are quantitative, with a catalytic quantity of cofactor. Note, however, in this example a high quantity of cofactor relative to the substrate ([propanal] = 0.50 mM, [NADH] = 0.37 mM; [NADH]/[propanal] = 0.74), which makes its regeneration less critical (Figure 39).

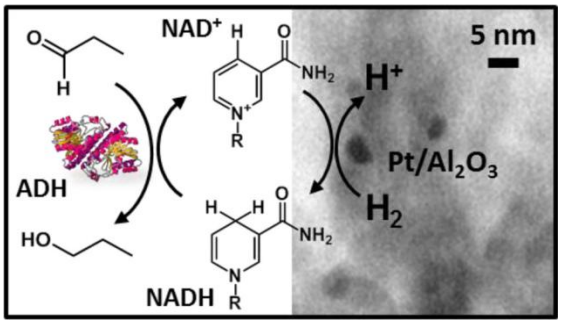

Figure 39. NADH regeneration by a heterogeneous catalyst Pt/Al_2O_3. Reprinted from Ref. [126] with the permission from American Chemical Society

The regeneration method is selective towards NADH despite the various possible hydrogenation sites leading to the formation of 1,6-NADH. Bimolecular coupling of NAD^+ under an electrical overpotential may also result in another degradation product (written NAD_2) *via* radical coupling (Figure 40).

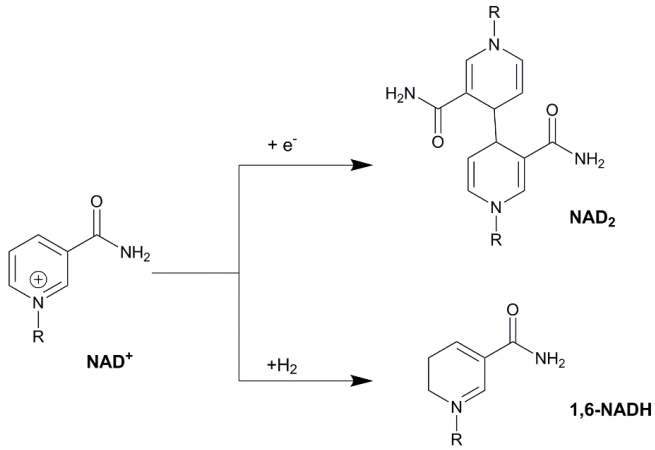

Figure 40. NAD^+ into NAD_2 or 1,6-NADH degradation pathway.

Direct hydrogenation of propanal to propanol by supported platinum can also be achieved. Higher temperatures are required, however, and selectivity is not excellent. In this case a phenomenon similar to hydrogen spillover between the platinum and the nicotinamide cofactor is observed.

More recently, Godwin *et al.*also studied the regeneration of NAD^+ using a platinum-seeded cadmium sulfide (Pt@CdS) photocatalyst [127]. This process is carried out by using three types of catalysis (enzymatic, photo- and organocatalysis) the butanol was not only transformed into *2*-ethylhexanal, but also molecular hydrogen was formed, which could be recovered and utilized in hydrogenation process. The authors showed also that, upon photo-irradiation, Pt-decorated CdS nanorods (NRs) oxidize NADH back to NAD^+ and reduce protons to H_2, thereby pushing enzymatic equilibrium toward butyraldehyde (BA) formation. In the studies carried out in the absence of NAD+ regeneration by the Pt@CdS, the production of BA decreased significantly due to the rapid back reaction by the enzyme to butanol.

5.2.4. Electrocatalysis and photocatalysis

These two pathways are based on the same principle of electron transfer between a charge transfer agent from a light or electrical energy source. In all the examples, the regeneration studied is reduction of NAD^+ into NADH.

Electrochemical regeneration is well documented and consists of two pathways: a direct pathway on electrode [128] or an indirect pathway *via* an organometallic complex acting as mediator to accelerate the charge transfers between the cofactor and the electrode. The first systems were described as early as 1987 by Steckhan *et al.*, pioneer in non-enzymatic regeneration of cofactors, with the first rhodium-based organometallic complexes [129]. Formate is used as co-substrate to generate the active species cationic rhodium hydride $[H\text{-}Rh(III)]^+$. This system can be improved by immobilizing the complex and the cofactor on a PEGylated membrane [130]. To achieve good performance levels, overpotentials must often be applied. Electron transfers may lead to radical coupling pathways to form NAD_2 for example. The systems reported in the literature are also rarely tested in the presence of a cofactor derived from an *in situ* enzymatic reaction (Figure 41, [131]). The concept has been validated but perspectives are limited for hybrid catalysis.

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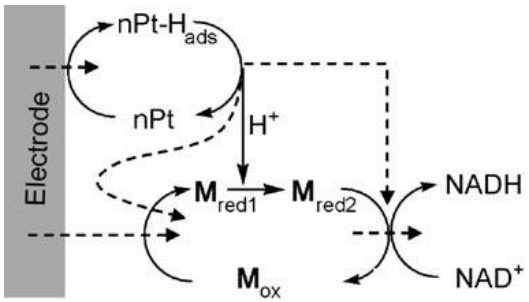

Figure 41. Schematic diagram illustrating NADH indirect electrochemical regeneration with Pt electrode and organometallic mediator (dotted arrows = electron transfer). Reprinted from Ref. [131] with the permission from Wiley.

Following the recent progress made in photocatalysis, new systems are available to regenerate cofactors. Mediators based on rhodium complexes can transfer the electrons produced by photochemical stimulus received, for example, by a carbon nitride [132]. A supported version was developed by coordinating the rhodium-bipyridine complex on a photosensitive polyarylene polymer (Figure 42) [133]. In this case, acceptor and mediator form a single entity to favour charge transfer and post-regeneration separation. For these photoactivated pathways, improvements are expected in terms of productivity and electron transfer selectivity.

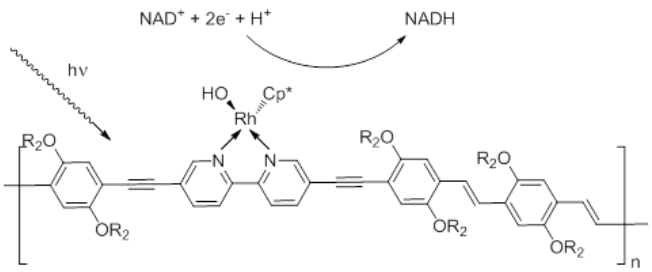

Figure 42. NADH regeneration photocatalysed by a photosensitive polymer/supported rhodium system. Adapted from Ref. [133].

These first examples demonstrate that transition metals play a major role in charge and hydrogen transfer. Over the last decade, organometallic chemistry in aqueous phase has developed exponentially, especially with the transition metals of groups 8, 9 and 10. Photochemical reduction of water to produce hydrogen [134] and oxidation of water to produce dioxygen, mimicking the plant photosystem, have led to the emergence of several organometallic catalysts active in aqueous phase based mainly on iridium [135], but also on platinum and ruthenium [136]. The complexes of these metals therefore become interesting candidates to be implemented in combination with enzymes: we will now describe the first studies involving such complexes to regenerate nicotinamide cofactors coupled with oxidoreductase enzymes.

5.2.5. Cofactor regeneration by organometallic complexes: enzyme mimic and cofactor regeneration

In natural photosynthesis, the electrons and protons extracted from water by solar energy are used to reduce $NAD(P)^+$ by the action of ferredoxin-NADP reductase to generate NAD(P)H which reductively fixes CO_2 as sugars in the Calvin cycle [137]. The protein ferredoxin comprises iron-sulfur clusters whose metal atoms oscillate between oxidation degrees +II and +III. The transition metals therefore play a major role in enzymatic catalysis [138]. First, the enzyme may depend on one or more metal ions to main a stable three-dimensional structure and/or a functional active site, without the metal being involved directly in the catalytic action. Second, the metal may itself be responsible for catalytic activity within the active site: for example, abundant metals such as nickel and iron, integrated within a protein matrix, are the active sites of enzymes from the hydrogenase family, whose artificial synthesis has experienced tremendous development over the last few years with the work of Fontecave, Artero *et al.* [139].
Researchers turned naturally towards biomimetic (or bioinspired) catalysis, attempting to replace metalloenzymes by simpler, often polynuclear, organometallic complexes, which would perform the same functions [140]. Artificial synthesis of these complexes, also called "biohybrids", may itself also draw inspiration from the natural maturation pathways. Consequently, a Ni-Mn binuclear complex mimicking the active site of a hydrogenase [NiFe] can be prepared by bioinspired maturation of a $Mn(CO)_3$-lysozyme cluster (the manganese complex interacts with a histidine residue of the lysozyme) with a nickel-thiolate precursor of type Ni(xbsms) (H2xbsms = 1,2-bis(4-mercapto-3,3-dimethyl-2-thiabutyl)-benzene) [141].
The pioneering studies in the field date back to the late 80s with the first organometallic version of cofactor regeneration by Steckhan *et al.* using rhodium complexes. The objective was to replace formate deshydrogenase (FDH) by an organometallic complex (Figure 43), which would itself act as NADH regenerator, but would be:

- less sensitive than the enzyme to inhibition by the reduced cofactor;
- capable of entirely converting the oxidized cofactor;
- transposable indifferently between NAD and NADP systems.

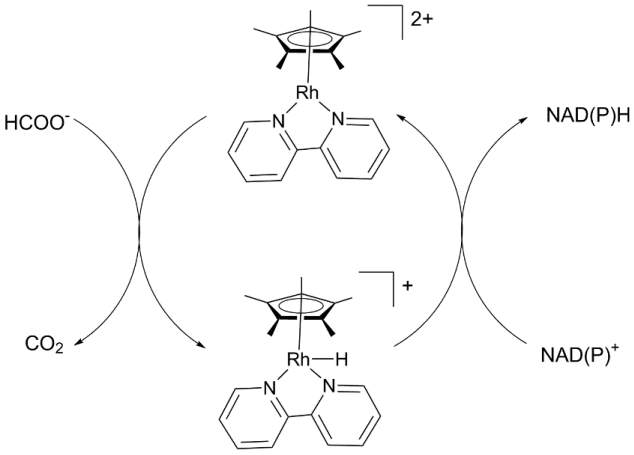

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Figure 43. First example of cofactor (NADH) organometallic regeneration with a rhodium cationic complex.

The mechanistic aspects were then studied by Fish *et al.* [142] on cofactor models. Organometallic complexes can in fact be used to work with non-natural cofactors, simplified models of the conventional NAD^+ and NADH cofactors. We will discuss later the information supplied by these mechanistic studies. This field experienced a revival of interest in the years 2000. Sadler *et al.* tried to replace enzymes of the hydrogenase/dehydrogenase class by ruthenium and iridium organometallic complexes to catalyse hydrogenation/dehydrogenation redox reactions whose catalytic cycle would involve the use of NAD as cofactor. These complexes were initially developed for their anti-cancer activity: their interaction with the cofactors of the nicotinamide family used by the enzymes of cancer cells causes the cells to apoptosis [143]. During these initial cytotoxicity studies, the authors discovered that these complexes were capable of mimicking an activity of lactate dehydrogenases, for example, by using NADH as a source of hydride for hydrogenation of pyruvate into lactate. The structures of the ruthenium complexes used are based on pyrimidine ligands whose structure has been optimized in a preliminary study for the selective oxidation of NADH into NAD^+ (Figure 44) [144].

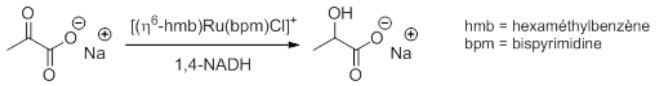

Figure 44. Ru organometallic catalyst using NADH as cofactor

As a conclusion to their studies, the authors announced that they had conducted the same reaction from NADH produced by an alcohol dehydrogenase in a first step, without regenerating the NAD^+ cofactor. The NADH thus produced can be used by the ruthenium complex as hydride source to hydrogenate pyruvate. During this conversion, a ruthenium hydride was detected by ^1H NMR with a characteristic signal at -7.5 ppm. This intermediate allowed the authors to propose a reaction mechanism detailed in Figure 45.

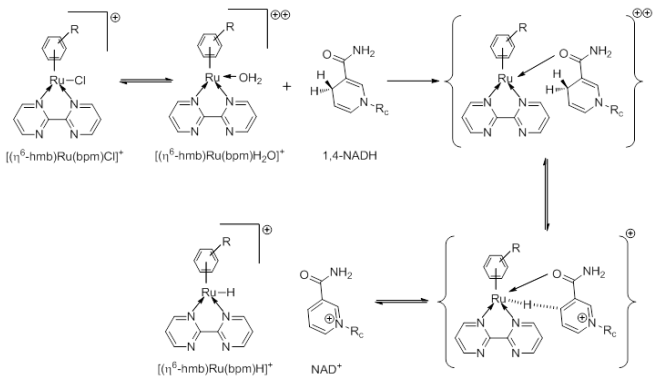

Figure 45. Mechanism proposed for the production of NAD^+ from NADH involving the formation of ruthenium hydride.

A similar mechanism involving an Ir-H intermediate was proposed by the same team with $[Cp^*Ir(phen)H_2O]$ complexes. Hydride was transferred by this complex from NADH to reduce benzoquinone to hydroquinone. Consequently, the reaction catalysed by the iridium complex produced NAD^+ from NADH (Figure 46) [145].

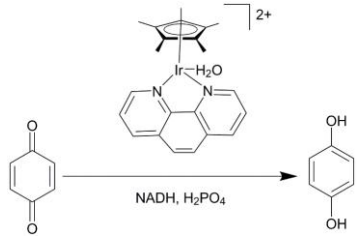

Figure 46. Reduction of benzoquinone by an iridium complex in the presence of NADH. Adapted from Ref. [145].

In this article, the team demonstrates the catalytic activity of the iridium complex and identification of NAD^+ by ^1H NMR. The reaction was monitored using NMR and formation of a metallo-hydride is detected by a signal at -11 ppm. The iridium complex also acts as an enzymatic mimic. As with the ruthenium complex of the previous article, it demonstrates that the iridium complex can catalytically transfer hydride between NADH and benzoquinone (2 mol.%). Rather than using reduction of an organic compound (pyruvate or benzoquinone) to regenerate NAD^+, why not taking advantage of this ruthenium or iridium hydride to couple a reduction of H_2O, H_3O^+ or O_2 into H_2 or H_2O_2, for example? Water could therefore be used as a source of protons or electrons, depending on the redox direction, as in natural photosynthesis, without suffering from the presence of organic compounds which would then have to be separated. The system therefore moved in this direction with the next article published by Sadler *et al.*: no organic molecule is used to regenerate the catalyst. Catalyst regeneration occurs with water, the reaction solvent, and generates dihydrogen. This is the first example of a nicotinamide cofactor regeneration system without adding organic molecules to the system. The regeneration mechanism with the iridium complex is shown in Figure 47.

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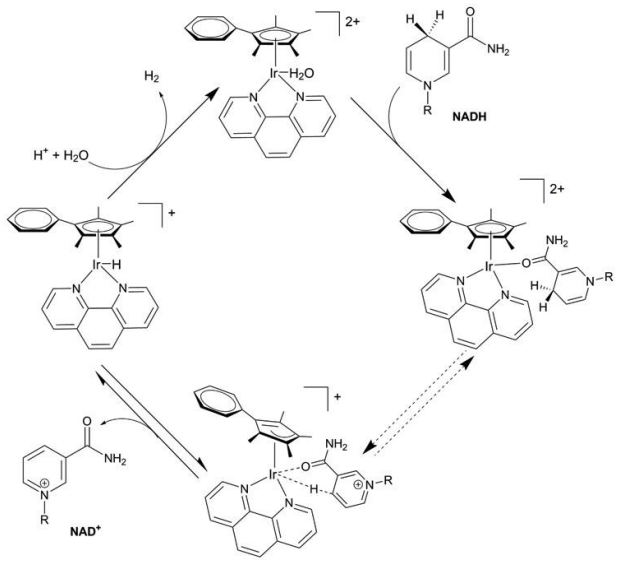

Figure 47. Mechanism proposed by Sadler *et al.* for NAD^+ regeneration with an iridium organometallic complex. Adapted from Ref. [144].

To the best of our knowledge, this is the only mechanism proposed in the literature. The main advantage of this catalyst is the use of the solvent as regeneration substrate. With a view to combining this catalyst with a biochemical catalyst, this property is extremely important since it avoids the problem of compatibility between the enzyme and the regeneration substrate.

The mechanism proposed by Sadler *et al.* shows the formation of an iridium hydride species (Figure 47) and the consumption of a proton to form dihydrogen and regenerate the initial aqua iridium complex [146].

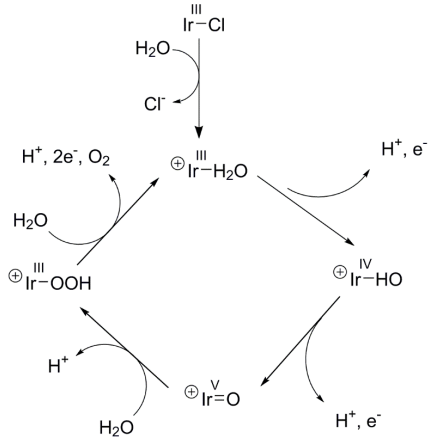

Figure 48. Mechanism proposed for oxidation of water by an iridium complex. Reproduced from Ref. [146] with the permission from the American Chemical Society.

Iridium therefore retains its degree of oxidation +III throughout the mechanism, taking mono- and di-cationic states. The mechanism for reformation of the active species is not studied however: the mechanism involved is quite different from the water oxidation reaction. No comparison can therefore be made and the mechanism remains to be studied. The water oxidation mechanism removes two protons from the aqua ligand, changing the degree of oxidation of iridium from +III to +V. The iridium +V species is sufficiently electron-deficient to undergo a water nucleophilic reaction and therefore form an O-O bond, as shown in Figure 48 [146].

Despite the mechanistic differences, the proposals of iridium complexes for water oxidation remain a source of inspiration for the regeneration of cofactors by organometallic catalysis. By reviewing the literature, a list of synthesizable, characterized complexes can be drawn up. In addition, the synthesis intermediates obtained provide information on the catalyst's oxidizing properties.

Note also the complementary studies conducted with iridium complexes and nicotinamide cofactors by Fukuzumi *et al.* This team studied the interconversion between NADH and NAD^+ using an iridium complex with a bidentate ligand [C,N], as shown in Figure 49 [147].

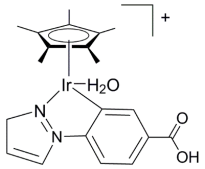

Figure 49. Iridium [N,C] complex used by Fukuzumi *et al.* Adapted from Ref. [147].

When converting NADH to NAD^+ under slightly acid conditions, the formation of NAD^+ was confirmed by ^1H NMR analysis. The reaction was followed by the formation of H_2 according to the reaction described in Figure 50.

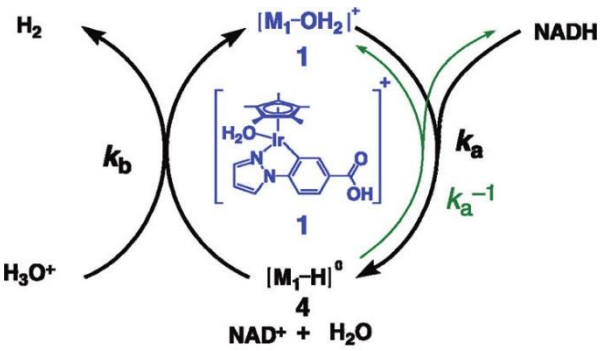

Figure 50. Conversion of NADH into NAD+ under slightly acid conditions (pH = 4-6). Adapted from Ref. [147] with the permission from the American Chemical Society.

The impact of varying the pH between 4.1 and 6.1 has been investigated. The Turn Over Frequency (TOF), *i.e.*, the number of moles converted per unit of time and per mole of complex increases with pH, and, therefore, the proton concentration decreases (Figure 51).

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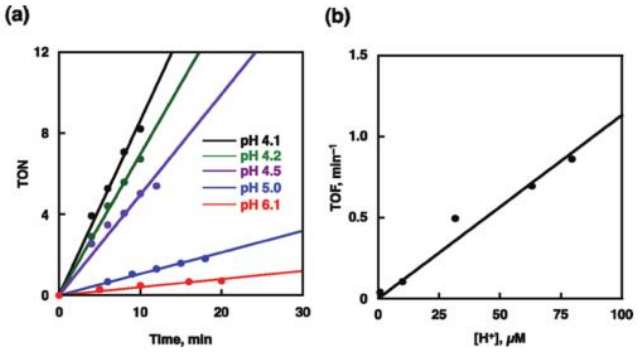

Figure 51. TON and TOF variation depending on the pH with the catalyst of Fukuzumi's team for the formation of NAD^+ from NADH.). Adapted from Ref. [147] with the permission from the American Chemical Society.

At a pH of 6.1, the reaction is very slow: the authors demonstrate in fact that at a pH close to neutral and under hydrogen atmosphere, the opposite reaction occurs with consumption of hydrogen and reduction of NAD^+ to NADH.

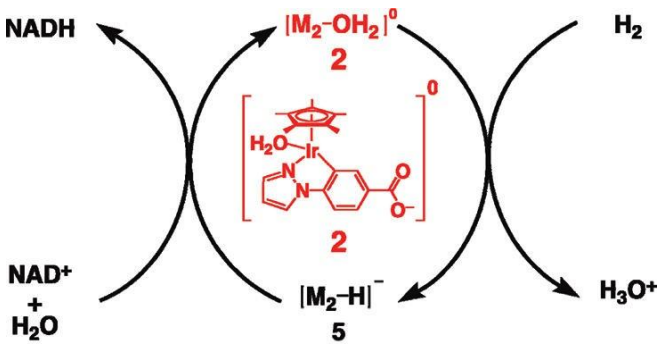

Figure 52. Conversion of NAD^+ into NADH under neutral conditions (pH = 7.0). Adapted from Ref. [148] with the permission from American Chemical Society.

Fukuzumi *et al.* conducted further investigations on the use of these complexes to reduce NAD^+ to NADH catalysed by two families of [N,C] and [C,C] iridium complexes, with a sacrificial alcohol as a hydrogen donor (Figure 52) [148]. (The [C,C] complex is obtained by simple photo-irradiation of the [N,C] complex at λ = 330 nm).

These results demonstrate a critical effect of the pH on the reaction and confirm an assumption of the mechanism proposed by Sadler *et al.* [149] (Figure 53), where a proton is consumed to return to the active species and to release H_2. However, no detailed mechanistic study of the NAD^+ regeneration step was provided. Iridium complexes based on equivalent structures have also recently been studied as mimics of oxidases by the same team [149].

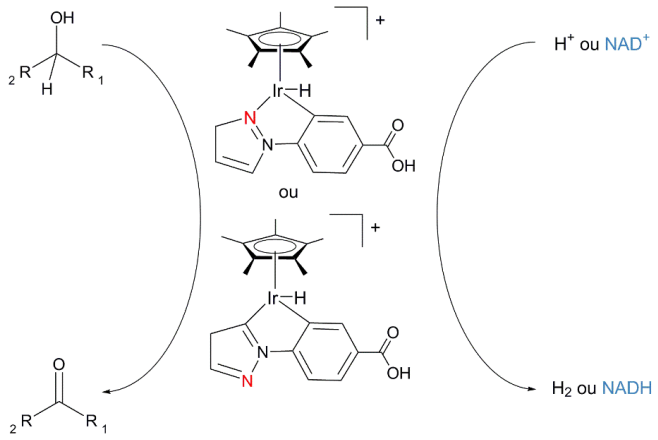

Figure 53. Conversion of NAD^+ to NADH with an iridium complex. Adapted from Ref. [149] with the permission from the American Chemical Society.

These studies conducted by Sadler *et al.* and Fukuzumi *et al.* demonstrate that iridium, rhodium and ruthenium complexes bearing Cp*and [N,N] or [C,N] ligands can regenerate NAD^+ cofactor under conditions which would be compatible with an enzymatic reaction namely in water, at ambient temperature, possibly with a non-pressurized hydrogen atmosphere, without organic co-substrate to transfer protons and electrons. Depending on the pH conditions, the opposite reaction of NADH regeneration may also occur. Considering these articles from the point of view of developing hybrid catalysis in the field of cofactor regeneration, the following question arises: Sadler *et al.* example shows that a ruthenium complex can hydrogenate pyruvate into lactate by converting NADH into NAD^+. Could it be used to regenerate the NAD^+ cofactor during an enzymatic dehydrogenation coupled reaction? The activity of hydrogenase/dehydrogenase type oxidoreductases is governed, amongst other things, by the pH, which can be adjusted in order to work in one direction or the other. A common optimum pH working range will therefore be the *sine qua non* condition to allow a hybrid system to operate.

The hybrid approach for organometallic regeneration of cofactors was first considered in 2007, but has experienced a revival of interest over the last few years. All the studies on cofactor regeneration by organometallic complexes have been described using the nicotinamide family. Amongst the examples of chemoenzymatic and hybrid synthesis reported so far in the present review, irrespective of the regeneration pathway considered, most of the oxidoreductases (alcohol dehydrogenase (ADH) of *Rhodococcus sp., Rhodococcus ruber, Lactobacillus brevis* and *Lactobacillus kefir)*, operate in the reduction direction. The NADH cofactor is therefore oxidized concomitantly into NAD^+ and the regeneration required is the reduction of NAD^+ into NADH. Few examples describe the use of oxidoreductases in the oxidation direction (in other words, in the dehydrogenation direction), Sadler *et al.* example is the most representative, even though it is not implemented in hybrid catalysis but in a sequential chemoenzymatic approach. Regarding the conversion of products obtained from biomass such as sugars, carbonylated compounds or (poly)hydroxylated compounds, both reaction directions are likely to be of interest to our opinion to develop a hybrid catalysis system suitable for the valorisation of biomass.

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5.2.6. NADH regeneration hybrid catalysis.

Süss-Fink *et al.* were the first to use rhodium, iridium and ruthenium catalysts with a phenanthroline ligand for NADH regeneration in combination with an enzymatic reaction [150]. Organometallic regeneration reactions involve the same substrates as a FDH type enzyme, *i.e.*, the HCO_2^-/CO_2 couple (Figure 54).

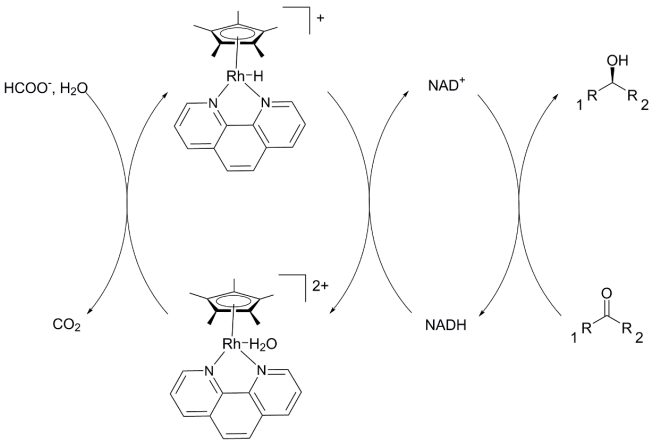

Figure 54. Ketone enzymatic reduction/NADH organometallic regeneration hybrid reaction. Adapted from Ref. [150] with the permission from Wiley.

The studied complexes bear a phenanthroline ligand functionalized or not with an amine or nitro moiety and a pentamethylcyclopentadiene or hexamethylbenzene ligand for ruthenium, inspired by the pioneering work of Steckhan *et al.* [151] and Fish *et al.* [152]. Rhodium and iridium catalysts exhibit total conversions under conditions, which would be compatible with an enzymatic reaction (T = 38 °C; pH = 7). The article published by Fish *et al.* proposed a mechanism suggesting the appearance of a rhodium hydride from formate forming carbon dioxide then the transfer of this hydrid to the pyridinium group of NAD^+ to form NADH. Substitution by an amino moiety has no significant electronic impact whereas the nitro moiety is involved in one-electron reductions making the ligand non-innocent and modifying the redox potentials of the corresponding complexes.

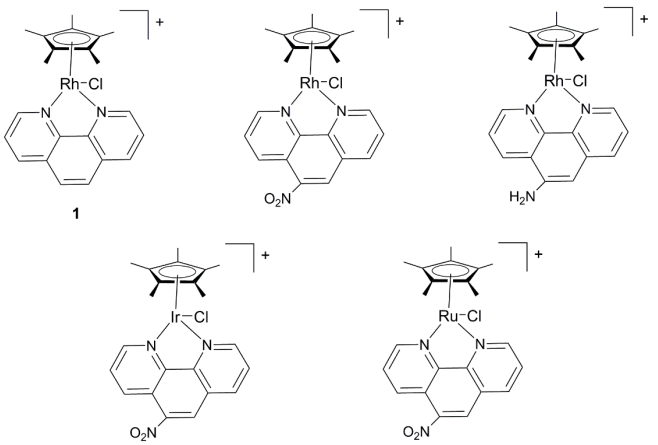

Figure 55. Organometallic complexes studied used for NADH regeneration.

After selecting a rhodium complex exhibiting the best activity (complex **1** of Figure 55) to produce NADH from NAD^+ amongst the studied complexes, the authors implemented it in the presence of an enzymatic conversion. The selected hybrid system involve hydrogenation of a ketone to a secondary alcohol using an alcohol dehydrogenase and simultaneous NADH regeneration using the rhodium complex. The yield of the hybrid reaction is not as good as when the enzymatic reaction is carried out alone with an excess of cofactor (62 %yield compared to80 %). Note that under hybrid conditions, the ketone is introduced with an initial concentration of 33 mM in the presence of 1 mM of NADH and 0.5 mM of complex: a 62 % yield implies a net use of 20.46 mM of NADH. This shows that the complex was able to regenerate the cofactor nearly twenty times under the enzyme operating conditions. In addition, the complex did not inhibit the enzyme. We may also mention the studies conducted by Fish *et al.* [153], which describe *in situ* regeneration of cofactor models demonstrating the versatility of organometallic regeneration for a wide range of cofactors. Enzymatic regeneration of such cofactors is not straightforward due to the specific key/lock recognition between the enzyme and the cofactor. Once again, this rhodium complex (Figure 56) implements the formate ion as a hydrogen donor. Regioselective oxidation of *2*-hydroxybiphenyl by hydroxylation into the corresponding catechol is catalysed by the enzyme *2*-hydroxybiphenyl *3*-monooxygenase (HbpA) of *Pseudomonas Azelaica HBP1*. The natural NADH cofactor is replaced by a biomimetic cofactor *N*-benzyl-*1,4*-dihydronicotinamide. Nevertheless, deactivation of the system by a possible interaction between aminated and thiolated residues with the rhodium complex has been observed. A stable and productive regeneration system has been developed by protecting these $–NH_2$ and –SH functions.

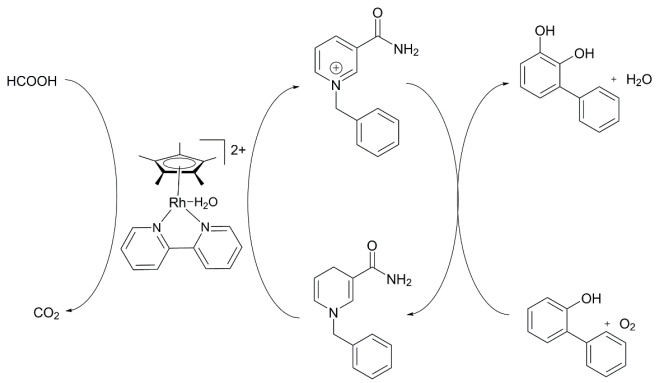

Figure 56. Hybrid catalysis of oxidation of phenol into catechol and organometallic regeneration of the cofactor biomimetic. Adapted from Ref. [153] with the permission from Elsevier.

We therefore see that this type of organometallic complex is compatible with enzymatic hydrogenation/dehydrogenation.

5.2.7. NAD^+ regeneration: example by oxidative mechanism

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The only example of NAD^+ regeneration coupled with an enzymatic oxidation reaction in hybrid catalysis is described by Gröge *et al.* [154] with sulfonated iron porphyrin complexes (Figure 57). This team tested various porphyrins but only one exhibits true activity for NAD^+ regeneration. The oxidizing agent used is dioxygen from the air dissolved in water.

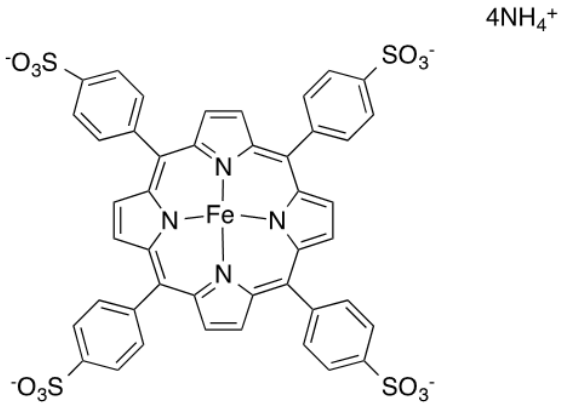

Figure 57. Sulfonated iron tetraphenylporphyrin.

Regeneration was implemented *in situ* with two different enzymatic reactions: first, the conversion of glucose to gluconic acid with glucose dehydrogenase of bacterium *Bacillus sp.* in the presence of a sodium salt, and, second, the conversion of cyclooctanol to cyclooctanone using alcohol dehydrogenase.
The porphyrin regeneration activity is 0.11 $mol.mol^{-1}.s^{-1}$ at ambient temperature in distilled water buffered at pH 7. This remains an isolated example of NAD^+ regeneration by oxidative mechanism (Figure 58).

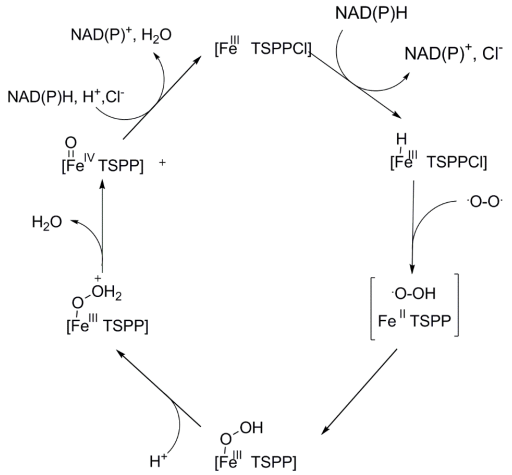

Figure 58. Proposal of oxidative mechanism for NAD^+ regeneration according to Gröger *et al.*.Reprinted from Ref. [155] with the permission from Wiley.

Apart from these two mechanisms, we may mention the stoichiometric regeneration of a NADH model by an iron-based organometallic complex $[Fe_3(CO)_{12}]$ [155], which was described by Beller *et al.*, although the reaction was only tested on the cofactor without associated substrate. In addition, the conditions do not seem to be adapted to cofactor regeneration under enzymatic reaction conditions as it occurs at 65 °C in toluene and the yields are increased in the presence of iron(II) triflate, $Fe(OTf)_2$, which is poisonous for enzymes if the concentrations are too high. In this example, the concentration is 35 mol.% compared with the NADH model, which would suggest that the enzyme will be inhibited by iron triflate at these concentrations.

6. Conclusion and outlook

Efficient integration of chemical and biological catalysis is playing an increasingly important role in the field of valorisation of bioresources and in catalysis in general. Many chemoenzymatic synthesis implemented successively that are reviewed in the first part show the diversity of transformations that can be envisioned and highlight the compatibility issues between chemical and biological catalysis. Nevertheless, the first hybrid examples show the high potential of such combinations by decreasing the number of reaction and separation steps in many synthetic fields, in particular in biomass valorisation. In this context, the use of chemical catalysis may remove barriers inherent to biological - in this case enzymatic - catalysis with firstly decomposition of hydrogen peroxide H_2O_2 which inhibits enzymes, and secondly cofactor regeneration by applying the concept of hybrid catalysis with one-pot/one-step implementation. Decomposition of hydrogen peroxide has been extensively studied and concerns a limited range of enzymes. Note, however, that a revival of interest is to be expected concerning the Cetus reaction to decompose the hydrogen peroxide formed which limits the production of fructose, an ideal intermediate for the formation of HMF. Enzymes of the oxidoreductase family can selectively convert biosourced feedstocks into platform molecules or high added-value molecules. These oxidoreductases nevertheless exhibit the major disadvantage of requiring a cofactor, which they reduce or oxidize depending on the substrate present in the reaction medium. This cofactor is therefore stoichiometrically modified by the enzymatic reaction, which generates a high cost when considering implementing this enzymatic process without regeneration on industrial scale (in a "biorefinery"). Cofactor regeneration methods are currently being studied and the results report the efficiency of organometallic complexes both as regards cofactor regeneration and respect of green chemistry principles to reduce separation energy costs, for example. Studies on chemical NADH regeneration have been reported in the literature, but few examples have been published concerning NAD^+ regeneration. Chemical catalysis developed for the chemical regeneration of these cofactors has focused in organometallic complexes, based mainly on rhodium and iridium. Mechanistic studies of the regeneration action modes involved are ongoing in order to clearly understand the critical parameters of the reactions and optimize the systems. Amongst the most promising complexes, an iron porphyrin and an iridium organometallic complex have demonstrated their ability to reduce the NAD^+ cofactor, first in the absence of any other organic molecule, and then, more recently, in a full hybrid catalytic approach. However, this is a preliminary study and work to improve the chemoenzymatic process including

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screening to obtain more efficient and selective complexes as well as a more in-depth study of compatibility with the enzyme are still required.

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Keywords: Hybrid catalysis • chemoenzymatic integration • cofactor regeneration • oxidoreductases • cascade reactions

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