Combining bio- and chemo-catalysis: from enzymes to cells, from petroleum to biomass

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In the future, biomass will continue to emerge as a viable source of chemicals. The development of new industries that utilize bio-renewables provides opportunities for innovation. For example, bio- and chemo-catalysts can be combined in ‘one pot’ to prepare chemicals of commercial value. This has been demonstrated using isolated enzymes and whole cells for a variety of chemical transformations. The one-pot approach has been successfully adopted to convert chemicals derived from biomass, and, in our opinion, it has an important role to play in the design of a more sustainable chemical industry. To implement new one-pot bio- and chemo-catalytic processes, issues of incompatibility must be overcome; the strategies for which are discussed in this opinion article.

One-pot chemoenzymatic reactions
The ability to combine certain steps of bio- and chemo-catalytic reactions suggests an important role for these processes in the conversion of biomass into valuable chemicals. Unfortunately, the combination of bio- and chemo-catalysis into a ‘one-pot’ process is not straightforward; each chemo-catalyst has an optimum set of conditions, and these conditions are highly variable with respect to solvent, temperature, pH and pressure. Various strategies have been employed to overcome these compatibility problems, as discussed herein, which will serve as important lessons to researchers interested in this field.

Dynamic kinetic resolution
A variety of reactions that employ an enzyme and chemical catalyst into one pot have been reported (Figure 1). Amongst these, dynamic kinetic resolution (DKR) has been the most intensely studied [1–4]. DKR is the conversion of a mixture of stereoisomers (usually a racemic mixture of enantiomers) into a pure, or virtually pure, isomer. An enzyme is employed to generate the desired enantiomer at a much faster rate than the unwanted enantiomer, and this kinetic resolution ensures the purity of the product. From the racemate, the action of the enzyme results in conversion of 50% of the material into the product, which is then separated from the unreacted material. In DKR, an additional (often chemical) catalyst is added to set up a dynamic equilibrium between substrate enantiomers, which ultimately results in a theoretical yield of 100%.

The most common example of DKR is the combined use of Candida antarctica lipase B (CALB) and a hydrogen transfer catalyst to prepare a chiral ester from a racemic mixture of secondary alcohols (Figure 1a) [1]. For example, the combination of a homogeneous precious metal catalyst and CALB has resulted in the multiple-ton-per-year production of a chiral ester in high purity (>99% enantiomeric excess) [5]. Moreover, the concept of DKR has been extended to polymers, thereby enabling the synthesis of chiral polyesters, polystyrene and polycaprolactone [6]. The success of these combined chemo- and bio-catalytic processes is partly a result of the solvent- and temperature-tolerance of the lipase, which enables the chemo-catalytic step to be operated in conventional solvents, such as toluene, and up to temperature of 70 °C with good chemo-catalytic performance.

In our DKR studies [7], metal complexes (e.g. Figure 2a) are designed with a high potential to racemize secondary alcohols in the presence of CALB, immobilized in the commercial product Novozym 435°C (Novozymes, Denmark). Common structural features of these catalysts include a precious metal cation, commonly rhodium, iridium or ruthenium, ligated by a strongly coordinating and highly chelating ligand, such as the pentamethyl cyclopentadienyl anion, which helps to tune the reactivity of the metal and control reactivity, and one or two more labile ligands, such as chlorides, which are substituted by the substrate to initiate the reaction. The activity of the bio- and chemo-catalytic system is highly dependent on the enzyme used; this could be attributed to interaction between the ‘soft’ metal centers of the catalyst and sulfur or aromatic nitrogen donors on the enzyme, and interactions that lead to bond formation are likely to render the catalyst inactive. Highly successful and versatile chemo-catalysts often contain bulky ligands, such as phenyl-substituted cyclopentadienyl ligands (e.g. Shvo’s complex; Figure 2b), and we posit that bulky groups on the chemo-catalyst sterically impede interaction with the enzyme.

Homogeneous catalysts
Homogeneous catalysts can also assist in the recycling of cofactors for NADH-dependent enzymes [8]. In this way, the activity of alcohol dehydrogenase (ADH) can be coupled with other oxidation or reduction reactions. For example,
Figure 1. One-pot chemo-enzymic reactions involving an isolated enzyme and a chemical catalyst. (a) An example of DKR [1]: 1-phenylethanol is converted to (R)-1-phenylethanol acetate using lipase B from CALB coupled to a racemization catalyst. (b) Chemo-catalytic formate oxidation to recycle NAD(P)+ to NAD(P)H and enable ADH-catalyzed ketone reduction [9,10]. (c) Application of chemo-catalyst [Pd(PPh₃)₂Cl₂] to convert an aryl substrate to a biaryl followed by ADH to reduce the ketone substituent into a chiral alcohol [12]. (d) Combination of amination catalyzed by soluble Pd complexes and biocatalytic amidation catalyzed by lipase B [13]. (e) Conversion of D-galactose to 4-deoxy-D-glucose by combined bio- and chemo-catalysis in water [15,17].
ADH reduction activity can be coupled to the oxidation of formate to CO$_2$ (Figure 1b), with NADH recycled via NAD$^+$. The reactions are mediated by a soluble metal complex of similar properties to those used in DKR, but which contain a ligand to help confer water solubility, such as 2,2'-bipyridyl (e.g. Figure 2c). The catalyst consumes the chemical oxidant or reductant and recycles the enzyme cofactor [9,10]. Alternatively, a soluble catalyst can be used as a substitute for NADP$^+$ to facilitate the operation of FAD-dependent monooxygenases [11]. Currently, these recycling methods are not competitive with enzymatic regeneration methods but, as they offer relatively simple and potentially stable methods, further development is worthwhile [8].

Recent reports of reactions that result from the combination of an enzyme and a homogeneous catalyst include the combination of Pd-catalyzed Suzuki C–C bond-forming reactions with ADH activity; all in the aqueous phase (Figure 1c) [12]. Initial tests were not promising and pointed towards compatibility problems between the catalysts. Upon further investigation, one of the major sources of enzyme inhibition has been found to be triphenylphosphine (PPh$_3$), a promoter of the chemo-catalyst, and boronic acid. Surprisingly, the enzyme can operate in the presence of bound PPh$_3$ in the form of the catalyst [Pd(PPh$_3$)$_2$Cl$_2$]. The incompatibility issues have been addressed by ensuring that the phosphine promoter is bound to the metal and by limiting the boronic acid. This is achieved by carrying out the C–C coupling reaction first using [Pd(PPh$_3$)$_2$Cl$_2$], adjusting the pH to 7, and then adding the enzyme to carry out the reaction. Secondary biaryl alcohols have been prepared in water with excellent conversion (up to 91%) and stereoselectivity (>99% enantiomeric excess).

The combination of Pd-catalyzed C–N bond formation and lipase amidation has been used to generate new amide and amine functional groups [13], which are important groups in pharmaceuticals. Immobilized CALB has been used in conjunction with a soluble Pd complex to convert benzyl amines into products in toluene solution (Figure 1d). In the transformation, a new amine group is attached and the primary amine function is converted to an amide. The authors have reported a synergy between the bio- and chemo-catalytic reactions and have suggested that the Pd-catalyzed reaction is promoted by methanol that is generated by biocatalytic amidation. In the same study, one-pot C–C bond-forming reactions were also coupled to amidation [13].

**Heterogeneous catalysts**

The combination of traditional heterogeneous solid catalysts and enzymic bio-catalysis was pioneered by Kieboom and co-workers, as discussed below. Moreover, the combination of hydrogenation activity of a heterogeneous catalyst (Pd on alumina) with DKR by immobilized CALB has been investigated recently, to convert acetophenone into $R$-1-phenylethanol [14].

**Chemo-enzymatic biomass conversion**

Kieboom and co-workers have combined enzymatic and chemo-catalysis to achieve the chemical transformation of carbohydrates [15,16]. Reactions are carried out in
water, and the enzymes employed include glucose isomerases and galactose oxidases. Chemical catalysts are heterogeneous (solid) or homogeneous (soluble) chemo-catalysts, including Pd and Pt metals on carbon supports. In a representative example, D-galactose is converted into 4-deoxy-D-glucose derivatives by an enzyme, an organic chemo-catalyst, and a heterogeneous catalyst in one pot (Figure 1e) [15,17]. The overall transformation, which is carried out in water, is achieved by oxidation, catalyzed by D-galactose oxidase, followed by L-proline-catalyzed dehydration, and finally hydrogenation catalyzed by Pd on carbon. A yield of >95% has been achieved by simple stepwise addition of the catalysts and reagents under suitable conditions.

The work performed by Kieboom et al. is highly relevant to the sustainability of the chemical industry. Currently, the majority of chemicals are derived from petroleum; yet, within a few decades, petroleum will become a limited and increasingly expensive resource [18]. To prevent a rapid increase in the prices of manufactured products, new and sustainable sources of chemicals, such as biomass, must be exploited. One approach to obtaining chemicals and fuels from biomass is the ‘biorefinery’ concept. Biorefineries meet the market demand for several products that start from a biological source. The emphasis is placed on the sustainability and self-reliance of the refinery, and to this end, the energy to drive the process is generated in-house from biomass. The United States Department of Energy has outlined a strategy for coping with the transition from fossil fuels to biomass feeds [19]. In their approach, biomass is converted into several different renewable platform chemicals that can feed into existing supply chains. The main route from biomass to useful chemicals is through sugars, which can be obtained from starch, hemicellulose and cellulose. These sugars can act as precursors to a wide range of building blocks; examples include lysine, a precursor for caprolactam, which is used in the production of nylon 6, and sorbitol, a potential source of glycols, which are useful chemicals in their own right (e.g. as antifreeze) and are precursors of polyurethanes. Using biomass-derived building blocks in this way will help to smooth the transition from fossil fuel to a biomass-driven chemical industry. Several recent reviews have summarized efforts to convert biomass to chemicals [20–22].

In the mid- to long-term, chemical industries driven by the availability and utility of biomass will emerge. In such an environment, the most efficient route from a biorenewable feed to a valuable product should be sought. One-pot reactions, which combine more than one catalytic step into cascade or tandem processes, have great potential in this area. The one-pot approach is both biomimetic, because it mirrors the natural synthesis of chemicals, and highly efficient, because it avoids intermediate separations and greatly reduces the use of solvents, reagents and energy [15]. The most sustainable route to a chemical target could theoretically be constructed by combining the best methods for achieving the individual steps into one pot. In many cases, the most efficient transformations are a mixture of bio- and chemo-catalyzed steps [23].

**Whole-cell bio-catalysis and chemo-catalysis**

The preceding examples illustrate how chemo- and bio-catalytic methods can be used to add considerable value to chemical products; most notably by using an enzyme to control stereoselectivity and a chemical catalyst to control chemo- and regioselectivity. In these studies, the compounds used are of high purity, which fits well into the current paradigm of the chemical industry. With the bio-renewable feedstocks, such as biomass, crude, impure and highly oxidized material must be converted into products. We believe that fermentation is a sensible first step. Microbes (e.g. bacteria, microalgae and microfungi) feed on complex natural materials. In the laboratory, these microbes can be cultured and developed to digest biomass in bioreactors. If the metabolism of the cells is carefully controlled, the microbes can be coerced to produce specific chemicals as the products of fermentation. To convert biomass into chemicals of commercial value, efforts should be directed towards the combination of whole-cell biocatalysis and chemo-catalysis – an approach in which relatively few examples have been reported.

Whole-cell bio-catalysis and solid-state heterogeneous catalysis is a considerable challenge because the chemo-catalyst is free in solution to diffuse to and interact with the bio-catalyst, and the nutrients of the fermentation broth might interact with the chemo-catalyst as well. It has been found that the bacterium *Clostridium butyricum* is highly sensitive to the presence of a soluble Ir catalyst [25]. When a soluble chemical catalyst is toxic to a whole-cell bio-catalyst, a two-step, one-pot process can be considered, in which the fermentation is first carried out to generate the chemical intermediate, and the chemo-catalyst is added in an immiscible solvent. Adoption of a biphasic approach (Figure 4) should reduce poisoning and improve product separation. In addition, this provides the potential to keep the fermentation active, thus enabling different chemo-catalytic phases to be sequentially layered with the same fermentation, but yielding different chemical products.

We investigated the integration of homogeneous chemo-catalytic processes with *C. butyricum* fermentation of a biorenewable, glycerol, formed in the preparation of biodiesel [25]. In our initial study, glycerol was transformed into secondary amines via 1,3-propanediol using the microbial activity, followed by the action of a soluble Ir complex (Figure 2a) dissolved in toluene or a water-immiscible ammonium ionic liquid (Figure 3b). A biphasic aqueous/ionic liquid solvent system has also been used to combine whole-cell bio-catalysis with Pd-catalyzed C–C bond formation [26]. The chemo-catalytic reaction is performed first in an imidazolium ionic liquid; rehydrated *Escherichia*
coli ADH-A cells are then added to effect the highly enantioselective reduction of a ketone group, which yields a chiral secondary alcohol (Figure 3c). The aqueous biocatalytic phase can then be separated and recycled. Moreover, the use of the ionic liquid allows the chemo-catalyst to be reused.

**Compartmentalization: separating the bio- and chemo-catalysts**

One persistent theme in the literature is the challenge of finding compatible reagents, solvents and reaction conditions. Compartmentalization can help minimize poisoning of the catalysts that arises from interactions between the bio- and chemo-catalysts, or their associated reagents. A simple way to do this is to separate bio- and chemo-catalysts into different liquid phases. To this end, ionic liquids have shown promise and have been noted as good solvents for biomass processing [27] and good media for running enzymatic processes [28,29]. Useful properties exhibited by ionic liquids include low volatility and the ability to alter toxicity and biodegradability by changing groups attached to the cation or anion [30–32].

When compatibility problems are too acute, the catalysts can be supported in organic or inorganic solid-state materials to isolate them from each other. Compartmentalization by carriers can enable enzymes and chemical catalysts to operate together [33,34]. In an example of this strategy, an incompatible enzyme (lipase) and chemical catalyst (Rh) have been entrapped in silica gels for enzymatic esterification and Rh-catalyzed hydrogenation in one pot [35]. The extension of this sol–gel technique to include ionic-liquid-mediated ‘ionogel’-forming reactions opens up the possibility that the materials could be imparted with different characteristics (e.g. hydrophobicity). This could enable the gels to be easily separated from each other and to be used independently [36].

The issue of compatibility between the bio- and chemo-catalyst might be addressed more directly by rational design. The concept of chemo-catalyst design is mature, and catalysts can be chosen to maximize biocompatibility, as observed for DKR. With the advent of directed evolution, engineering of optimized biocatalysts has also become a possibility [37], and tolerance to chemical reagents can be used as a design criterion [38].
Concluding remarks
One-pot processes that combine bio- and chemo-catalysis provide attractive routes to valuable chemical products. Examples of one-pot bio- and chemo-catalytic reactions contain many approaches to overcome problems in compatibility. Some combinations are relatively simple to achieve and only involve the avoidance of certain reagents [2,7], but others are more challenging. In these cases, researchers can adopt various strategies, including the two-step introduction of the bio- and chemo-catalysts under controlled conditions [12,25], biphasic solvents [25,26], or the use of solid materials to entrap the catalysts [35,36]. The application of these technologies opens up new possible combinations. We believe that one-pot bio- and chemo-catalytic processes have an important role to play in future technologies, and point particularly at the potential of whole-cell bio- and chemo-catalysis to convert biomass directly from crude bio-renewable feeds into chemical products, with no intermediate separation [25]. Combined bio- and chemo-catalysis has proven successful in chemical synthesis, and now has an important role to play in building a more sustainable chemical industry.

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References