Design of biocatalysts for efficient catalytic processes

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Highlights

- Important recent trends in the biocatalyst design have been identified.
- Functionalized porous materials with large pores are promising biocatalyst supports.
- Magnetically recoverable biocatalysts are in forefront of biocatalyst design.
- Multienzyme biocatalysts are for sustainable and efficient cascade reactions.
- Biohybrid catalysts combining enzyme(s) and nanoparticles allow for cascade reactions.

ABSTRACT

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Biocatalysts based on immobilized enzymes received considerable attention due to important applications in syntheses of value-added chemicals, pharmaceuticals and drug intermediates with great catalytic efficiency and high yields of target molecules. The important advantages of such biocatalysts are enhanced stability in tolerant pH and temperature range, separation from reaction solutions, stability in repeated use, etc. In this review, we discuss recent findings in biocatalyst design, in particular, types of promising supports, the biocatalyst surface modification, and incorporation of magnetic nanoparticles for facilitated magnetic recovery. Furthermore, we highlight the development of multienzyme and enzyme/nanoparticle catalysts for cascade reactions, which are carried out in a one-pot process and allow elimination of isolation and purification of intermediates.

Introduction

Biocatalysts, i.e., catalysts based on native enzymes, received considerable attention because of their applications in numerous biochemical and chemical reactions including syntheses of pharmaceuticals and drug intermediates. The major advantages of native enzymes are (i) high chemo-, enantio-, and regioselectivity, (ii) mild reaction conditions with regard to temperature, pressure, and pH, and (iii) low energy consumption [1]. On the other hand, enzymes are quite expensive and can be used only once because of non-feasible separation. In addition, enzymes can partially denature in aqueous solutions which leads to a lower activity. These deficiencies are eliminated or at least diminished for immobilized enzymes [2]. The benefits of immobilized enzymes are the increase of the catalyst stability, control of the reaction rate, possibility of the catalyst separation from reaction products and further reuse, option of stopping the reaction at a desired time, modulation of catalytic properties by a proper modification (varying pH, temperature, ionic strength, etc.), and the prevention of the reaction product contamination which is crucial for pharmaceutical and food industries [2,3]. All these factors lead to the decrease of the process cost and to the improvement of the quality of target products [4].

Enzyme immobilization has been carried out using numerous approaches such as adsorption on solid supports [5], a covalent attachment via functional moieties [6], entrapment of cross-linked enzyme aggregates in 3D ordered macroporous silica [7], encapsulation inside MOFs [8], etc. The immobilization method can improve the pH and temperature range of the enzymatic activity, thus increasing the reaction rate and the product yield. Among all immobilization methods, the direct adsorption has shown least promise as it is normally accompanied by enzyme denaturing, unless the support has specially designed textural properties or in the case of nanoparticles (NPs) due to NP curvature [9]. A covalent attachment of enzymes to functional supports allows easy variations of an immobilization density and a linker length which could strongly affect the enzyme properties [10]. Among functional supports organic and inorganic solids have been explored such as polymers [11], NPs [12], various scaffolds including proteins and microbes [13], porous oxides [14], etc. Currently, the research in biocatalysis is mainly focused on evaluating the influence of (i) the nature of the support, modifier and crosslinking agent; (ii) the introduction of two or more enzymes; (iii) conditions of the enzyme immobilization on the catalytic process.

Considering the fast development of the biocatalyst field, in this paper we will discuss accomplishments in the biocatalyst design for 2015-2019. We will focus on most promising supports, methods of enzyme attachment as well as on multienzyme biocatalysts and biohybrid catalysts for cascade reactions. The cascade reaction catalysts represent an emerging technology inspired by Nature where the spatial localization allows for the high chemo-, regio- and stereoselectivity [15].

Enzyme immobilization on porous supports

Porous supports including oxides and polymers have been employed for enzyme immobilization for many years. In recent times, the research focus has been placed on hierarchical structures [16,17], dependencies on pore sizes [18], and the use of magnetic porous oxides for magnetic recovery [19]. The presence of pores or cavities where enzymes could locate to create an optimal degree of crowding achieved in cells is not a prerequisite, but it could significantly enhance catalytic activity of biocatalysts [20].

Bolivar et al. reported a new approach where luminophor-doped oxygen-sensing moieties in silica were combined with an integrated approach of enzyme immobilization to create an oxygen-dependent biocatalyst [21]. Zbasic2, positively charged mini-protein (7 kDa), was attached to D-amino acid oxidase (79 kDa), after which the resultant chimeric protein was connected to silica in a certain orientation. Enzyme immobilization on mesoporous silica described in this work resulted in more than 10-fold increase of enzyme effectiveness at high loading compared to porous glass and was almost as effective as the native enzyme. This was attributed to the selection of supports which intensify O_2 mass transport, the importance of which was established using an internal oxygen sensing capability, allowing further biocatalyst optimization. We believe this is a seminal work, which initiated a series of follow-up papers where mono- and bienzymes immobilized on porous supports have been reported [22,23].

The important recent advance in biocatalysts based on porous supports is the development of hierarchical porous materials such as MOFs [24], polymers [25], agarose hydrogels [26], and inorganic oxides [16,17] containing sufficiently large pores (at least ~25 nm) which could accommodate enzymes without restrictions and denaturation. The large surface area is also beneficial to increase the enzyme loading at the lowest catalyst volume. Among hierarchical materials, alumino-siloxane aerogels (ALS-PG) with unusual structural features were obtained via templating with pollen grains of Hibiscus rosa-sinensis [16]. The distinctive shape of the Hibiscus rosa-sinensis pollen as a sacrificial template (Figure 1) allows for the development of both macro- and mesoscale pores, as well as funnel-shaped macrochannels within the mesoporous framework.

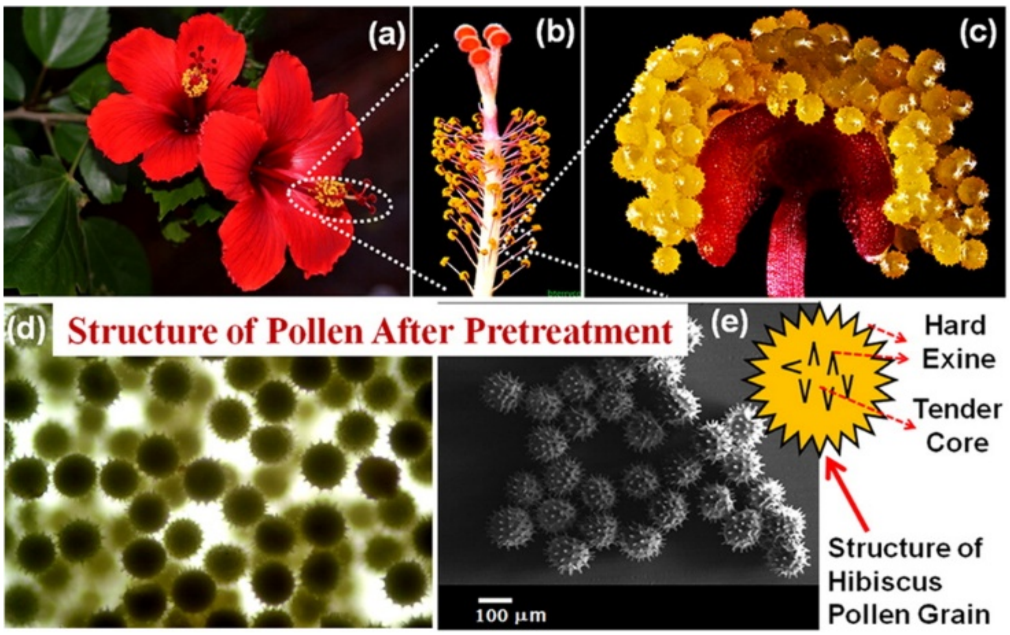

Figure 1. (a–c) Photograph of hibiscus flower (Hibiscus rosa-sinensis) and its pollen grain (d) optical and (e) SEM images of freeze-dried pollen grain used for the experiment obtained after pretreatment process. Reprinted from ref. [16]. Copyright (2016) American Chemical Society.

Hierarchical ALS-PG allowed high loading of steapsin lipase and excellent immobilization efficiency. The biocatalyst was further improved by functionalization with methyl and amino propyl groups and demonstrated enhanced catalytic performance in hydrolysis, esterification, and transesterification.

An interesting approach has been utilized for modification of biocatalysts based on porous supports. Such a modification was carried out via PEGylation which significantly increases stability of biocatalysts [27]. The authors assumed that PEG creates a viscous medium around enzymes on the surface, thus preventing the enzyme structure distortion due to heat or denaturing agents. An alternative pathway, where porous silica was functionalized with phenyl groups, i.e., made more hydrophobic, was shown to result in the optimal combination of activity, thermal stability and immobilization efficiency of Thermomyces lanuginosus lipase, in the case of low density of phenyl groups [28].

Magnetically recoverable biocatalysts

For the last five years, there was an explosion of interest in magnetically recoverable catalysts including biocatalysts. This can be attributed to easy magnetic recovery of such biocatalysts for a repeated use, which, in turn leads to energy savings and cheaper target products. Magnetic properties are achieved due to magnetic NPs, most frequently iron oxide or ferrites, imbedded in the biocatalyst support. The major designs of magnetically recoverable biocatalysts include functionalized magnetic NPs, magnetic NPs or NP clusters coated with polymers [29,30], silica [5], imbedded in the pores of mesoporous oxides [31], etc. Magnetic microreactors with enzymes immobilized on magnetic beads for continuous flow processing on microscale have also been reported and represent a promising alternative to conventional reactor designs [32].

In the majority of cases, the iron oxide surface is isolated from an enzyme by a polymer or a silica shell, thus a magnetic NP role is limited to enabling easy separation. However, if enzymes are attached in the vicinity of iron oxide NPs, the enzyme catalytic activity can be enhanced as was recently demonstrated for glucose oxidase attached to mesoporous silica and alumina containing magnetic NPs [31]. This was attributed to enzyme-like properties of iron oxide.

A combination of a one-step purification and an attachment of extracellularly expressed enzymes to a magnetic support has been reported by several groups [30,33]. This constitutes an important advance in a biocatalyst design making it less expensive and more sustainable. As an example, extracellular His-tagged Thermomyces lanuginosus lipase (His-TLL) was immobilized on core/shell iron oxide NPs containing long-armed nickel-nitrilotriacetic acid surface groups (Ni-NTA-MNPs), using the cell culture supernatant of Pichia pastoris (h-TLL) (Figure 2) [30]. This allowed high loading of the enzyme and its specific activity in the one-pot conversion of waste grease to biodiesel with a remarkable 94% yield and exceptional recyclability.

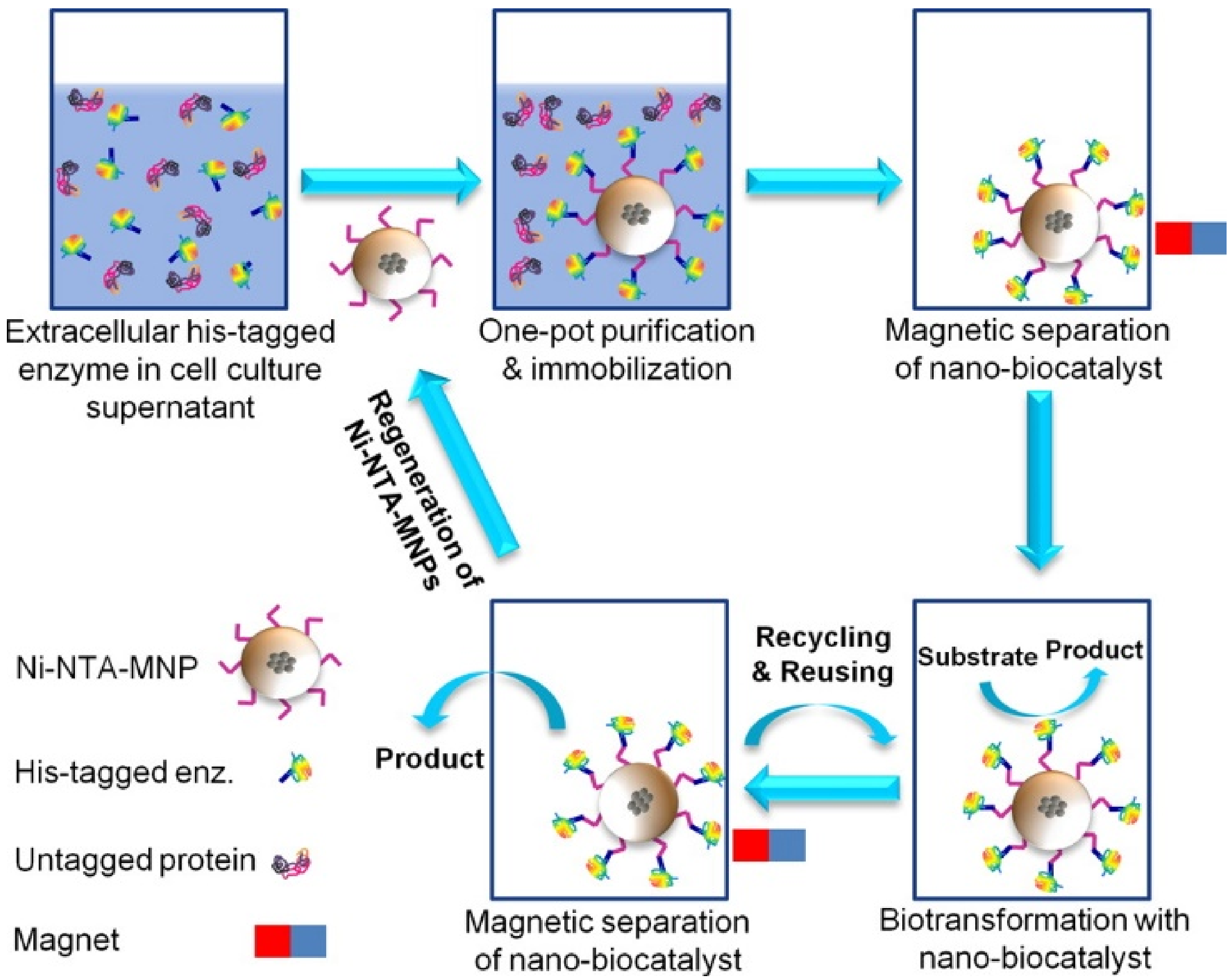

Figure 2. Immobilization of His-tagged extracellular enzyme directly from cell culture supernatant on magnetic nanoparticles (Ni-NTA-MNPs), biotransformation with and recycling of the biocatalyst, and regeneration of Ni-NTA-MNPs from the used catalyst. Reprinted from ref. [30]. Copyright (2016) American Chemical Society.

Multienzyme biocatalysts for cascade processes

Cascade reactions existing in Nature, where different enzymes are located in vicinity of each other, inspire researchers to implement a similar approach for the design of diverse biocatalysts, combining multiple enzymes on the same support [34,35]. The major advantages of multienzyme biocatalysts are robustness of one-pot syntheses as well as elimination of the steps of isolation and purification of intermediates. In turn, cascade reactions are highly promising for sustainable processes to obtain environmentally sound chemicals and materials without hazardous side products. The important parameters to follow in multienzyme catalysts are the enzyme ratio, their distribution on the support surface as well as enzyme access for substrates [36].

Zore et al. suggested to employ a polymer, poly(acrylic acid), to covalently conjugate glucose oxidase (GOx) and horseradish peroxidase (HRP) followed by adsorption on graphene oxide [37]. Pitzalis et.al. proposed covalent immobilization of GOx and HRP on amino functionalized mesoporous SBA-15 [38], allowing for increased thermal stability and recyclability in detoxification of agricultural wastewaters, which contain both glucose and recalcitrant phenolic compounds. Two- or three-step cascade reactions were carried out with multienzyme films formed by self-assembly of protein-polymer surfactants containing GOx and HRP, or β-glucosidase [39]. Despite some loss of catalytic activity compared to native enzymes, the easy manipulation of biocatalyst films as well as an ON-OFF function of chemical reactions by easy changing the film location, make these multienzyme biocatalysts promising as controllable multifunctional biomaterials.

Inverse opal hydrogel particles have been used as support for immobilization of HRP and urease [40]. The particle assembly created a perfect ordered porous structure for enzyme immobilization, allowing easy penetration of substrates. Due to ordering this support possesses unique photonic band gaps, resulting in bright colors for encoding of immobilized enzymes. The encoded supports with different immobilized enzymes could be mixed in various manner to carry out cascade reactions. Despite these structural advances, the activity of this biocatalyst did not exceed 50% of the activity of the native enzymes, indicating that there is a room for improvement.

Crosslinking of GOx and HRP with a DNA scaffold via self-assembly and encapsulating this construct into MOFs based on zeolite imidazolate framework-8 (ZIF-8) (Figure 3) allowed a decrease of the biocatalyst enzyme loss by a factor of ten compared to conventional encapsulation into MOFs [41]. This biocatalyst first performed glucose oxidation with the formation of H_2O_2 followed by oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)$^{2-}$ ($ABTS^{2-}$) by HRP to form $ABTS^{-}$. It showed excellent catalytic efficiency, stability, and reusability due to encapsulation in the DNA scaffold.

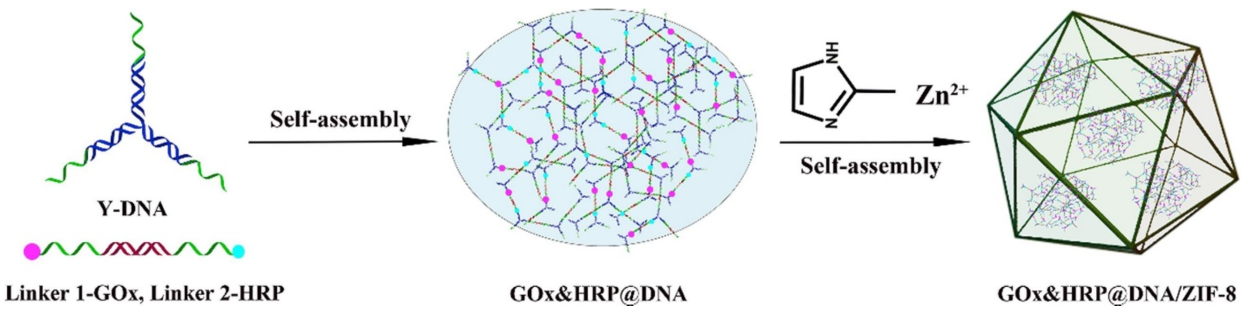

Figure 3. Schematic for cross-linking multiple enzymes network by Y-scaffold DNA and entrapment in zeolitic imidazolate framework. Reproduced from Ref. [41] with permission from Elsevier (2019).

There are a few examples where magnetic supports have been employed for cascade reactions. Magnetic bienzyme biocatalyst was synthesized by the covalent attachment of α-amylase and glucoamylase to Fe_3O_4/SiO_2 core-shell NPs to carry out a one-pot starch hydrolysis [42]. It is noteworthy, that a significant increase of the biocatalyst activity was achieved when a shielding film formed by a coordination of Fe^{3+} ions with multifunctional tannic acid was employed to protect enzymes from denaturation and detachment. With a similar magnetic support but using DNA modification before the attachment of GOx and HRP to obtain enzyme-DNA conjugates

[43], the authors were able to significantly enhance catalytic properties compared with mixtures of free enzymes and the biocatalysts formed via non-specific adsorption on the support.

An interesting example of the bienzyme biocatalyst support was reported by Rezaei et. al. [44]. GOx and glucoamylase (GA) have been covalently attached to amino functionalized Ag dendrites (prepared in the presence of Ni Raney catalyst), creating a large surface area for enzyme immobilization (Figure 4). The catalyst was tested in a cascade transformation of starch to gluconic acid and showed better activity and stability over a wide pH and temperature range compared to native enzymes. Surprisingly, however, despite Ag could also play a catalytic role in conjunction with enzymes, the discussion of the Ag dendrite influence on catalysis is absent in ref. [44] as well as the comparison with non-metallic supports.

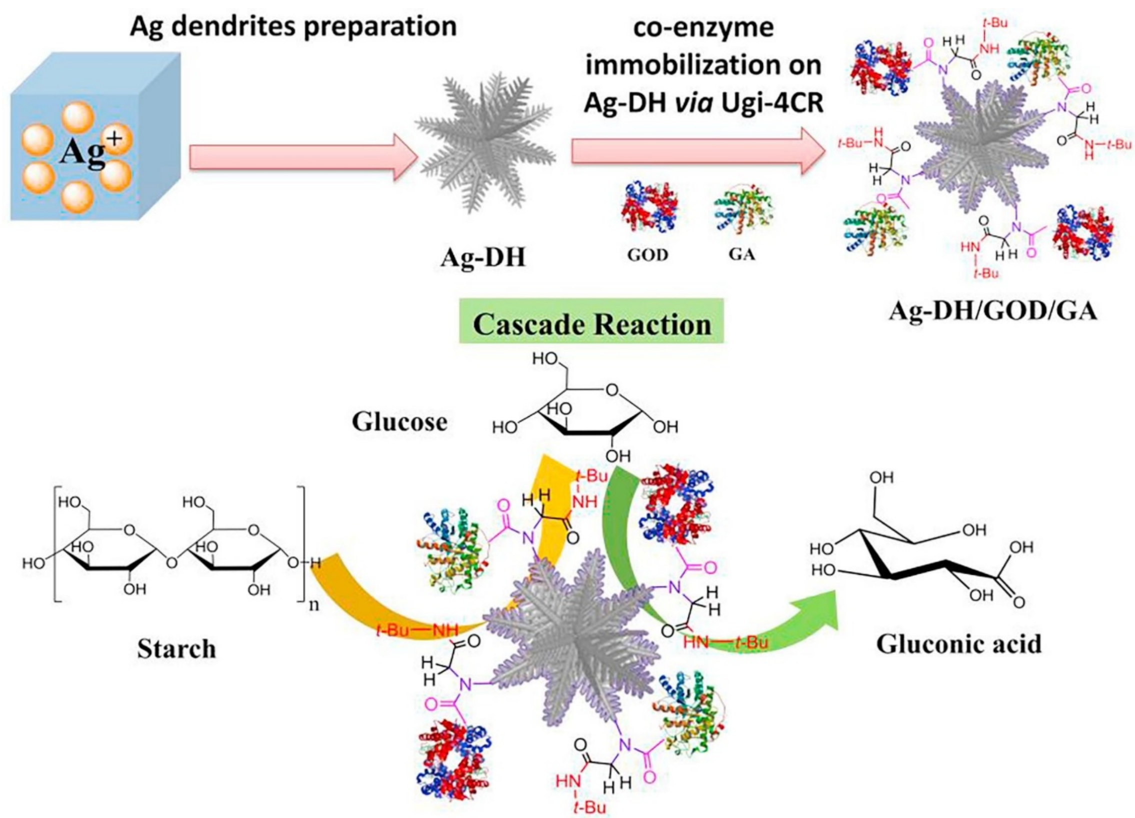

Figure 4. Schematic illustration of the preparation of double enzyme microsystem (Ag–DH/GOD/GA) for the one-pot biotransformation of starch to gluconic acid. GOD stands here for GOx, Ag-DH stands for hierarchical dendrites, and Ugi-4CR stands for the Ugi four-component reaction. Reproduced from Ref. [44] with permission from Elsevier (2019).

Combination of enzyme(s) and metal NPs for cascade reactions

In addition to cascade reactions performed with multienzyme biocatalysts, enzymes can be combined with catalytic NPs to carry one-pot reactions which otherwise would be impossible or ineffective. In ref. [45] crosslinking of aggregated lipase B enzyme of Candida antarctica (CalB) created an active support for stabilization of Pd NPs. The hybrid catalyst showed good catalytic activity in a one-pot reaction, where first Pd NPs catalyzed cycloisomerization of 4-pentynoic acid to produce a lactone that was utilized as an acyl donor to obtain enantiomerically pure 1-phenylethyl 4-oxopentaoate derivatives. The catalyst was easy to prepare and showed good stability in five consecutive experiments.

Wang et al. reported immobilization of Pd NPs and CalB on carbon nitride (C_3N_4) as biohybrid catalyst for the one-pot cascade transformation of benzaldehyde to benzyl hexanoate [46]. In this reaction, benzaldehyde is first reduced to benzyl alcohol with hydrogen in the presence of Pd NPs followed by the reaction with ethyl hexanoate to form benzyl hexanoate (target product). The latter transformation is catalyzed by CalB. To prevent the enzyme denaturation during the catalyst synthesis, Pd NPs were formed in C_3N_4 first (using impregnation and reduction) followed by the attachment of CalB using glutaraldehyde. The optimized biohybrid catalyst containing 0.5% Pd and 2% CalB showed the best catalytic activity with the highest yield of benzyl hexanoate of 81.7%. When mesoporous silica NPs (MSN) were utilized as support, Pd NPs were formed in the pores, followed by surface modification with long-chain alkanes (using trimethoxy(octadecyl)silane) and immobilization of CalB [47]. The resultant biohybrid catalyst afforded almost 95% yield of benzyl hexanoate. The authors emphasized the importance of loading of Pd NPs and CalB into different locations of the mesoporous structure to avoid mutual deactivation. A modification with hydrophobic tails allowed a variation of catalyst hydrophobicity to control dispersibility.

In continuation of this approach, the same group used hard scaffold MOFs to immobilize Pd NPs and CalB [48]. In this case, the authors adjusted biohybrid hydrophobicity using a ligand exchange with lauric acid, whose amounts (20 and 50 mM) dramatically influenced the biohybrid catalyst dispersibility and catalytic properties. For this catalyst, the yield of benzyl hexanoate was 100%. Apparently, tailor-made hydrophobicity and a rigid scaffold for NP and enzyme immobilization determine the excellent catalytic properties of the biohybrid catalyst.

An innovative photoswitchable biocatalyst for cascade reactions was fabricated using tyrosinase (TYR) which catalyzes the generation of dihydroxyphenylalanine (DOPA) attached to TiO_2 NPs to create a light responsive trigger that can activate the HRP activity using light [49]. The pioneering feature of this work is the TiO_2/DOPA trigger formed by *in situ* TYR catalysis, which, in turn, leads to the combination of TYR and HRP for the development of the novel and efficient cascade biocatalyst.

Conclusions

In this review, we identified the most important recent trends in the biocatalyst design to achieve advanced catalytic properties in syntheses of value-added chemicals, pharmaceuticals, and intermediates. Among supports for biocatalysts, functionalized porous materials with a hierarchical pore structure containing large mesopores or macropores appear promising as they possess high surface areas and can accommodate large amounts of enzymes in a controlled manner. However, for better performance, often an additional modification is needed for protecting enzymes or making a biocatalyst more hydrophilic or hydrophobic. Magnetically recoverable biocatalysts are clearly a hot topic as they allow easy magnetic separation without loss of catalytic performance and in some instances, show the catalytic property enhancement.

The most prominent trend in the biocatalyst design is the development of catalysts for cascade reactions using multiple enzymes or combining enzymes with catalytic NPs. Both areas of research are still very new but they already showed promise for the development of biocatalytic processes, where several reactions occur in a one-pot reactor. The best biocatalysts display exceptional catalytic efficiency and a minimal loss of activity compared to native enzymes and require low energy consumption. For the cascade reaction catalysts, there is no need for isolation of intermediates, no hazardous chemicals are formed, and the waste is minimized compared to conventional consecutive reactions, resulting in the same target products. We believe this is the most promising direction for the development of biocatalysis.

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Conflict of Interests

The authors declare no conflict of interests.

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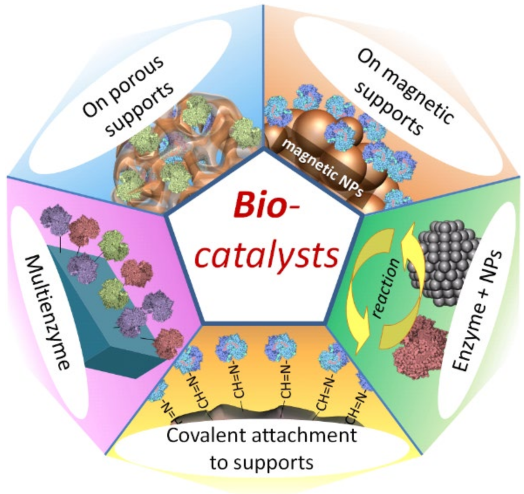
Graphical abstract figure


On porous supports
On magnetic supports
magnetic NPs
Bio-catalysts
Multienzyme
reaction
Enzyme + NPs
CH=N-
Covalent attachment to supports