Biocatalysis refers to the utilization of enzymes, either in purified form, or existed as part of crude cell lysate or intact cells, to catalyze single- or multi-step chemical reactions, converting synthetic molecules or natural metabolites into high-value products. In recent decades, with the abundant information of genome sequences and better understanding of metabolic pathways, the enzymes involved in biochemical reactions can be modified using genetic techniques to improve the yield of products, modify the substrate specificity and manipulating the metabolic networks to satisfy specific industrial needs [1].

The Special Issue Recent Advances in Biocatalysis and Metabolic Engineering presents twelve original research and review articles involving triterpenoid synthesis and catalysis, β-glucan and lignin hydrolysis, nanomaterials, enzyme immobilization and fluorescence-based characterization, in vitro peptide/protein phosphorylation, and enzymatic dye decoloring, along with novel practices relating to enzymes using synthetic biology and metabolic engineering. The published articles demonstrate experimental and simulated data which can be applied in pharmaceuticals, diagnosis, functional foods, materials and biofuel production.

Triterpenoids from several natural sources are of interest due to their various biological activities for medicinal purposes [2,3]. However, most triterpenoids are hydrophobic, which limits their practical applications. Glycosylation and hydroxylation reaction are the common approaches to increase the bioactivity and the aqueous solubility of these triterpenoid compounds [4]. To facilitate the development of new nutraceuticals and pharmacological formulations of triterpenoids, Chang et al. [5] attempted to apply an enzymatic synthesis to produce a novel Ganoderma triterpenoid saponin by glycosylation of ganoderic acids via a cascade bi-enzymatic synthesis of BtGT_16345, and Toruzyme®. The glycosylation synthetic strategy can be employed on other small molecules to produce novel bioactivity compounds [6].

Oleanolic acid (OA), a triterpenoid found in plants and foods, exhibits beneficial bioactivities for humans [7]. Cytochrome P450 enzymes are a large enzyme family that play important roles in the metabolism of drugs, carcinogens, and steroids [8]. To efficiently synthesize a hydroxylated OA derivative, Cao et al. [9] screened a set of cytochrome enzymes, and found recombinant CYP3A4 capable of regioselectively hydroxylating the methyl group of OA at the C-23 position to synthesize 4-epi-hederagenin.

Enzymes could be applied in various fields such as food processing, probiotics production, paper processing, and biofuel production [10]. To apply cellulases for substrate hydrolysis is a common practice in food processing [11]. β-glucanases can be produced via the fermentation of cellulolytic fungi [12]. However, enzyme production by fungi cultivation is complicated and time-consuming, limiting its application in industry. Zhong et al. [13] attempted to construct a recombinant β-glucanase extracellularly with E. coli host via a kil-km secretion cassette and overexpress it on a bioreactor scale. Factors affecting fed-batch cultivations including various feeding strategies and nutritional supplements were conducted to yield higher biomass and β-glucanase activity. These fed-batch strategies for
enzyme production from *E. coli* can be used as a reference to facilitate the production of other bioproducts.

Immobilization of enzymes shows superior effects on, for example, enzyme stability in reaction, enzyme reusability, and bioproducts separation [14]. Various methods can be employed for enzyme immobilization [15]. For preparing a recyclable biocatalyst system for the synthesis of L-theanine, Chi et al. [16] proposed a direct way to cross-link aggregated γ-glutamyl transpeptidase (GGT) onto a magnetic nanoparticle. The targeted recombinant GGT was derived from *Bacillus licheniformis* with the help of site-specific mutagenesis techniques (N450D) to increase the transpeptidation/hydrolysis ratio, which could facilitate the biosynthesis to L-theanine in an efficient way.

Immobilization can also be carried out with membranes as a supporting material [17]. Wong et al. [18] applied a modified regenerated cellulose membrane to couple metal ions to construct an immobilized metal ion affinity membrane/Co$^{2+}$ (IMAM-Co$^{2+}$). This system was utilized to immobilize the recombinant scaffolding protein (CipA) and xylanase derived from a *Clostridium thermocellum* cellulosome [19]. The co-immobilization was performed via applying an IMAM-Co$^{2+}$ membrane to adsorb CipA first, followed by adsorbing xylanase in series. The two-step approach enhanced xylanase activity, heat and pH resistance, and reusability.

Graphene quantum dots (GQDs), a nanostructured material with high surface area and surface energy, have attract great interest in catalytic reactions due to their special physiochemical characteristics [20]. Glucose oxidation reaction (GOR) is a very common redox reaction performed by many electrochemical conversion devices [21]. Gu et al. [22] attempted to perform the GOR with synthesized boron–nitrogen-codoped GQDs as the electrode. The stability and catalytic activity of the GQD electrodes was analyzed in the GOR. In this way, an inexpensive and high-performance catalyst for the GOR was constructed.

Fluorescence techniques have been employed to study enzyme kinetics and identify human diseases [23,24]. Two articles in the Special Issue focus on the modeling of enzyme activity and detection of enzymes as a diagnostic tool using fluorescence-based approaches. Vorob’ev published an enzymatic modeling study of trypsin taking the unfolding of protein substrate, termed demasking, into account [25]. In the study, the intrinsic fluorophore tryptophan was employed to monitor the demasking rate of substrate β-Lactoglobulin (β-LG), by which hydrolysis of peptide bonds is remodeled. In the process of demasking, the intrinsic tryptophan is exposed to the aqueous solvent resulting in a red shift of wavelength of maximum fluorescence (Amax). Gong et al. demonstrated a diagnostic tool for butyrylcholinesterase (BChE), which is a key enzyme involved in diabetes, cardiovascular disease, cancer and chronic liver disorders [26]. The assay relies on the detection of an enzymatic production using fluorescence resonance energy transfer (FRET). The chemical probe, termed 11, is synthesized using exo-6, a derivative of 5-(2-aminoethylamino)-1-naphthalenesulfonic acid (EDANS), and 4-[4-(dimethylamino)phenylazo]benzoic acid (DABCYL) with a disulfide link, causing the FRET effect while the probe is intact. The presence of BChE cleaves the probe and gives rise to the fluorescence signal of EDANS. The assay provides a specific detection of BChE superior to Ellman’s colorimetric detection, which is interfered easily with by glutathione (GSH), a thiol group containing biomolecule abundant in human plasma.

In this Special Issue, we included four comprehensive reviews on the aspects of enzymatic catalysis and practices of metabolic engineering. Slovakova and Bilкова [27] outlined the current achievements for the manufacturing phosphorylated and multiphosphorylated peptides and proteins of synthetic or recombinant origin. Enzymatic methods used for in vitro phosphorylation of peptides and recombinant proteins are proposed. The availability of various kinases with different activity, specificity, and stability make it feasible to manipulate and modulate the phosphorylation reaction in vitro. The phosphorylation performed by immobilized kinases with all the advantages of immobilization is highlighted. This review also discussed the kinase-related phosphorylation pathways, which
are essential for understanding the pathogenesis of ailments such as Alzheimer’s disease, inflammation, and bacterial infection. The bulk production of phosphorylated recombinant proteins and peptides can be exploited for diagnostic and therapeutic applications.

Molina et al. [28] reviewed the recent advances in enzyme metal–organic framework (MOF) encapsulation by one-pot, in situ approaches in mild conditions. The advantages of using MOFs to entrap enzymes within their micropores, including minimal enzyme leaching, high enzyme loading, and activity preservation, are exploited by one-pot synthesis of the biocatalysts. In addition, the methodology entails key sustainability issues: quick synthesis, preparation at room temperature with water as the sole solvent and moderate pHs, cheap and non-toxic metals and linkers, etc. However, few enzymes immobilized on MOFs can be prepared in situ because of the lability of the enzymes and the harsh conditions of most MOFs synthesis. Although MOF materials such as zeolitic imidazole frameworks are widely reported [29], this review emphasized other less common ones such as Fe-BTC, NH2-MIL-53(Al), HKUST-1(Cu), and Mg-MOF-74 which are traditionally prepared under harsh conditions incompatible with enzymatic activity. Therefore, the information on the preparation of these composites using mild synthesis conditions, as well as their performance in trapping active enzymes are crucial for one-step enzyme immobilization applications using MOFs.

Although lignocellulosic biomass is the most abundantly available source of raw material on the planet, the conversion of biomass to biofuels is often hindered by the notorious recalcitrance of this material. Substrate hydrolysis into fermentable sugars is a rate-limiting step in the whole biofuels production process, due to the lack of effective and cost-effective cellulase enzymes. In this special issue, Ha-Tran et al. [30] discussed the *Clostridium thermocellum* cellulosome, which is a robust cellulase nano-machinery that contains over 70 enzymes on its scaffolding protein CipA. It is a promising biological material utilizing biomimetic approaches to construct artificial designer cellulosomes and cellulase cocktails. In addition, the hyper-modular property of the *C. thermocellum* cellulosome is of great interest from a mechanistic viewpoint and its potential for industrial applications. For cellulase assembly and surface display, novel protein pairs have been detected as an alternative approach to the conventional *C. thermocellum* cohesion-dockerin system. Due to the slow growth and strictly anaerobic culture condition of *C. thermocellum*, the low yield of cellulosome triggers the development of conversion of non-cellulosolytic biofuel microbes into consolidated bioprocessing microbes using metabolic engineering strategies. Successful cases have been demonstrated in *Bacillus subtilis, Saccharomyces cerevisiae, Pichia pastoris,* and *Kluyveromyces marxianus*. These crucial issues were updated and discussed in this review.

In a review, Xu et al. [31] summarized the structure and catalytic characteristics of dye decoloring peroxidases (DyPs) from different heme peroxidase families on the basis of amino acid sequence, protein structure, and enzymatic properties. The catalytic ability of dye decoloring and lignin degradation varies greatly among DyP classes. Furthermore, potential applications of DyPs in paper manufacturing, environmental protection, biomedicine, and biofuel are also discussed. In the past decades, novel research strategies based on genetic engineering and synthetic biology in optimizing the yield, stability, and catalytic activity of DyPs, along with industrial applications have been developed. Nevertheless, according to the current research findings, improvements in catalytic efficiency, production level, and alkali resistance remain necessary to bring DyPs to the industrial level.

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