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REVIEW ARTICLE

Nano-Biocatalysts: Potential Biotechnological Applications

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Abstract Biocatalysts are a biomolecule of interest for various biotechnological applications. Non-reusability and poor stability of especially enzymes has always limited their applications in large-scale processing units. Nanotechnology paves a way by conjugating the biocatalysts on different matrices. It predominantly enables nanomaterials to overcome the limited efficacy of conventional biocatalysts. Nanomaterial conjugated nanobiocatalyst have enhanced catalytic properties, selectivity, and stability. Nanotechnology extended the flexibility to engineer biocatalysts for various innovative and predictive catalyses. So developed nanobiocatalyst harbors remarkable properties and has potential applications in diverse biotechnological sectors. This article summaries various developments made in the area of nanobiocatalyst towards their applications in biotechnological industries. Novel nanobiocatalyst engineering is an area of critical importance for harnessing the biotechnological potential.

Keywords Nanobiocatalyst · Nanobiotechnology · Nanocatalysts · Nanotechnology · Nanomaterial's · Biocatalysts · Immobilization

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Introduction

Biocatalysts especially enzymes are the essential component for cellular functioning. They have been isolated from all possible life forms and characterized for their biocatalytic properties. The biocatlysis trasfiormation has been widely reported by either whole-cell or cell-free system [1–4]. Enzymes as a cell-free system catalyze specific reactions in ambient environmental conditions without influencing product quality. Their particular activity, specificity, and selectivity make them promising biocatalysts for numerous industrial [5–8], environmental [9, 10], and diagnostic applications [11, 12]. Though usage of enzymes in industrial application promotes green chemistry, however, their poor stability and non-reusability enhance their operational cost in large-scale industrial processes. Immobilization of biocatalysts, including enzymes on the solid support, was observed as a technical solution to resolve these issues [13–15]. Significant efforts were made to immobilize enzymes on insoluble supports to enhance their stability, as well as reusability. Engineering of the immobilization supports and optimization of immobilization chemistry paved the way towards their recovery and reusability in large-scale industrial applications [16, 17]. The essential efforts made in the line of development were engineering of novel immobilization supports, optimal immobilization chemistry, and direct immobilization of the whole cell as the enzyme source (Table 1). Despite initial success, none of the technologies were able to answer all the issues. At the same time, nanotechnology has emerged as a promising material science technology.

Nanobiocatalysts were engineered for desirable properties towards their applications in the field of energy, synthesis, diagnostics, therapeutics, environmental

Table 1 Immobilized nanobiocatalysts for biotechnological application

Enzyme	Supports	Immobilization method	Application	References
*Hj*LAD and SpNox	Cu metal	Encapsulation	L-xylulose production	[18]
Cholesterol oxidase	TiO_2–MWCNT@Inulin nanocomposite	Encapsulation	Determination of cholesterol in spiked blood serum and milk samples	[19]
Alcohol dehydrogenase	Carboxymethyl dextran-coated magnetic nanoparticles	Covalent	Determination of ethanol	[20]
Glucose dehydrogenase	Silica support (MM-SBA-15)	Encapsulation	Gluconic acid production	[21]
(*S*)-Mandelate dehydrogenase (SMDH) and laccase	Chitosan	Covalent	Stereoselective biotransformation of racemic mandelic acid	[22]
Laccase	rGO–Fe_3O_4	Adsorption	Oxidation of phenolic compound	[23]
	Cu and Zn-metals	Encapsulation	Degradation of bisphenol A	[24]
	Cu metal	Encapsulation and cross–linking	Decolorization of synthetic dyes	[25]
	Fe_2O_3 yolk-shell	Covalent	2,6-Dimethoxyphenol biosensor	[26]
	Fe_2O_3 yolk–shell	Covalent and cross-linking	Decolorization of synthetic dyes and degradation of bisphenol A	[13]
	Fe_3O_4	Covalent	Degradation of bisphenol A	[27]
	Fe_3O_4–MWCNTs@SiO_2	Covalent	Decolorization of azo dyes	[28]
Lipase	Magnetic rice straw	Adsorption and cross-linking	Fatty esters production	[29]
	Fe_3O_4 nanoparticles coated with 3-aminopropyltriethoxysilane	Covalent	Fatty acid ethyl ester production	[30]
Cellulosic enzymes	Fe_3O_4	Covalent	Ethanol production	[31]
α-Amylase, pectinase and cellulase	Fe_3O_4 magnetic nanoparticles	Cross linking	Juice clarification	[32]

management, agriculture, medicines, food processing, etc. (Fig. 1). Cumulatively, the usage of nanotechnology has expressed the true potential of nanobiocatalyst in biotechnological applications [33]. The current review enlists various biotechnological applications of the nanobiocatalyst for human welfare.

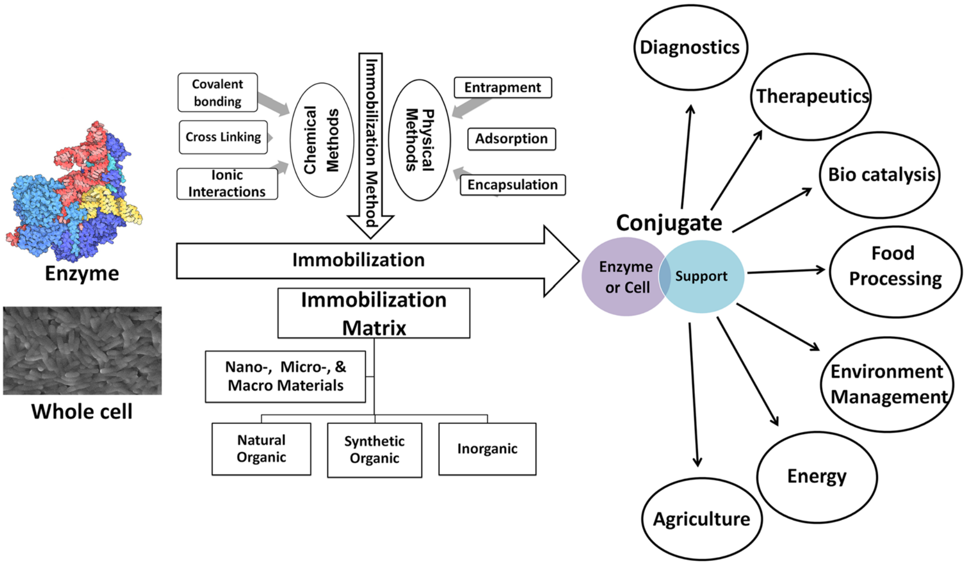

Fig. 1 Enzyme technology for biotechnical applications. Enzymes or organisms could be immobilized on the various matrices using varied immobilization chemistry for diverse biotechnological applications

Immobilized Biocatalyst: Supports and Characteristics

Immobilization of the enzymes on suitable supports improves their stability as well as activity during the transformation reactions [34–36]. A wide variety of materials are used for the immobilization of enzymes (Table 1). Among them, polymeric materials are well-reported support matrices for the immobilization of enzymes. In natural polymers, the most common are polysaccharides [31, 37], and in synthetic polymers, frequently used polymers are polystyrene, polyacrylate, polyacrylamide, etc. [38]. Conducting polymers are good for enzyme immobilization due to their unique properties that enhance the efficiency of biocatalysts [38]. Other reported materials for immobilization are carbon heterostructures [23, 39], silica [40–43], clays, metal oxides [44], and glasses. Alginate is also a well-reported material for immobilization [45]. Recently, hybrid organic–inorganic materials within the size range of 1–100 nm and large surface area, functional moieties for covalent linkage, biocompatibility, as well as with improved catalytic properties, were proved as excellent support materials for immobilization [24, 46–49]. Nanomaterials are fabricated either with top-down approaches (Milling, laser ablation, explosion, sputtering, etching, etc.) or bottom-up approaches (chemical reduction, spinning, sol–gel process, molecular condensation, green synthesis, supercritical fluid synthesis, etc.[27]. These nanomaterials were characterized as nanoparticles, nanoreefs, nanoboxes, nanofibers, nanotubes, and nanoflowers. Enzymes are immobilized through covalent linkage, entrapment, crosslinking, electrospinning, electrodeposition, coating, adsorption, co-precipitation, etc. Immobilization of enzymes on these nanostructures was found to enhance enzyme performance, stability, and reusability. These nanomaterial coupled enzymes have been characterized as nanobiocatalyst that holds the promising strengths of both nanotechnology and biotechnology (Fig. 1). Selection of the support surface or the matrix for immobilization is decided primarily by their biocompatibility and resistance to microbial attack. Furthermore, cost, stability, hydrophobicity, surface area, and compression behavior are also critical factors towards the industrial utilization of the materials [34]. In comparison to non-porous support, porous supports are more desirable. Biocalysts, including enzymes immobilization can be demonstrated with the help of different strategies like covalent [50], crosslinking [25], adsorption, encapsulation, and metal ions-based hybrid formation [48]. The strategy to choose is also dependent upon the mechanical, chemical, and kinetics characteristics of biocatalysts (Fig. 1).

Potential Biotechnological Applications

Environmental Remediation

Biocatalysts are applied successfully for the transformation of micropollutants to less or nontoxic moieties. Various prominent biocatalysts have been reported for the degradation of organic contaminants including laccases, hydrolases, peroxidases, and oxidoreductases etc. [51]. Nanoflower-linked laccase was characterized for residual decolorization of the synthetic dyes with an efficiency of up to 84.6% [25]. Immobilized *Pleurotus ostreatus* laccase was characterized for transformation of Bisphenol A into nontoxic product [51]. Several microbial sources such as fungi (*Tramates versicolor, Pleurotus eryngii),* bacteria (*Pseudomonas aeruginosa, Rhodococcus erythropolis*), and algae (*Monoraphidium braunii, Chlamydomonas reinhardtii*) have been concluded to have a catabolic mechanism for pollutant cleanout [52]. Many organic pollutants including phenols, 2,4,6-trinitrotoluene (TNT), nitroaromatic compounds, chlorophenol, dyes (malachite green, bromophenol blue), and polychlorinated biphenyls can be degraded using different classes of biocatalysts [52, 53].

Chemical Synthesis

A wide variety of reactions like esterification, trans-esterification and chemoselective transformations are catalyzed by biocatalysts [54]. Several industrially useful compounds such as alkyl levulinates and glycerol carbonates can be produced through biocatalysis. Microbial lipolytic enzymes are well known for their capability to catalyze biotransformation reactions of compounds containing ester-bonds e.g. conversion of waste into high-energy products like biofuel and other value-added products via energy-efficient pathways [55–57]. *Methylosinus sporium* produced a maximum methanol concentration of 6.45 mM In a methanotrophic reactor [56]. Bioplastics like polyhydroxyalkanoates were obtained by biocatalysis and considered an alternative to the non-biodegradable plastic [58]. *Bacillus cereus* EGU43 was characterized for its potential to produce polyhydroxyalkanoates with an optimal output of 195 mg PHA/l using effluent from the H2 production stage [58].

Pharmaceuticals and Healthcare

Biocatalysis use in the pharmaceutical industry is speeding up [59]. For this, the biocatalyst should have specificity and good activity. Sitagliptin is one of the most popular processes in pharmaceuticals showing the applicability of biocatalysis [60]. Chondroitin sulfate lyase, a biocatalyst applied for the synthesis of chondroitin sulfate, is used in a variety of therapeutic uses such as osteoarthritic treatment. Statins, a big class of medicine used in the treatment of hypercholesterolemia also works through biocatalysis [61]. Laccase, a biocatalyst used in the polymerization of phenolic compounds produces compounds with enhanced physicochemical properties and is used as nutraceuticals. Biocatalysts are also highly applicable in the synthesis of chiral pharmaceuticals [61].

Agriculture and Food Industry

A strong correlation exists between enzymology and agricultural technology. Initially, enzymes were reported by agricultural chemists and even many of their characteristics were also elucidated by them. Primary sources of enzymes are agricultural plants, animals, and microorganisms. These biocatalysts are being widely applied in food industries where they are employed to modify the properties of raw products for their conversion into food [62]. Biocatalysts regulate the appearance and texture of the food materials which in fact influence the product value. A big family of enzymes mainly α-amylase, β-amylase, glucoamylase, pullulanase, and transglutaminase along with some more enzymes are also involved in the starch industry [62]. Xylanases which are produced by different species of *Trichoderma* and *Aspergillus*, are extremely valuable in the baking industry to increase the bread volume, reduce stickiness, and crumb structure [63]. The enzymes pectinases, cellulases, and tannases are the most widely used ones in the fruit juice industry [63]. Lipases are used for the production and ripening of cheese that provides a lipolytic flavor to the product [62, 63].

Biomass Conversion and Fuels

The major constituent of biomass is a polysaccharide which could be bio- catalyzed to achieve fuel or fine chemicals. Polyols, syngas, glycerol, cellulosic alcohols, ethers, and various fatty acids are the product or by-products of the conversions processed through bio-catalysis [29]. Biomasses rich in cellulose and hemicellulose are the primary source to produce biofuels. Lignocellulosic materials like wood chips, municipal wastes, and crop residues are also the prime sources to produce biofuels. Cellulose catabolism proceeded through the biocatalyst can lead to the production of fuel precursors [64]. The availability of wastes biomass especially as biowastes in large quantum such as from agricultural and municipal origin has been considered as low-cost feed-stock to produce fuels [65–67]. Anaerobic digestion and fermentation are the generally employed biochemical processes in fuels and value-added product production from complex feed such as biomass [4]. The fermentative process is a less energy-intensive process for the production of H_2. Although the efficiency is not much and is mainly influenced by the feedstocks, besides other environmental conditions. The various individual microbial culture [68], co-culture [69], and mixed cultures were reported for the biological hydrogen production [70, 71]. Immobilized *Methylocystis bryophila* showed an enhanced methanol production up to 52.9 mmol/L [72]. Co- or mixed cultures influence the H_2 production rates synergistically. Immobilized co-culture of *Bacillus* and *Enterobacter* was observed as an efficient H_2 producer (6.4-fold improvement) than individual strain [69]. Glycerol which is a byproduct of biodiesel production is also found a good feedstock for the production of hydrogen [68]. Immobilzed *Bacillus thuringiensis* EGU45 was characterized for an H_2 yield of 0.386 mol in reference to per mol of consumed pure glycerol [68]. Methanol is found as a primary component in various synthesis reactions and is also considered a component in gasoline blends. Methane is a major greenhouse gas other than carbon dioxide. So bio-conversion of methane to methanol is not only economically important but also environment friendly. Synthetic gas mixture (CH_4, CO_2, and H_2) is more efficient in comparison to CH_4 for H_2 production [72, 73]. Production of methanol by using biological methods is regarded as a cost-effective method in comparison to the chemical methods of synthesis at ambient conditions [74, 75]. Methanotrophs which are aerobic and gram-negative bacteria successfully produce methanol by utilizing CO_2 and CH_4 as carbon sources [76, 77]. The process proceeds through a complex metabolic mechanism involving different enzymes such as methanol dehydrogenase (MDH), methane monooxygenases (MMOs), formaldehyde dehydrogenases, and formate dehydrogenases [78].

Analytical Chemistry

Engineered biocatalysts are efficient detectors for many prominent biomarkers. Enzymes in the immobilized state possess great potential to act as sensors and can detect their respective target molecules [79]. In this process, the analyte molecule moves towards the enzyme and is converted into a product by the catalytic action of the enzyme with a simultaneous release of electrons. The released electron gets transferred to the transducer through an electrode and can be detected [79]. Materials like metal nanoparticles, graphene materials, and carbon nanotubes have excellent electrical and mechanical properties that provide rapid electron transfer rates. Oxidoreductases are commonly used in the detection of phenols [26]. Several compounds including triglycerides, glucose, heavy metals, urea, and catechol have been recognized by bio-sensing where lipase, glucose oxidase, urease, tyrosinase are the respective enzymes involved in the processes. Bilirubin oxidase, cholesterol oxidase, and glutamate oxidase are used for the detection of bilirubin, cholesterol, and L-glutamate for liver diagnosis.

Carbon Dioxide Utilization

Generally, CO_2 is regarded as a pollutant that disturbs the environment and causes the greenhouse effect. However, in recent years it has been treated as a chemical feedstock to produce different kinds of carbon-based materials. Among the different strategies involved to convert CO_2 into useful compounds, bio-based materials such as microorganisms and enzymes are of particular interest for redox reactions of CO_2. The major advantage of utilizing biocatalysts in the CO_2 conversion process is high yields and selectivity to the generated products. In this field, dehydrogenase enzymes are of special focus as these are especially known to transform CO_2 into carbon monoxide or hydrocarbons like methanol [80], formate, or formaldehyde [81] where nicotinamide adenine dinucleotide (NADH) acts as a co-factor in the involved redox reactions. In addition to dehydrogenases, carbonic anhydrase is also able to transform CO_2. In hydrogenation reactions, carbonic anhydrase converts CO_2 into bicarbonates. As already stated, supported enzymes are more stable and active as compared to the native ones, encapsulation in gels and gel beads is a better approach. Silica sol–gel supported dehydrogenase enzyme can have a 90% yield in methanol production from CO_2 [82]. Polymeric sheets can also be used for the same purpose where efficient amounts of methanol are produced from CO_2 [82].

Conclusion and Future Prospective

Catalytic selectively and specificity of enzymes make them a potential candidate for biotechnological applications. Conjugation of enzymes with smart nanomaterial's have significantly enhanced their catalytic performance and made them suitable candidate for biotechnological applications in the area of nutraceuticals, diagnostics, drug designing, energy, synthesis, environment management. Despite advancement, further efforts are still required to improve Nanocatalysts stability, biocompatibility, environmental safety, and reusability for wider applications.

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