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# Engineering magnetic nanobiocatalytic systems with multipurpose functionalities for biocatalysis, biotechnology and bioprocess applications

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ABSTRACT

Enzymes are high-performance natural biological catalysts with wide-ranging applications in agricultural, medical, food and environmental sectors. Nevertheless, lack of efficient recovery, reusability, and high cost of the soluble form of enzymes are the most daunting challenges rendering biocatalytic systems inadequate for industrial exploitation. In order to deal with these inadequacies, immobilization appears to be a prodigious approach for enhancing the stability and catalytic efficiency of enzymes, as well as enabling their separation and reusability in continuous reaction batches. Among different nanostructures, magnetic nanomaterials have garnered supreme interest as support matrices for biomolecules and enzymes immobilization because of their substantial surface area, larger surface-to-volume ratio, modifiable surface, and adjustable surface particle size, stability, and high mass transferring ability. In addition, they can be quickly recovered from the complex reaction system by a simple external magnetic field. Magnetic nanoparticles incorporated biocatalysts demonstrated a broad-working temperature and pH profile and augmented storage and thermal stabilities compared to their native derivatives. This paper provides a recent and state-of-the-art overview of the development and application of multifunctional magnetic nanobiocatalytic systems for an array of biotechnological purposes. In the first half, the development, functionalization, and use of nanostructured magnetic materials as enzyme immobilization supports are delineated. Then the prospective applications of magnetic nanobiocatalytic systems in different industrial sectors, including wastewater treatment, biodiesel

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and butanol production, hydrolysis of lignocellulosic biomass, glucose monitoring, fruit juice extraction and clarification, and synthesis of non-natural benzylisoquinoline alkaloid are comprehensively vetted with representative examples. Finally, the conclusive points and future prospects in this evolving field are also directed.

## 1. Introduction

The recent years have witnessed a dramatic surge in the use of biocatalysis in different industries, such as the biomedicine, food, energy, pharmaceuticals, and drug industries, and it plays a crucial role in environmental protection (Ellis et al., 2022; Sun et al., 2018; Truppo, 2017; Wu et al., 2021). Given important properties, including selectivity, specificity, low toxicity, and lack of secondary reactions, the use of enzymatic processes is considered a cost-effective, competitive, and more promising technology over chemical methods. Despite broad application prospects, the use of free enzymes is generally limited on a large scale due to low operational stability, high cost and challenging recovery from the reaction systems (Ren et al., 2019, 2020; C. Zhang et al., 2020; Zhong et al., 2020). Immobilization onto solid supports is a prospective way to enhance the catalytic stability and performance of the enzymes, which also solves the challenges of recovery and recycling (Federsel et al., 2021; Gan et al., 2021; Tan et al., 2021a,b). Based on the immobilization support, enzymes are often likely to improve their kinetic and biochemical properties and can be employed in the scale-up of bioprocess (Di Fabio et al., 2022).

Among various supporting matrices, $Fe_3O_4$ magnetic nanoparticles are fascinating materials for protein and enzyme attachment because of their magnetic properties, easy handling, biocompatibility, reuse, and recovery (Bilal et al., 2018; Vaghari et al., 2016). Recently, they have gained exceptional interest in enzyme biocatalysis, mainly due to their ability to carry substantial amounts of enzymes due to larger surface area and augmented enzyme stability (Hamid et al., 2022). Herein, an exhaustive effort has been made to portray the latest and state-of-the-art progress in designing and applying multifunctional magnetic nanobiocatalytic systems for an array of biotechnological purposes.

## 2. Magnetic nanomaterials as enzyme immobilization supports

A number of novel solid supports, including microchips (Bao et al., 2012; Fan et al., 2013; Gasilova et al., 2014; Nunes-Miranda et al., 2014), monoliths (Krenkova et al., 2009; Ma et al., 2008; Sproβ and Sinz, 2010; Yuan et al., 2014), magnetic nanoparticles (MNPs) (Cheng and Zheng, 2014; Khoshnevisan et al., 2011; Mu et al., 2014; Shi et al., 2014; Woo et al., 2015; T. Zhang et al., 2013), and microstructures (Ahn et al., 2012), have been employed recently to successively immobilize enzymes. Concerning these supporting materials, magnetic nanoparticles have a range of benefits, such as significant surface areas that enable the immobilization of large numbers of enzymes, simplicity in separating from the digested peptides with just a magnet, strong biocompatibility, and renewability (Fig. 1). For instance, Mu et al. successfully synthesized magnetic $Fe_3O_4$ NPs with poly(2-vinyl-4,4-dimethyl azlactone) functionalities as well as utilized them to covalently immobilize L-asparaginase by reacting the azlactone functionals with the enzyme's amine groups (Mu et al., 2014). This compound was used in the simulating cure of acute lymphoblastic leukaemia because it prevented the variations of native L-asparaginase. Cheng and colleagues developed a magnetic enzyme nanosystem with a magnetic core of $Fe_3O_4$ and polydopamine (PDA) cover. Trypsin was immobilized on this support due to PDA's adhesive capabilities (Cheng and Zheng, 2014). The protein digestion process took 30 min because of this magnetic enzymatic technology.

Immobilization method is another important factor that significantly affects digestion utilizing immobilized enzymes in addition to the characteristics of the solid supports. The considerable literature, which includes outstanding recent evaluations, has outlined its impacts (Rodrigues et al., 2011; Secundo, 2013; Sheldon and van Pelt, 2013). Physical methods for immobilizing proteolytic enzymes include adsorption, encapsulation, etc.; chemical approaches include van der Waals force, chelating with metals, and covalent bonding. Encapsulation and enclosing methods are frequently used to immobilize objects; however, they have limitations such as

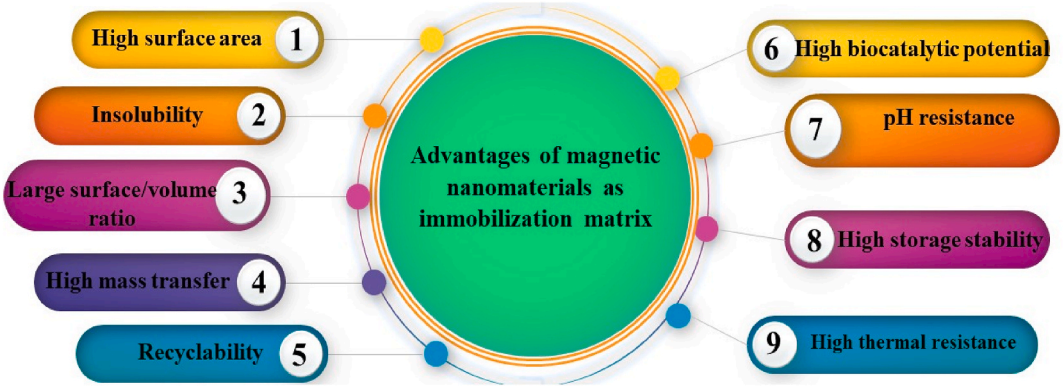


**Fig. 1.** Representative advantages of magnetic nanomaterials as enzyme immobilization supports.

enzyme leaking and delayed mass transfer of the products and substrates inside the support matrix. Physical adsorption and van der Waals forces-based techniques are simple, but these are associated with risks from non-specific binding affinity and enzyme loss during function. Covalent interaction is the best effective method for preventing enzyme separation from the supports during operation. The probable denaturation and inactivation brought on by the protein's 3D structure being altered by multiple access binding is the cost to be paid for this advantage (Ozyilmaz and Yağız, 2012). Additionally, if the immobilized enzyme loses function, the common solid supports utilized for covalent immobilization are not easily regenerated. Therefore, it is essential to create new immobilization techniques and supporting components that allow for robust yet reversible attachment of enzymes.

Porcine lipase was immobilized on a monolithic polymer substrate with thiol functionals produced inside the boundaries of a fused silica capillary and engineered with gold nanoparticles (AuNPs). In contrast to standard physical or chemical immobilization approaches, their innovative strategy used stronger but reversible Au–$NH_2$ as well as Au–S interactions. The transesterification of triacylglycerides from cooking oil to fatty acid methyl esters was the final possible use of this bioreactor that showed its capacity to make biodiesel (Lv et al., 2014). Cao et al. proposed renewable support for enzyme immobilization using magnetic $Fe_3O_4$ nanoparticles covered with a coating of AuNPs. The AuNPs functioned as an intermediary ligand for the reversible immobilization of trypsin. The Au–S and Au–$NH_2$ bindings, which are powerful but reversible, immobilized the enzyme. This immobilized trypsin bioreactor was utilized for conventional protein digestion, which was completed in 15 min. After digestion, a magnet was used to remove the trypsin-loaded NPs from the reaction medium (Cao et al., 2016). Wastewater from the dyes factories is a significant generator of contamination. The enzyme peroxidase is crucial in breaking down phenolic substances like azo dyes. The immobilization of the peroxidase enzyme was done on $Fe_3O_4$ MNPs, and the modification was done by utilizing glutaraldehyde via co-precipitation to make the enzyme stable, efficient, and recyclable. Studies revealed that peroxidase-MNPs are incredibly stable in an environment of fluctuating pH and temperature. Hence, researchers find the potential application of peroxidase-MNPs for bio-remediating the green azo dye and red azo dye from the wastewater from the textile industry (Darwesh et al., 2019).

Chloroperoxidase (CPO) is significant in both ecological and societal terms because of its numerous potential uses, which range from the production of pure optical molecules to activities relating to the environment (Campbell et al., 2013; Mumbo et al., 2013). A site-specific association between avidin and biotin allowed for layer-by-layer (LBL) directed assembly to accomplish the molecular configuration of CPO on the surface of $Fe_3O_4$ MNPs. To assess the catalytic effectiveness of immobilized CPO (I-CPO), soluble aniline blue underwent enzymatic oxidative decoloration. I-CPO's decoloration efficacy was greater than 90% within 10 min.

The enzyme-assisted extraction (EAE) method is regarded as an environmentally acceptable, efficient, and potential alternative to standard solvent procedures. EAE offers the quickest and least amount of solvent necessary to extract biological components. Furthermore, this can be done under moderate parameters, which is advantageous for extracting thermo-sensitive compounds, including oil, flavors, pigments, etc. Several research has been conducted to investigate EAE for various bioactive compounds in the food sector (Puri et al., 2012). Recently, scientists co-immobilized enzymes (α-amylase and glucoamylase) on MNPs utilizing glutaraldehyde as a bridging component for the pretreatment of Curcuma longa powder. Furthermore, the turmeric-root powder was pre-treated for the extraction of curcuminoids using a combination of produced biocatalyst and reduced power ultrasound. Compared to the solo strategy, the cumulative impact increases the extraction efficiency under the optimal solvent extraction procedure by 1.3–1.5 times. With the use of crystallization, the curcuminoids were extracted, yielding 54% (w/w) isolation and the purification of 91% (Patil and Rathod, 2022).

The ideal immobilization technique is site-specific as well as covalent linkage; however, conventional techniques sometimes need either genetic engineering or complex material development, increasing operating complications and difficulties. Tang et al. have developed a unique site-specific and covalent approach for immobilization, based on carefully chosen immobilization sites on lipase. For the site-specific immobilization of lipase, natural polyphenol epigallocatechin gallate (EGCG)-modified $Fe_3O_4$ NPs were developed. The immobilized lipase resulted in a greater biodiesel yield of 92.1% than unbound and randomized immobilized lipases. The findings demonstrated that synthesized site-specific immobilization carriers were advantageous in sustaining the natively charged catalytic site conformation and improving the rigidness of the immobilized lipase. After eight iterations, the site-specific immobilized lipase can retain up to 75.3% biodiesel output, suggesting it an excellent nanocatalyst for sustainable biodiesel synthesis (Tang et al., 2022).

## 3. Development and functionalization of magnetic nanomaterials

It is crucial to create chemiluminescent functionalized nanomaterials (CF-NMs) with excellent chemiluminescence (CL) efficacy, high catalytic activity, high stability, and quick magnetic separation capabilities in CL biosensing. Owing to its high CL content, simple assembly, and high biocompatibility, CF-NMs have attracted significant attention in bioassays during the last decade (He and Cui, 2012; Y. Huang, Gao and Cui, 2018; Zhong et al., 2019). Functionalization of $CuFe_2O_4$ MNPs with N-(4-aminobutyl)-N-ethylisoluminol (ABEI) resulting in the formation of ABEI/$CuFe_2O_4$ with enhanced catalytic performance was prepared using a post functionalization and solvothermal technique. At room temperature, 0.2556 g $CuC1_2.2H_2O$ and 0.81 g $FeC1_36H_2O$ are mixed in 20 mL ethylene glycol solution. The solution was allowed to clear, and then 1.64 g NaAc and a measured quantity of $Na_3Cit_2H_2O$ were introduced and agitated for 2 h. After collecting the solution, it was sealed in an autoclave and heated to 200 °C for 16 h. Deionized water and ethanol were used to wash the product after the process. The precipitates were vacuum dried for 6 h and kept at 4 °C for further use. The next step included ultrasonication of 10 mg of the resulting powder into a solution of deionized water. Then a 2:1:1 volumetric mixture of $CuFe_2O_4$ suspension, deionized water, and ABEI solution was prepared. After 12 h of shaking, the mixture is separated magnetically. After three washes, the resulting ABEI/$CuFe_2O_4$ was dissolved in deionized water and stored at 4 °C until further usage. A parallel approach for efficient catalysis of $H_2O_2$ may have been enabled by the peroxide-like reactivity and $Cu^{2+}$ and $Cu^0$ abundance on the

surface of ABEI/$CuFe_2O_4$. A sensor free from enzymes to detect the $H_2O_2$ was created due to the responsive reaction of ABEI/$CuFe_2O_4$ to $H_2O_2$ (Y. Huang et al., 2018).

$Fe_3O_4$@$SiO_2$-creatine, an ecofriendly and practical magnetic organometallic nanobiocatalyst, was efficiently synthesized using the naturally occurring creatine molecule. Co-precipitation was used to synthesize $Fe_3O_4$ nanoparticles (Eivazzadeh-Keihan et al., 2020). Using a variation of the Stöber technique, a layer of $SiO_2$ was deposited over the magnetic $Fe_3O_4$ core (Hui et al., 2011). Firstly, 40 ml distilled water as well as 160 mL solution of ethanol was used to disperse 2.00 g of as-prepared $Fe_3O_4$ NPs for 15 min in an ultrasonic water bath. Later, 10 mL of a 25-wt percent ammonia solution was added dropwise to the reaction medium while vigorously mixed using a magnetic stirrer. A syringe was then used to gently inject 1 mL of TEOS into the mixture over 20 min, and the solution was then stirred for 12 h at ambient temperature. At last, the resulting dark solid was magnetized and collected, washed multiple times with distilled water and ethanol, and dry at 60 °C. To bind the (3-chloropropyl)-trimethoxysilan (CPTMS) as a bridge with silica-coated MNPs, one ml of CPTMS reagent was mixed with one 100 ml of dry toluene, following the addition of 1 g of $Fe_3O_4$@$SiO_2$, the solution was stirred for 18 h at 60 °C. The brown precipitate formed ($Fe_3O_4$@$SiO_2$–Cl) was rinsed in toluene, magnetically isolated, and then vacuum dried at 70 °C. For the first step in synthesizing $Fe_3O_4$@$SiO_2$-creatine, 80 mL of ethanol. Initially, a single gram of $Fe_3O_4$@$SiO_2$–Cl NPs and 1.31 g (10 mmol) of creatine were combined. The synthetic route of $Fe_3O_4$@$SiO_2$-creatine catalysts is also shown in Fig. 2. The solution was stirred for 10 h in a round-bottom flask in refluxing settings. The resulting material was then magnetically isolated, washed with acetone, distilled water, and ethanol many times, and dried in an oven at 70 °C. Using this functionalized MNPs catalyst, 2-amino-4H-chromene derivatives were prepared using a greener synthetic route (Eivazzadeh-Keihan et al., 2022).

Sonochemical and co-precipitation techniques have been used to synthesize spinel cobalt ferrite MNPs with an approximate diameter of 40–50 nm in an aqueous phase without any organic stabilizing agent or surfactant. The dispersions of nanoparticles in alcoholic or aqueous media are stable. In an aqueous medium containing ethanol (1:3), the uncapped nanoparticles were subsequently used in the Knoevenagel reaction as a reusable catalyst. An outside magnet was used to help in the catalyst separation and collection from the reaction media. At merely 5 mol% of the catalytic concentration at 50 °C, high yields of the appropriate Knoevenagel compounds were achieved quickly (Senapati et al., 2011). Cobalt ferrite MNPs ($CoFe_2O_4$) with mean diameters of 25 nm were created as a catalyst for the oxidation of a variety of alkenes in the vicinity of *tert*-butyl hydroperoxide (t-BuOOH). As revealed in this work, an alkene-to-aldehyde or -to-epoxide conversion with almost quantitative yields was achieved using $CoFe_2O_4$ nanoparticle catalysts. In five successive runs, the catalyst showed no evident activity loss while being easily collected utilizing a magnet (Kooti and Afshari,

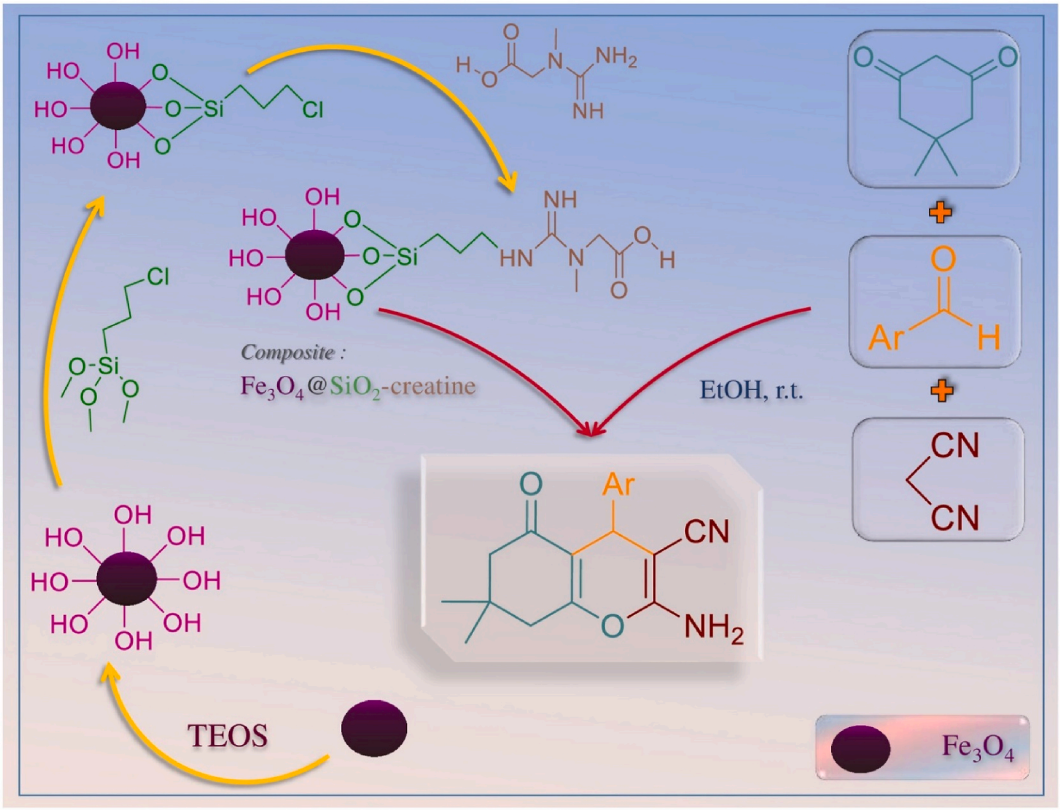


**Fig. 2.** Synthetic rout of $Fe_3O_4$@$SiO_2$ NPs with a creatine functionalization and development of 4H-chromene derivative. Reprinted from Eivazzadeh-Keihan et al. (2022) with permission from Springer Nature. This is an open access article distributed under the terms of the Creative Commons CC BY license (http://creativecommons.org/licenses/by/4. 0/).

2012).

A new and easy way to make spinel cobalt ferrite ($CoFe_2O_4$) NPs in one pot has been found. The hydroxylating agent tributylamine (TBA) is used as homogenous chemical precipitation and hydrothermal heating in the synthesis. Transmission electron microscopy (TEM) and electron diffraction (ED) patterns were used to analyze the developed $CoFe_2O_4$ NPs. The alcohols are converted to their respective aldehydes by catalytic oxidation reaction using $CoFe_2O_4$ MNPs that are magnetically recoverable. Using this oxidation method, they were able to get high yields and excellent selectivity (Paul et al., 2016).

Activated carbon was shown to be an efficient sorbent for the purification and separation of gaseous and liquid phase mixtures due to its large surface area, diverse surface groups on the surface, and intrinsic micropores (Filippou et al., 2017; Rivera-Utrilla et al., 2011). Composites of active carbon and magnetic materials like magnetite ($Fe_3O_4$) or maghemite (g-$Fe_2O_3$) form magnetic activated carbons (Lompe et al., 2016). Magnetite NPs were first prepared by co-precipitation strategy following an altered Massart Technique. In this case, a clear yellow solution was obtained by dissolving 1.13 g (5.6 mmol) of $FeCl_2.4H_2O$ and 3.03 g (11.2 mmol) of $FeCl_3.6H_2O$ in 150 ml of DI water and then heating the solution to 60 °C under rapid mixing and an inert $N_2$ environment. The pH was then adjusted to 10 by adding an aqueous ammonia solution. $N_2$ was employed as a shielding gas while the suspension was heated to and maintained at 90 °C for 1 h. The magnetic field was used to collect the black precipitate, and then it was washed in water and ethanol before being freeze-dried (Filippou et al., 2017; Kyzas et al., 2014; Kyzas et al., 2013; Saroyan et al., 2017). The activated carbon based on wood BAX-1500 (B)15 (Filippou et al., 2017) was first rinsed in a Soxhlet system to eliminate soluble contaminants before being used to manufacture the magnetite-impregnated activated carbon. A homogeneous suspension was then achieved after 30 min of ultrasonic treatment of half a gram of the substance in 150 mL of water. Magnetite (0.25 g) was then introduced, and the whole material was sonicated for 30 min. Magnets were used to collect the resultant micro-meso porous carbon that is activated, which was then freeze-dried.

## 4. Application prospects of magnetic nanobiocatalytic systems

### 4.1. Biodegradation of dyes

Dyes-loaded wastewater produced by textile and various other industries is a major source of environmental pollution due to its very high organic loading (Oliveira et al., 2022). The presence of coloured components containing diverse synthetic dyes and chemicals, i.e., surfactants, mineral oils, salts, heavy metals, etc., is a severe problem of such wastewater (Dzumbira et al., 2021; Fei et al., 2022; Nawaz et al., 2022). These coloured effluents are highly toxic to the environmental matrices and exhibit an undesirable impact on water streams and living entities (da Silva et al., 2022; Q. U. Hassan et al., 2022; Khan et al., 2021). These detrimental pollutants should be treated and eliminated before findings way to water streams. In contrast to numerous physico-chemical dye removal methods, microbial bioremediation employing a diversity of microorganisms, such as fungi, yeast, bacteria, and microalgae, or their enzymatic system with efficient pollutants degradation abilities is a preferred and environmentally friendly technology in the remediation of industrial wastewaters, effluents, polluted water bodies and soils (Aslam et al., 2022; Kalsoom et al., 2022; Noreen et al., 2022; Saeed et al., 2021; Villalba-Rodríguez et al., 2022).

The use of nanoparticles and nanomaterials has recently received extensive interest in a wide range of biological applications due to their inimitable inherent properties (Rafeeq et al., 2022; Daphedar et al., 2022). Among different kinds of nanomaterials, magnetic nanoparticles have received widespread utilization and are considered impressive support materials for enzyme immobilization given their magnetic behavior that enables magnetic-assisted retrieval of the enzyme molecules from the complex reaction system (Alsaiari et al., 2021). synthesized magnetic copper ferrite ($CuFe_2O_4$) and iron oxide ($Fe_3O_4$) nanoparticles and employed them as catalytic support for immobilizing laccase in the presence of glutaraldehyde as a coupling reagent. In contrast to free laccase, $Fe_3O_4$ and $CuFe_2O_4$ nanoparticles-conjugated enzyme presented enhanced biocatalytic activity under an extensive pH and temperature range and incubating at 4.0 °C for up to 20 days. The $CuFe_2O_4$ nanoparticle-assisted nanobiocatalysts have a superior efficiency in catalyzing the biodegradation of Direct Red 23 (DR23) dye than the laccase immobilized $Fe_3O_4$ nanoparticles that might be ascribed to the occurrence of copper ions. The presence of copper ions boosts the biocatalytic properties of laccase by binding via the copper-binding sites. The immobilized nanobiocatalytic system revealed excellent reusability and stability, retaining over 70.0% decolorization of DR23 for up to 6 consecutive cycles.

A novel peroxidase was extracted from the bioremediated products of azo dye containing textile wastewater in a bioreactor. After purification, the enzyme was immobilized on MNPs to enhance the enzyme's efficiency, stability, and recycling ability. Compared to the free enzyme, the MNPs-immobilized derivative was remarkably stable towards pH and temperature perturbations and retained its activity following 100 reuse cycles and storage at 4 and 25 °C for 3 months. The newly isolated MNPs-immobilized peroxidase catalyzed the complete decolorization of direct green and reactive red dyes in a lab-scale bioreactor, indicating the possibility of scaling up the bioremediation process bioreactor level for potential utilization in various environmental biotechnology applications (Darwesh et al., 2019).

### 4.2. Biodegradation of phenolic compounds

Phenolic compounds are worrying pollutants liberated in wastewater from different industrial units, including petrochemicals, metallurgy, pesticides, pulp and paper manufacturing, pharmaceuticals, resin, and plastic production (Casillas et al., 2017; N. Liu et al., 2016; Singh et al., 2021). They posture significant threats to the environment and living organisms even at a very low concentration (Felshia et al., 2017; Bilal et al., 2019; Bhandari et al., 2021). The US Environmental Protection Agency (EPA) has declared phenol a priority pollutant (Doğan et al., 2015; Lončar et al., 2011). Hence, its effective mitigation in wastewater is an emerging health and environmental issue. For phenolic compounds removal, Qiu et al. (2020) developed an efficient nanobiocatalytic system by

immobilizing laccase onto MNPs modified with amino-functionalized ionic liquid using dialdehyde starch as a cross-linking agent (Fig. 3). The immobilized magnetic nanobiocatalyst effectively removed phenol, 4-chlorophenol and 2,4-dichlorophenol in a broad pH and temperature range with a maximum removal efficiency of 86.1%, 93.6% and 100%, respectively. After 6 consecutive cycles, it preserved over 80% of its initial catalytic activity, indicating an efficient and novel approach for biodegrading phenolic contaminants.

$Fe_3O_4$ nanoparticles modified by 3-aminopropyltriethoxysilane, and glutaraldehyde were fabricated to immobilize *Rhus vernicifera* laccase (RvLac) via covalent linkages. The resulting immobilized enzyme maintained 82.9% activity after 10 repeated cycles and 16-fold thermal stability enhancement at 60 °C. It presented 84.9 and 72.2% degradation of bisphenol A at a concentration of 50 and 125 μM, respectively, which was 1.9-fold higher efficiency than a free enzyme (Patel et al., 2021). (Tarasi et al., 2018) achieved 53.0 and 68% degradation of bisphenol A by immobilizing *T. versicolor* laccase on MNPs Fe@PA and Fe@-PA-CD, respectively. Likewise, 50% degradation was recorded by immobilized *B. subtilis*-derived laccase on magnetic carbon-based nanosupport (C. S. Zhang et al., 2020). In another study, polyethyleneimine (PEI)-coated MNPs immobilized laccase was found efficient in the continuous degradation of phenolic contaminants in a fixed bed reactor. After 18 h treatment, the degradation efficiency in the bed was 2.38-times greater than batch reaction after 6 consecutive operational cycles. Under the optimum conditions, the phenolic degradation was retained at over 70% in 48 h continuous treatment, offering a robust choice for eliminating phenolic pollutants in industrial wastewater (Xia et al., 2021).

(Yadav et al., 2021) fabricated silanization functionalized MNPs and applied them to covalently immobilize a recombinant small laccase (rSLAC) from *Streptomyces coelicolor*. The immobilized magnetic nanobiocatalyst MNP-rSLAC was employed for removing phenolic contaminants, including phenol, 4-fluorophenol (4-FP) and 4-chlorophenol (4-CP). In the presence of acetosyringone as a natural mediator, the MNP-rSLAC catalyzed the complete degradation and transformation of 80 μg/mL of all selected phenolics within a short time of 2 h. It retained over 70% of its original activity after 10 consecutive catalytic runs and was readily retrieved from the reaction system by applying a magnetic field. The growth inhibition assays using *Escherichia coli* showed that rSLAC-assisted treatment decreased the toxicity of phenol, 4-FP, and 4-CP by 90%, 55% and 60%, respectively. The findings suggest a high potential for immobilized enzyme-based nanobiocatalytic platform for the sustainable and green bioremediation of wastewater polluted with phenolic compounds (Yadav et al., 2021).

### 4.3. Membrane bioreactors for wastewater treatment

Recently, the implementation of membrane technologies in bioreactors has appeared as a prodigious choice to recover water from contaminated wastewater streams (Yadav et al., 2021) since membranes can retain contaminants of diverse origins and chemical nature (Crini and Lichtfouse 2019). Moreover, the efficiency of membranes can be augmented by integrating nanoscale particles, i.e., silver, or different enzymes (Yadav et al., 2021). The amalgamation of two technologies, like magnetic compounds and immobilization is also likely to develop a more robust treatment approach. Several researchers have reported the enhanced enzyme activity following immobilization onto magnetic nanomaterials even after many consecutive treatment cycles (Li et al., 2020; Rangel-Muñoz et al., 2020). Similarly, the water treatment can be improved using an external magnetic field in terms of pollutant elimination or dye decolorization (Rangel-Muñoz et al., 2020). (Sotelo et al., 2022) designed five different kinds of magnetic biofilters containing

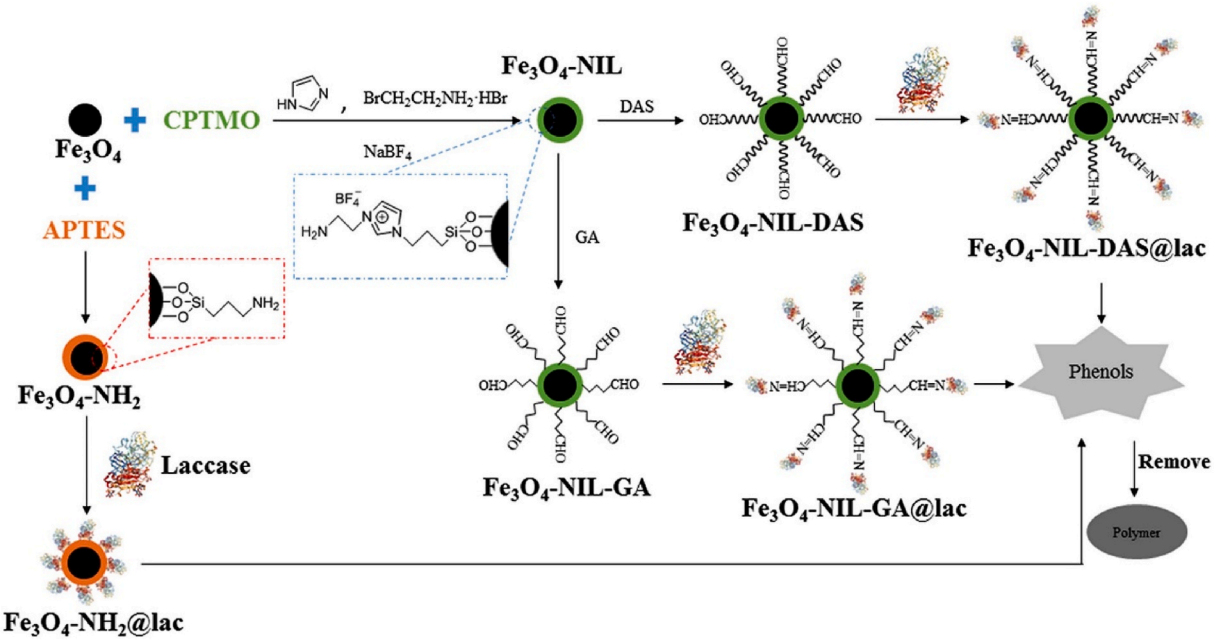


**Fig. 3.** Development of MNPs modified with amino-functionalized ionic liquid for laccase immobilization and its application for phenols elimination. Reprinted from Qiu et al. (2020) with permission from Elsevier.

MNPs-immobilized laccase and permanent magnetic elements, such as metallic meshes and neodymium magnets and applied them to decolorize Congo Red dye-loaded artificial wastewater in continuous flow bioreactors. Among these, filters containing laccase-immobilized magnetite, permanent magnets and metallic mesh catalyzed the maximum decolorization (27%) of Congo Red along with the largest half-life (seven cycles). Using permanent magnetic elements led to increased nanoparticle retention in the filters and facilitated the mass transfer between the biocatalyst and dye molecules to boost wastewater treatment.

### 4.4. Biodiesel production

Biodiesel is extensively recognized as a feasible, sustainable, and environmentally friendly substitute for fossil fuel for numerous technological applications owing to its outstanding qualities and environmental acceptance (Ajala et al., 2015; Mumtaz et al., 2022). Currently, the mainstream worldwide biodiesel supply is typically obtained from chemical processes using alkali catalysts, i.e., KOH or NaOH (Thangaraj et al., 2019). Nevertheless, the alkaline catalytic reactions produce a large amount of soap that hinders the effective recovery of biodiesel from glycerol. In addition, these processes explicitly consume a massive amount of water to eliminate alkali catalysts from the target products, causing environmental pollution (Alsultan et al., 2021; Tan et al., 2022a). In contrast to chemical methods, enzyme-based approaches offer a number of advantageous features (Tan et al., 2022b). For instance, the lipolytic enzyme can catalyze the transformation of triglycerides and free fatty acids to produce biodiesel without soap formation in a one-step reaction system (DelRe et al., 2021; Hossain et al., 2020; Z. Liu and Smith, 2021). These enzymes have demonstrated a good scope in polymer synthesis, the biomedical sector, and organic transformation. Therefore, extensive studies have recently been carried out on using immobilized biocatalytic systems as environmentally biocompatible alternatives to non-biocatalysts to produce a spectrum of chemicals and pharmaceutically relevant products. In a recent study, lipase from Bacillus subtilis was covalently attached to MNPs and used a green biocatalyst for producing biofuel from spent olive oil transesterification with methanol. Under optimal reaction conditions, results revealed about 45% conversion to methyl esters using the magnetite-immobilized lipase within 1 h at 37 °C with a

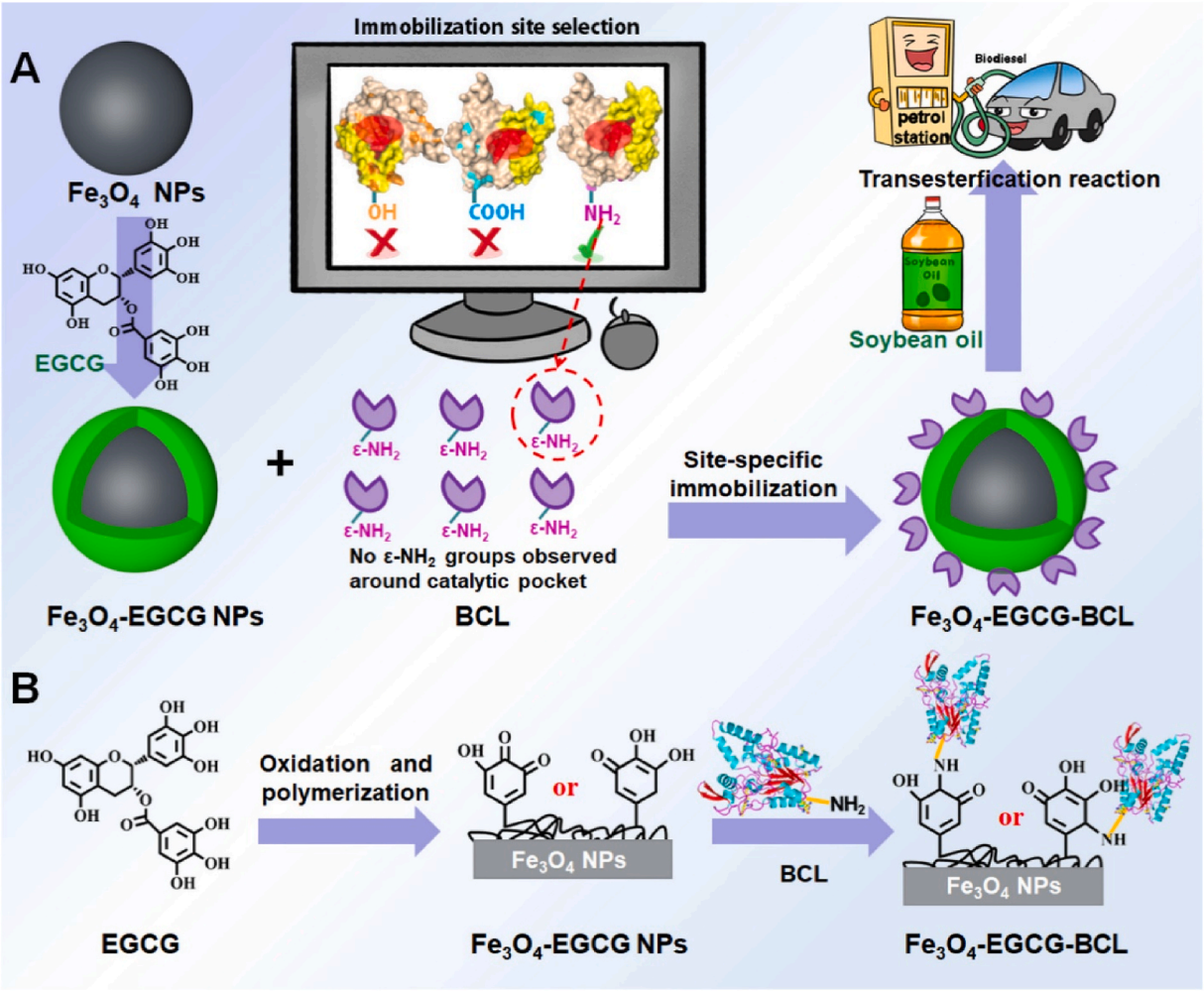


**Fig. 4.** (A) Schematic portrayal of $Fe_3O_4$-EGCG-BCL fabrication for biodiesel synthesis; (B) Mechanistic insight into BCL attachment with EGCG Coating. Reprinted from Tang et al. (2022) with permission from American Chemical Society.

methanol-to-oil molar ratio of 12:1 (Maroju et al., 2022). Magnetic hybrid sol-gel nanocomposite immobilized *Candida Antarctica Lipase B* (CALB) was used to produce biodiesel from waste cooking oil (WCO). For this, $Fe_3O_4$ MNPs were fabricated by the co-precipitation method, coated with silica, and functionalized with N-[3-(trimethoxysilyl)propyl] ethylenediamine (TSD) and organic-inorganic hybrid tetraethyl orthosilicate (TEOS). In addition to improved thermal stability, immobilized biocatalyst resulted in a 96% yield of biodiesel in 30 min at 40 °C, M/O molar ratio of 4:1, and a catalyst dosage of 1.0 g (Parandi et al., 2022).

A novel site-specific covalent immobilization technique was developed for lipase attachment onto natural polyphenol-modified MNPs. Computer-assisted structural analysis unveiled that lysine residues with free amino groups located distant from the catalytic pocket and lid were selected as the active immobilization regions to maintain the enzyme activity. In contrast to random immobilization, the tailor-made site-specific immobilization resulted in a substantial improvement in activity recovery. It also maintained the native conformation of the catalytic pocket and increased the rigidity of the immobilized lipase, exhibiting a significantly improved biodiesel yield compared to free and randomly attached immobilized counterparts. Preservation of over 75% biodiesel yield after eight successive cycles renders it a perfect nanocatalytic system for efficient biodiesel production (Fig. 4) (Tang et al., 2022).

### 4.5. Butanol production from waste textile

In recent decades, researchers have shifted their interest in producing higher alcohols to overcome the inadequacies of cellulosic ethanol fuel. Revitalizing the old acetone-butanol-ethanol (ABE) fermentation by *Clostridia* has gained increasing importance for large-scale biosynthesis of cellulosic butanol (Luo et al., 2019, 2020, 2021). The issue of expensive product separation has been overcome by integrating fermentation with efficient separation technologies, explicitly pervaporation and gas stripping (Cai et al., 2016). For butanol synthesis, enzyme-assisted cellulose hydrolysis plays an influential role as a selective stage (Ab Rasid, Shamjuddin, Rahman and Amin, 2021). It has recently been established that hybrid hydrolytic processes, such as cellulose regeneration, chemical hydrolytic conversion of regenerated cellulose into water-soluble oligomers and enzyme-based post-hydrolysis of oligomers offer significant advantageous merits over the typical enzymatic cellulose hydrolysis of waste textile (Ibrahim et al., 2019). Pretreatments with polyester solvents or cellulose solvents have been recommended to separate cellulose contents from non-cellulosic textile parts (Haule et al., 2016; Yousef et al., 2020). Nevertheless, a relatively considerable amount of cellulases is still needed even after reducing the crystallinity of cellulose fibers to catalyze the complete depolymerization of cellulose particulates. In addition, using enzymes in a suspension might raise the issue of irreversible enzyme adsorption onto the particulate surface that regulates the bioactivity of enzymes (Kristensen et al., 2009). As a potential alternative, a "hybrid" hydrolysis approach has been attempted on cotton fibers that depend on dilute phosphoric acid hydrolysis following enzymatic post-hydrolysis of water-soluble cellulose oligomers (Ibrahim et al., 2019). The post-hydrolysis of soluble oligomers may promote utilizing immobilized cellulase to perform cellulose biotransformation as a realistic biocatalytic strategy for enzyme separation and reuse. In this respect (Javid et al., 2022), developed a "hybrid" hydrolysis for the first time, which was based on the chemical degradation of cellulose polymers to their oligomers following enzyme-catalyzed hydrolysis of the resultant "soluble oligomers" by chitosan-coated MNPs immobilized cellulase. This novel hybrid hydrolytic process was employed for biobutanol synthesis from textile and jeans waste, leading to a high yield of glucose and butanol production by *Clostridium acetobutylicum*. Dilute acid treatment of regenerated cellulose at a high temperature of 120–180 °C for 1 h 60 min resulted in less than 10 g/L glucose formation, and enzymatic oligomers hydrolysis caused up to 51.5 g/L glucose. The hydrolysate fermentation gives rise to the production of 5.3 g/L acetone-butanol-ethanol (ABE), whereas the simultaneous co-saccharification and fermentation (SCSF) of insoluble and soluble cellulose oligomers led to 17.4 g/L productions of ABE.

### 4.6. Hydrolysis of lignocellulosic biomass

In recent years, global interest has intensified in the sustainable and greener production of high-value products like biofuels and speciality chemicals from bio-renewable lignocellulosic biomass (LCB) (Lu et al., 2022). Effective utilization of LCB-based viable feedstock has the potential to diminish the reliance on petro-derived fossil fuels (Zafar et al., 2022). It is considered cheaper, accessible in enormous amounts, and often available in the form of agro-industrial waste or by-products. Among different techniques, enzymatic hydrolysis has recently gained incredible interest as an important biorefinery process step, given its favorable impact on the bioprocess economy (Asgher et al., 2016; Li et al., 2019; Xia et al., 2019). Nevertheless, enzymatic hydrolysis encounters several challenges, including mass transfer resistances, enzyme inhibition by biomass-derived inhibitors, water restraint, undesirable lignin-enzyme interactions, etc., that restrict the cost-efficiency of LCB conversion. Rashid, Mustafa, Ab Rahim and Gunes (2022) carried out immobilization of cellulase on recyclable magnetic nickel nanoparticles to deal with these challenges. The immobilized nanobiocatalyst presented exceptional reuse capability with the retention of over 80% of its preliminary activity after 10 repeated reaction cycles, indicating a considerable potential in lignocellulose hydrolysis. For repeated usability and to make the process economical, a recombinant purified β-xylanase enzyme from *Thermotoga naphthophila* was immobilized onto silica-coated MNPs. After 8- and 13-times repeated use, the MNPs-biocatalyst conjugate showed 56% and 11% of residual activity. It also presented 17.32% and 15.52% cellulose hydrolysis of pretreated rice straw after 1st and 8th treatment usage, respectively (Hamid et al., 2022).

Microbial-derived lipids can be bio-transformed into biodiesel by the transesterification process and thus receive extensive importance. Although serval microorganisms, including filamentous fungi, oleaginous yeasts, and microalgae, can accumulate huge amounts of intracellular lipids such as single-cell oil within a short duration (C. Huang, Wu, Liu, Li and Zong, 2011; Jin et al., 2015). Nevertheless, the high processing cost of microbial lipids production restricts the broad-spectrum applications, which is mainly ascribed to fermentation feedstock. Therefore, exploring abundant and low-cost fermentation substrates is an imperative strategy for reducing the production cost of microbial lipids. Given renewability and cheapness, lignocellulosic biomasses, such as forest and agriculture residues, exhibit numerous advantages as fermentative substrates to synthesize lipids by microbial strains (Annamalai et al., 2018). However, the generation of various toxic inhibitors, such as furans, phenols, and organic acids, during the preparation of

lignocellulosic hydrolysate possess harmful effects on microbial growth and lipid accumulation. To overcome these issues (Annamalai et al., 2018), introduced a novel strategy for detoxifying lignocellulosic rice straw hydrolysate using carboxyl-functionalized MNPs-immobilized laccase and enhancing the lipid yield by *Rhodotorula glutinis* (Fig. 5). In addition to better pH and thermal stability profile, the immobilized nanobiocatalyst was repeatedly used to eliminate inhibitors in pre-treated rice straw via applying an external magnetic field. Experimental findings revealed that immobilized laccase catalyzed the elimination of most of the furans, phenols, and formic acids after the first batch. It displayed good reuse capability in multiple batches for detoxification, removing 78.2%, 43.8%, 30.4% and 16.5% of phenols, furfural, HMF, and formic acid, respectively, after the 4th consecutive batch. Compared to untreated hydrolysate, the lipid accumulation in the detoxified rice straw hydrolysate was significantly increased, indicating the potential applicability of MNPs-immobilized laccase in detoxifying lignocellulosic biomasses for enhancing microbial lipid production.

### 4.7. Monitoring of glucose

Diabetes mellitus with impaired glucose metabolism is among the most prevalent chronic diseases that cause metabolic complications, including nephropathy, retinopathy, dementia, and cardiovascular diseases (Mann et al.; Vanessa Fiorentino, Prioletta, Zuo and Folli, 2013; Wong et al., 2016). Screening and monitoring glucose concentration in the blood is imperative in diabetes management. The glucose level in the urine is directly proportional to the blood glucose level, particularly in patients with diabetic complications (Naveen Prasad et al., 2021). Therefore, the glucose concentration in urine is also a realistic indicator for diabetes monitoring. Generally, various methods such as capillary electrophoresis, conventional chromatography, colourimetry, fluorometry, chemiluminescence, liquid chromatography/tandem mass spectrometry (LC-MS/MS) have been used for measuring the concentration of glucose (Buriova et al., 2004; Du et al., 2004; Fereja et al., 2020; Xie et al., 2017; Yee et al., 2019). All these methods present their merits and limitations. For instance, capillary electrophoresis and LC-MS/MS demonstrate high sensitivity but require operational expertise and high-cost instrumentation. Likewise, electrochemical techniques typically require sophisticated electrode modification. Colorimetric sensing devices are still widely used owing to their simplicity, rapidness, low cost, and high throughput. Colorimetric strips offer a robust bio-platform for biochemical analyses.

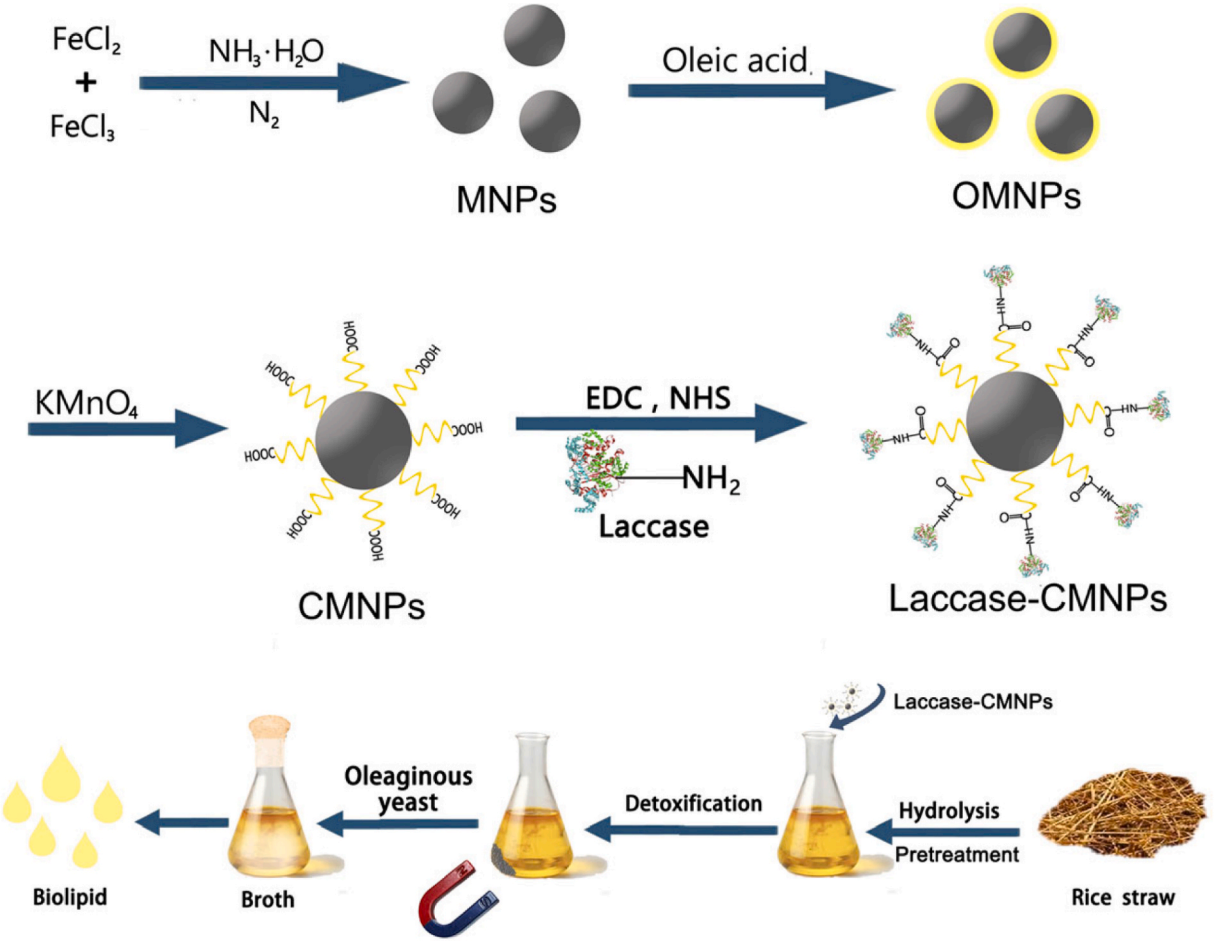


**Fig. 5.** Magnetic $Fe_3O_4$ nanoparticles immobilized laccase for rice straw hydrolysate detoxification and utilization in lipid production by *Rhodotorula glutinis*. Reprinted from Yin et al. (2021) with permission from Springer Nature.

In a current report (Luo et al., 2022), demonstrated the encapsulation of glucose oxidase into silica-capped MNPs by an easy self-assembly route, which does not involve any alkaline or acidic catalysts and organic solvents. The self-assembly approach ensures the maintenance of structure and bioactivity of enzymes, whereas the inclusion of MNPs enables fast separation and reprocessing of enzymes. They also proposed a new colorimetric-based biosensing method to determine glucose concentration in the urine based on the immobilized glucose oxidase and $H_2O_2$ test strip. For achieving the quantitative glucose determination, the signals were recorded by a smartphone in the colorimetric sensing method (Fig. 6). A recyclable MNPs-based cryogel optical biosensor was constructed for determining sucrose in sugar samples and sugarcane. For designing this biosensor, MNPs were entrenched in cryogel following the immobilization of multienzymes. Incorporating MNPs in cryogel ensured the separation of multienzyme cryogel biosensor by simply applying an external magnetic field, enabling subsequent repeated detections. The as-fabricated cryogel biosensor demonstrated exquisite selectivity for sucrose with a limit of detection of 3 mM and could be applied 32 times with excellent repeatability. Moreover, the developed optical biosensor was also effectively employed for sucrose determination and quantification of sucrose in sugarcane and sugar samples, indicating broad spectrum industrial deployment (Teepoo and Laochai, 2022).

### 4.8. Biomimetic production of benzylisoquinoline alkaloid

Amine oxidase enzymes belong to the oxidoreductases class and are widely spread from bacteria to humans. *Lathyrus cicera*-derived amine oxidases have recently emerged in the portfolio of biocatalysis and present significant potential in the green biosynthesis of aldehydes. This enzyme is responsible for catalyzing the oxidative deamination of a large number of primary amines into the aldehydes, but its broad-spectrum exploitation is restricted due to possible inactivation in the presence of high product concentrations. Immobilization is likely to enhance the overall biocatalytic properties, resulting in enzyme reprocessing and a reduction in the bioprocess costs (Di Fabio et al., 2022). evaluated the catalytic performance of amine oxidase immobilized on magnetic particles for biomimetic biosynthesis of new kinds of benzylisoquinolines, which are known to be structurally complicated alkaloids requiring a cumbersome chemical synthetic process (Magnus and Matthews, 2005). After immobilizing onto amino-functionalized MNPs, the immobilized biocatalyst retained its activity and significantly improved thermal stability (4 h at 75 °C). It can be reprocessed eight times with 90% efficiency after the last reaction cycle and aldehyde production ranging between 100 and 270 mg. One of the aldehydes formed was effectively utilized to biomimetic synthesis of benzylisoquinoline alkaloid. Fig. 7 and Table 1 enlists some recent examples of magnetic nanobiocatalytic systems for various biotechnological applications.

## 5. Conclusions and future outlook

Immobilization has been adopted to transform native biocatalysts into highly stable, recoverable and more robust derivatives for efficient utilization in biotechnology. Among the known nanosupport carriers, nanostructured magnetic materials are considered appealing carriers to constitute immobilized enzyme-based magnetic nanobiocatalytic systems because of their larger surface area,

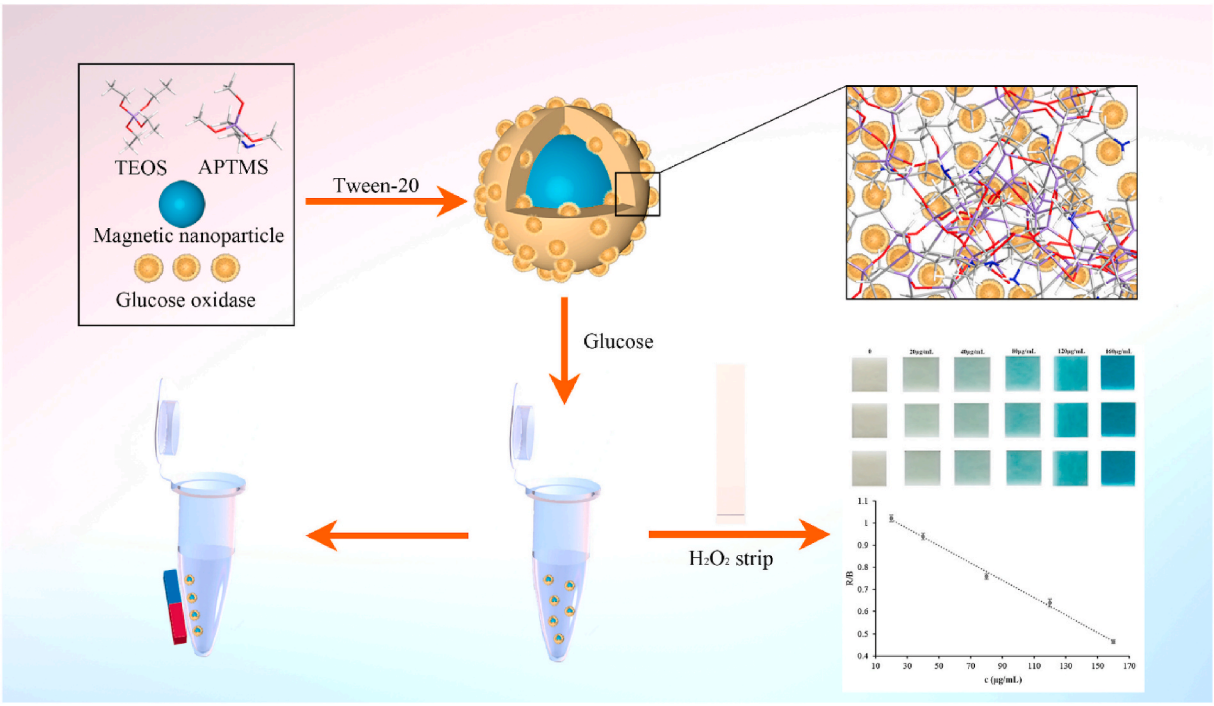


**Fig. 6.** Schematic of preparation and utilization of magnetic embedded enzymes Reprinted from Luo et al. (2022) with permission from MDPI. This is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

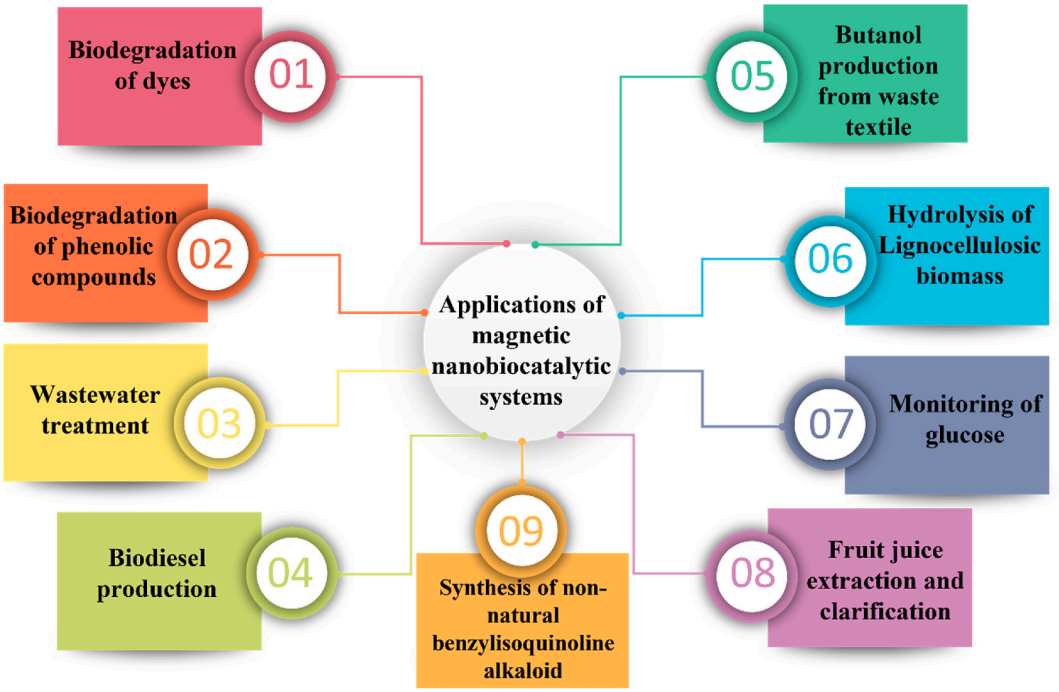


**Fig. 7.** Application of magnetic nanobiocatalytic systems in various biotechnological sectors.

modifiable surface, controllable particle size, easy recovery and separation using a magnetic field, and high mass transfer capability. Many opportunities can be created in biotechnology and catalysis by suitable incorporation of the enzyme with magnetic nanomaterials. Nevertheless, the large-scale implementation necessitates the development of optimized protocols to enhance the biocatalytic performance, stability, and recycling efficiency of enzymes. Under optimized immobilization parameters, magnetic nanobiocatalysts can be vividly applied to numerous fields, such as lignocellulosic biomass hydrolysis, biofuel production, environmental sensing, detection, and remediation and production of a spectrum of industrially pertinent compounds. Although extensive efforts have been attempted in the last few years to gain the in-depth immobilization understandings, intensive investigations are still needed to scrutinize the surface-function interfaces, nanomaterials-enzyme binding sites and the contribution of functional groups in the immobilization process to take full benefits of the immobilization system. For successful utilization, the industrial biocatalyst should be practically simple. To achieve this, intense research should be directed to multidisciplinary areas, as summarized below.

- Organic chemistry to introduce new reactive functionalities on the surface of the support.
- Material science to fabricate more novel support materials, which are physically inert, hydrophilic, chemically, and mechanical resistant, with controlled particle and pore size.
- Dynamic simulation and protein chemistry to envisage the type and intensity of interactions between the enzyme molecules and catalytic support.
- Bioprocess engineering for comprehending, modelling, and monitoring the underlying mechanisms, reactions, and mass transfer phenomena.
- Reactor engineering to develop new reactors compatible with immobilized supports, new approaches for biocatalyst retrieval, and effectual stirring systems.

## Author's contributions

**Muhammad Bilal, Jakub Zdarta:** Conceptualization, Data analysis and curation, Validation, Writing - original draft, review & editing. **Ehsan Ullah Rashid, José C. S. dos Santos, Pedro C. B. Fernandes:** Methodology, Data analysis and curation, Validation, Writing - review & editing. **Hairong Cheng, Teofil Jesionowski:** Data analysis and curation, Validation, Writing - review & editing. **Muhammad Bilal, Teofil Jesionowski:** Software, Supervision, Project administration, Validation, Visualization, Writing - original draft, review and editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Table 1**
Representative examples of magnetic nanobiocatalytic systems for various biotechnological applications.

| Magnetic nanocarrier | Enzyme | Improved biocatalytic properties | Application | Reference |
|---|---|---|---|---|
| Chitosan-coated MNPs | Alcalase | After 1 h of hydrolysis at 65 °C, the maximum oil yield was 20.55% (88.30% recovery). | Oil extraction from Atlantic salmon | (Y. Liu and Dave, 2022) |
| Magnetic nanoparticles | Glucoamylase and α-amylase | Enhanced extraction yield of curcuminoids by 1.3–1.5 fold using the concerted action of ultrasound and enzymes@AMNPs than the individual pre-treatment.<br>Separation of curcuminoids by crystallization resulted in 54% (w/w) isolation with a purity of 91%. | Extraction and purification of curcuminoids from *Curcuma longa* | Patil & Rathod (2022) |
| Magnetic dialdehyde cellulose | Laccase | Excellent performance for the decolorization of crystal violet.<br>90% decolorization of crystal violet at pH 6.0 and 45 °C for 21 h. | Dye decolorization | Qiao et al. (2022) |
| $Fe_3O_4$ magnetic nanoparticles | Fibrinolytic protease | Immobilized nanobiocatalyst did not induce any cytotoxicity against J774A.1 and HEK-293 cells. It was non-hemolytic and substantially minimized the hemolysis (from 2.07% to 1.37%) caused by MNPs.<br>Efficient thrombus degradation by immobilized enzyme system substantiated the thrombolytic efficiency of immobilization process.<br>Immobilized fibrinolytic protease catalyzed the complete degradation of the γ chain of human fibrinogen in 1.5 h. | Treatment of cardiovascular diseases | da Silva et al. (2022) |
| MNPs | Lipase | Under optimal reaction conditions, results revealed about 45% conversion to methyl esters using the magnetite-immobilized lipase within 1 h at 37 °C with a methanol-to-oil molar ratio of 12:1 | Biofuel production from food waste | Maroju et al. (2022) |
| MNPs-incorporated poly (vinyl) alcohol - chitosan cryogel | Invertase, mutarotase, glucose oxidase and peroxidase | The fabricated cryogel biosensor demonstrated exquisite selectivity for sucrose with a limit of detection of 3 mM and could be applied 32 times with excellent repeatability. The optical biosensor was also effectively employed for sucrose determination and quantification of sucrose in sugarcane and sugar samples. | Determination of sucrose in sugarcane and sugar | Teepoo & Laochai (2022) |
| Magnetic $Fe_3O_4$ and $CuFe_2O_4$ nanoparticles | Laccase | $CuFe_2O_4$ nanoparticle-assisted nanobiocatalysts a superior efficiency to catalyze the biodegradation of Direct Red 23 dye than the laccase immobilized $Fe_3O_4$ nanoparticles.<br>The presence of copper ions boosts the biocatalytic properties of laccase by binding via the copper-binding sites. | Degradation of organic dyes | Alsaiari et al. (2021) |
| Superparamagnetic iron oxide nanoparticles | Pectinase and xylanase | Improved storage stability and reusability of the enzymes | Fruit juice extraction and clarification | Hassan et al. (2022) |
| Chitosan-coated $Fe_3O_4$ nanoparticles | Cellulase | High yield of glucose and butanol production by *Clostridium acetobutylicum*.<br>The hydrolysate fermentation produces 5.3 g/L acetone-butanol-ethanol (ABE), whereas the simultaneous co-saccharification and fermentation of insoluble and soluble cellulose oligomers led to 17.4 g/L production of ABE. | Butanol production from waste textile | Javid et al. (2022) |
| Silica-capped MNPs | Glucose oxidase | A new colorimetric-based biosensing method was proposed to determine glucose concentration in urine, based on the immobilized glucose oxidase and $H_2O_2$ test strip.<br>The method showed an excellent linear performance in the concentration range of 20–160 μg $mL^{-1}$ and satisfactory recovery ranging from 94.3 to 118.0%. | Glucose monitoring in urine | Luo et al. (2022) |
| Silica-coated MNPs | β-xylanase | MNPs-biocatalyst conjugate presented 17.32% and 15.52% cellulose hydrolysis of pretreated rice straw after 1st and 8th treatment usage, respectively | Bioethanol industry | Hamid et al. (2022) |
| | Laccase | | Degradation of bisphenol A | |

(*continued on next page*)

**Table 1** (*continued*)

| Magnetic nanocarrier | Enzyme | Improved biocatalytic properties | Application | Reference |
|---|---|---|---|---|
| 3-aminopropyltriethoxysilane $Fe_3O_4$ nanoparticles | | Immobilized presented 84.9 and 72.2% degradation of bisphenol A at a concentration of 50 and 125 μM, respectively, which was 1.9-fold higher efficiency over the use of free enzyme. | | Patel et al. (2021) |
| Magnetic $Fe_3O_4$ nanoparticles | Laccase | Immobilized laccase displayed good reuse capability in multiple batches for detoxification, removing 78.2%, 43.8%, 30.4% and 16.5% of phenols, furfural, HMF, and formic acid, respectively, after the 4th consecutive batch.<br>The lipid accumulation in the detoxified rice straw hydrolysate was significantly increased compared to untreated hydrolysate. | Lipid production | Annamalai et al. (2018) |
| Amino-functionalized ionic liquid modified MNPs | Laccase | The immobilized magnetic nanobiocatalyst effectively removed phenol, 4-chlorophenol and 2,4-dichlorophenol in a broad pH and temperature range with a maximum removal efficiency of 86.1%, 93.6% and 100%, respectively. | Biodegradation of phenolic compounds | Qiu et al. (2020) |
| $Fe_3O_4$ magnetic nanoparticles | Peroxidase | MNPs-immobilized peroxidase catalyzed the complete decolorization of direct green and reactive red dye pollutants in a lab-scale bioreactor. | Bioremediation of textile wastewater dye | Darwesh et al. (2019) |
| Polyethyleneimine-coated MNPs | Laccase | After 18 h treatment, the degradation efficiency in the bed was 2.38-times greater than batch reaction after 6 consecutive operational cycles.<br>Under the optimum conditions, the phenolic degradation was retained at over 70% in 48 h continuous treatment. | Phenol degradation | Xia et al. (2021) |
| Amino-functionalized magnetic microparticles | Amine oxidase | Immobilized biocatalyst can be reprocessed eight times with 90% efficiency after the last reaction cycle and aldehyde production ranging between 100 and 270 mg.<br>One of the aldehydes formed was effectively utilized for the biomimetic synthesis of benzylisoquinoline alkaloid. | Synthesis of a non-natural benzylisoquinoline alkaloid | Di Fabio et al. (2022) |
| Magnetic hybrid sol-gel nanocomposite | Lipase | Immobilized biocatalyst resulted in a 96% biodiesel yield in 30 min at 40 °C, M/O molar ratio of 4:1, and a catalyst dosage of 1.0 g. | Biodiesel production from waste cooking oil | Parandi et al. (2022) |
| Magnetic nickel nanostructure | Cellulase | The immobilized nanobiocatalyst presented excellent reusability retaining over 80% of its original activity after ten repeated reaction cycles, indicating a considerable potential in lignocellulose hydrolysis | Hydrolysis of lignocellulosic biomass | Rashid et al. (2022) |
| Magnetic polymeric filters | Laccase | Filters containing laccase-immobilized magnetite, permanent magnets and metallic mesh catalyzed the maximum decolorization (27%) of Congo Red along with the largest half-life. | Dye decolorization in bioreactors | Sotelo et al. (2022) |
| $Fe_3O_4$ magnetic nanoparticles | Glyceraldehyde-3-phosphate dehydrogenase | The application of immobilized GAPDH Immobilized enzyme showed a potential effect in the actual food, removing over 80% of histamine in grape and black raspberry wines with a negligible influence on wine composition. | Degradation of histamine | Wang et al. (2021) |
| APTES-functionalized magnetic nanoparticles | Laccase | In the presence of acetosyringone as a natural mediator, the MNP-rSLAC catalyzed the complete degradation and transformation of 80 μg/mL of all selected phenolics within 2 h. | Treatment of phenolic contaminants | Yadav et al. (2021) |
| Polyphenol modified MNPs | Lipase | Higher reaction efficiency with over 90% biodiesel yield within 16 h | Biodiesel production | Tang et al. (2022) |

## Data availability

Data will be made available on request.

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