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Advanced applications in enzyme-induced electrospun nanofibers

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Electrospun nanofibers, renowned for their high specific surface area, robust mechanical properties, and versatile chemical functionalities, offer a promising platform for enzyme immobilization. Over the past decade, significant strides have been made in developing enzyme-induced electrospun nanofibers (EIEN). This review systematically summarizes the advanced applications of EIEN which are fabricated using both non-specific immobilization methods including interfacial adsorption (direct adsorption, cross-linking, and covalent binding) and encapsulation, and specific immobilization techniques (coordination and affinity immobilization). Future research should prioritize optimizing immobilization techniques to achieve a balance between enzyme activity, stability, and cost-effectiveness, thereby facilitating the industrialization of EIEN. We elucidate the rationale behind various immobilization methods and their applications, such as wastewater treatment, biosensors, and biomedicine. We aim to provide guidelines for developing suitable EIEN immobilization techniques tailored to specific future applications.

1. Introduction

Despite the promising features of enzymes, their low activity and stability often present significant barriers to large-scale applications, making it challenging for them to compete with traditional chemical processes.[1–3] A long-standing goal in biotechnology is to develop and understand design rules for stabilizing enzymes upon immobilization to materials.[4–6] Nanobiocatalysis, the integration of enzymes into nanostructured materials to enhance their resilience under harsher operational conditions, has rapidly emerged as a burgeoning field.[7–10] Nanostructures such as nanoporous media,[11–13] nanofibers,[14–18] carbon nanotubes,[19–21] and nanoparticles[22–24] demonstrate great efficiency in manipulating the nanoscale

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environment of enzymes, promising exciting advances in enzyme technology.

Nanofibers stand out due to their unique structure, high surface area-to-volume ratio, tunable porosity, superior mechanical properties, and ease of separation, making them ideal for enzyme immobilization.[25–30] According to research on Lens.org, adding the terms "enzyme", "immobilization", and "nano" yields 61 411 patents and 7088 articles, with 4574 patents and 1771 articles specifically related to nanofibers and nano-scaffolds. Notably, approximately 70% of these were released in the last ten years. Electrospinning is one of the simplest methods for fabricating nanofibers, ranging from one-dimensional to three-dimensional structures, using only a polymer matrix, syringe, and high voltage technique.[31,32] Therefore, the enzyme-induced electrospun nanofibers (EIEN) technique stands out as a highly promising method. In 2002, the first study immobilized α-chymotrypsin on the surface of modified polystyrene electrospun by covalent bonding, achieving over a 65% activity improvement compared to the free enzyme.[33] Combining the advantages of enzymes and nanofibers, EIEN have broadened their scope of use, including biomedical applications,[34–36] chemical production,[37–39] pollutant management,[40–42] food industry,[43–45] biosensor[46–48] and biofuels.[49,50] The use of electrospun nanofibers in these applications highlights their versatility and the advantages they offer over traditional materials.[27,51–53]

Immobilization techniques of EIEN can be divided into non-specific and specific methods.[52–54] Non-specific methods involve enzymes immobilization onto or into electrospun nanofibers. At present, due to the direct reaction, the interfacial immobilization methods (physical adsorption and covalent bonding) have the most extensive research.[48] Encapsulation of enzymes in nanofibers allows for uniform distribution and controlled release, suitable for applications like controlled drug delivery and sustained enzymatic reactions.[55,56] Non-specific enzyme immobilization can indiscriminately bind various enzymes, making them susceptible to adsorbing impurities during use, which may cause material contamination and decrease the efficiency of repeated applications.[57,58] In contrast, specific immobilization selectively targets a single enzyme or a particular class of enzymes, improving the quality, precision, and reliability of repeated use.[59,60] However, specific immobilization methods have been less researched due to their complexity and time-consuming properties.[61] Nevertheless, they hold promise for complex enzyme purification and the production of high value-added products.[62,63]

This review aims to provide a comprehensive overview of EIEN including their advanced fabrication methods and applications (Fig. 1). We will discuss non-specific immobilization methods including interfacial immobilization (direct adsorption, cross-linking, and covalent binding) and encapsulation techniques, as well as specific methods (coordination and affinity immobilization). Each method will be evaluated based on its mechanism, advantages, and limitations. We will also highlight recent advancements and practical applications, providing insights into selecting the optimal immobilization strategy for specific needs.

2. Based on non-specific immobilization techniques

2.1 Enzymes immobilization onto electrospun nanofibers

Electrospun nanofibers, with their fine structure and highly porous characteristics, offer a large specific surface area. The multilayer structure enhances the number of immobilization sites, promoting the uniform distribution and stable fixation of enzyme molecules in extreme environments.[64,65] Enzyme immobilization onto electrospun nanofibers through Interfacial methods, such as direct adsorption, cross-linking, and covalent binding, significantly provide easier access for

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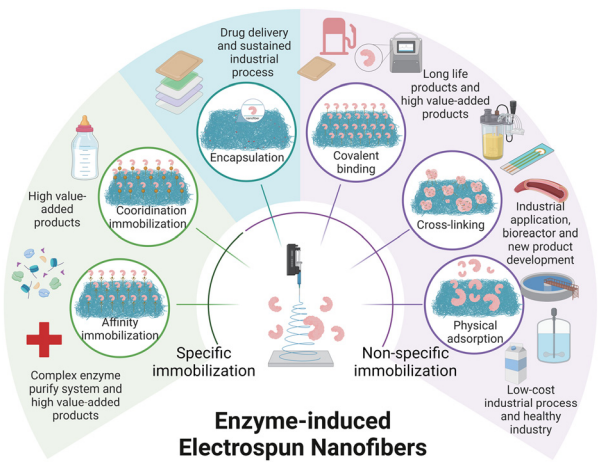


Fig. 1 Enzyme-induced electrospun nanofibers fabrication techniques and their related main applications in this review (2024 and created in BioRender).

substrates to active sites, making these methods simpler and more suitable for rapid reactions.[10,66,67]

2.1.1 Direct adsorption. The direct adsorption of enzymes does not alter their conformation, helping to retain enzyme activity as much as possible.[54] The mechanisms of adsorption involve weak interactions between enzymes and nanofibers, including electrostatic attraction, hydrophobic forces, and van der Waals forces.[68] However, weak interactions also mean that the binding is reversible and highly sensitive to process conditions like pH and temperature.[69,70] The unmodified interface of nanofibers lacks sufficient active sites (*e.g.*, hydroxyl, aldehyde, thiol, amino, carboxyl groups), which leads to limited adsorption capacity.[7,71] Sadly, electrospinning can increase the hydrophobicity of polymers, leading to the desorption of enzymes and causing them to shift towards an open conformation.

There are two main methods to enhance enzyme immobilization efficiency on the nanofibers through direct adsorption. (1) Mixing with natural polymers with functional groups. This affinity is driven by the polar interactions between the hydrophilic groups on both the enzyme and the substrate, facilitating a stronger and more stable binding.[72] Hydrophilic nanofiber supports, such as those made from chitosan or other polysaccharides, are particularly effective for immobilizing enzymes. Christ *et al.*[73] demonstrated that over 10 μg of eugenol oxidase per milligram of dry polymer matrix can be loaded onto UV-crosslinked chitosan nanofibers. Their studies further showed that bound enzyme activity was fully retained for over 7 days of storage under ambient conditions in aqueous buffer. (2) Surface modification of electrospun polymers. Atom transfer radical polymerization (ATRP) is a rising technology to graft various functional groups (hydroxyl and amino groups) onto the surfaces of nanofibers.[74] Zeng *et al.*[71] utilized ATRP to graft 2-(dimethylamino)ethyl methacrylate (DMAEMA) onto regenerated cellulose nanofiber (RC) to immobilize laccase. The highest immobilization amount achieved was 19.01 ± 0.87 mg mg^{-1}, approximately 3.3 times higher than the initial RC membrane. The method is also applied to immobilize galactose oxidase on vinyl sulfone (281 ± 20 μmol g^{-1}), carboxyl (560 ± 50 μmol g^{-1}), and laccase on amine groups (281 ± 20 μmol g^{-1}), respectively.[75] These results indicate that ATRP technology is a promising method to aid enzyme direct immobilization.

Direct adsorption methods are simple, low-cost, low-toxicity, and efficient for improving enzyme activity. Despite enhanced adsorption with modified supports, these methods also have several disadvantages, including high reversibility, weak adsorption force, limited adsorption capacity, and sensitivity to reaction conditions.[16] Based on these properties, they are suitable for disposable packages, food production, pollutant removal, and large-scale industrial applications.

One main advantage of this method is its non-toxicity, making it suitable for applications with strict safety requirements.[76] Chen *et al.*[77] designed and manufactured 0.5% gelatin-coated nanofiber peanuts immobilized with thrombin for use in hemostasis. The novel material exhibited a shorter blood clotting time and higher blood protein absorption capability compared to the other commercial Gauze, Gelfoam®, and Surgicel®. In addition to biomedical fields, this method is also used in food production. Zhu *et al.*[39] immobilized β-galactosidase on polystyrene (PS) electrospun nanofibers with functionalized graphene oxide (GO) to produce galactooligosaccharides (GOS). The enzyme adsorption rate of functional materials reached up to 87%, leading to improved catalytic behavior and transgalactosylation efficiency, with GOS synthesis and lactose conversion increasing to 72% and 81%, respectively. Sivas *et al.*[76] proved that the polycaprolactone and silk fibroin (PCL/SF) nanofibers for lactase immobilization slightly disrupted the oxidant–antioxidant balance without affecting zebrafish embryo mortality. Furthermore, Tunali-Akbay *et al.*[45] used the same materials, achieving hydrolysis of 42% of lactose in cow milk and 21% in goat milk.

For industrial applications, such as wastewater treatment and chemical production, cost is crucial. Kuang and coworkers[38] fabricated *Burkholderia cepacia* lipase (BCL)–SiO_2 nanofiber membrane as a bioreactor at the oil–water interface, exhibiting excellent enzyme capacity and retaining 83% activity after 5 catalytic cycles (Fig. 2a). And Zarei *et al.*[78] developed a conductive PCL-based nanohydrogel hybrid with cellulase enzymes to hydrolyze cellulose. The EIEN with a 96% enzyme immobilization efficiency retained 90% of its activity after 4 weeks and 73% after nine reuse cycles, exhibiting highly efficient catalytic conversion of cellulose.

In brief, the direct adsorption technique does not require complex chemical reactions or extensive modifications of the enzyme on the nanofiber surface, making it a straightforward, cost-effective, and non-toxic approach. Therefore, it is suitable for applications in food production and biomedical fields where safety is a priority, as well as in industrial processes where cost control is essential. Although various methods are applied to modify polymers with active sites, low immobilization efficiency, and high enzyme release remain the main challenges.

2.1.2 Cross-linking. Compared to direct immobilization, the cross-linking technique provides a stronger and more stable attachment of enzymes to the nanofiber surface, significantly reducing enzyme leaching and ensuring long-term stability under a wider range of conditions.[54] By using bifunctional or multifunctional reagents, covalent bonds are established between enzymes and a support matrix or between enzyme molecules, thus forming a network-like structure known as cross-linked enzyme aggregates (CLEAs).[79] CLEAs are easy to produce and can combine different enzymes for complex reactions. This approach enhances enzyme reusability and stability, minimizes the risk of enzyme loss, and preserves their high specificity.[80,81] For example, glutaraldehyde (GA), a commonly used cross-linker, forms CLEAs and immobilizes enzymes on supports by creating covalent bonds through Schiff base reactions with available amino groups. Kim *et al.*[82] immobilized lysozyme-CLEAs on the nascent chitosan/polyvinyl alcohol (CS/PVA) nanofibers by GA crosslinking retained more than 75.4% of its initial activity after 80 days of storage

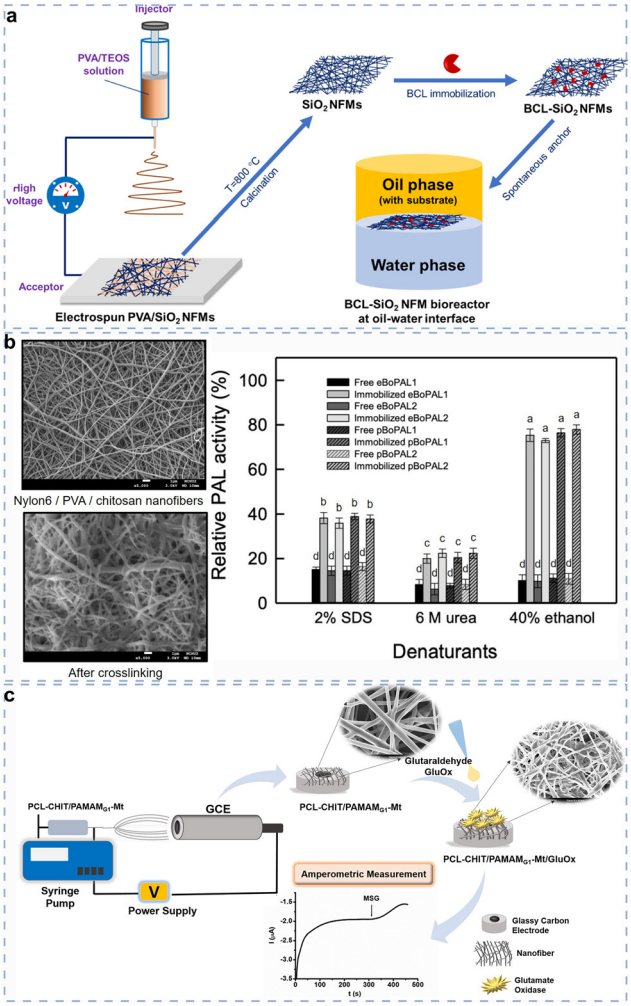


Fig. 2 (a) Construction of BCL–SiO_2 EIEN bioreactor and targeted catalysis at the oil–water interface.[38] Copyright © (2020 and ACS). (b) Images of EIEN nanofibers *via* cross-linking technique and denaturant tolerance of free and immobilized phenylalanine ammonia-lyases on electrospun nanofibers.[84] Copyright © (2021 and MDPI). (c) Glutamate oxidase ($GluO_x$)-induced biosensor *via* covalent binding on electrospun nanofibers.[47] Copyright © (2023 and MDPI).

at room temperature, while the free lysozyme lost all activity under the same conditions. Additionally, the immobilized lysozyme-electrospun nanofibers retained more than 76% of their activity after 100 catalytic cycles.

However, there are three main challenges in forming EIEN by cross-linking. (1) Absence of active sites. Similarly to adsorption, the lack of active sites (*e.g.*, amino groups) on the support matrix is a challenge. Natural polysaccharide polymers, such as cellulose triacetate, chitosan, gelatin, and chitin, can address this issue. de Melo Brites *et al.*[83] used cellulose triacetate to produce EIEN containing bromelain by cross-linking with glutaraldehyde, achieving an activity recovery of about 675%. *In vitro*, controlled release tests demonstrated a complete release process in three days. (2) Toxicity of cross-linker. GA is toxic and influences the growth of cells and organisms, which limits its usage. Polyalcohols can be good substitutes. For example, the immobilization efficiency of acetylcholinesterase (AChE) by sorbitol crosslinking on poly (acrylic acid) (PAA) nanofibers was 96%, and the nanofibers retained about 87% of their initial activity after 10 uses.[85] (3) Enzyme denaturation. Extensive cross-linking can result in enzyme denaturation. Optimizing enzyme concentration, glutaraldehyde concentration, and pH can mitigate this. For instance, optimizing the immobilization of laccase on polyethylene terephthalate (PET)-based EIEN resulted in the highest immobilization yield of 87.64%, an 81.2% increase from the yield before optimization.[86]

Due to its simple operation and higher stability, the cross-linking technique has wide application fields, including pollutant treatment, biosensors, chemical production, and biofuels, especially for industrial applications. Su *et al.*[87] developed a 3-layer cellulose membrane (RC10) bioreactor incorporating dextranase. The cross-linking technique significantly boosted low M_w oligosaccharide production to 11.5 μmol-isomaltose per min, far exceeding the 0.075 μmol-isomaltose per min achieved with adsorption-immobilized dextranase, maintaining a production rate of 11.3 μmol-isomaltose per min. This study underscores the potential of the designed system for efficiently producing stable low M_w oligosaccharides, offering valuable insights for optimizing enzyme immobilization strategies and membrane selection in enzymatic conversion processes. Moreover, Hsieh *et al.*[84] fabricated *trans*-cinnamic acid and ammonia to immobilize recombinant BoPAL1/2 phenylalanine ammonia-lyases onto nylon 6/PVA/CS electrospun nanofibers using dextran polyaldehyde as a cross-linking agent, retaining between 75% and 83% of their activity after storage at 4 °C for 30 days. The residual activities of free and immobilized PAL after treatment with 6 M urea, 2% sodium dodecyl sulfat (SDS), and 40% ethanol were 15%/38%, 8%/21%, and 10%/75%, respectively, indicating their strong potential for future industrial applications (Fig. 2b). This method is also used in industrial dye removal; PA6 loaded with horseradish peroxidase exhibited over 70% decolorization efficiencies after 60 minutes.[88] Additionally, cross-linking is applied in carbon capture and storage. Ng *et al.*[49] immobilized recombinant *Sulfurihydrogenibium yellowstonense* carbonic anhydrase (SyCA) on electrospun polyacrylonitrile (PAN) and PET nanofibers for CO_2 sequestration and pollution prevention. The EIEN exhibited 5.8-fold and 2.2-fold increases in $CaCO_3$ yields after 4 catalytic cycles compared to free enzymes, with excellent anti-interference capabilities, retaining 57% activity in the presence of 50 mM NO_x and 61% in the presence of 50 mM SO_x. Due to the enzyme network formation, high enzyme activity retention, milder reaction conditions, and significant flexibility with various types of enzymes and substrates, this method is widely used to provide a platform for enzyme loading. Alim *et al.*[46] reported a novel glucose biosensor utilizing co-immobilized glucose oxidase (GO_x) and horseradish peroxidase (HRP) on polymerized multiporous SnO_2 nanofibers with polyaniline. It displayed a linear response to glucose concentrations ranging from 5 to 100 μM, with a detection limit of 1.8 μM. The cross-linking method is also used in

vascular tissue engineering; Jia and coworkers[34] demonstrated that scaffolds immobilized with apyrase and 5′-nucleotidase improved antithrombotic performance and enhanced endothelialization, paving the way for more effective vascular grafts.

In short, compared to direct adsorption, the cross-linking method could form CLEAs between the enzymes, and covalent bonds between the enzyme and nanofibers, which significantly reduce enzyme leakage, and enhance stability, reproducibility, and anti-interference capabilities. The process is simple, leading to wide applications, including industrial production, pollutant removal, biosensors, and biomedical fields. However, certain crosslinking agents, especially GA, can be toxic or negatively impact enzyme activity. Additionally, the process may lead to uneven enzyme distribution on the carrier, and suboptimal crosslinking conditions could result in a loss of enzyme activity. Furthermore, crosslinked enzymes are difficult to regenerate or recycle, and identifying suitable substrates and agents often demands extensive experimentation and optimization.

2.1.3 Covalent binding. Covalent binding, like crosslinking, forms covalent bonds between enzymes and nanofibers. The nanofiber surface is first activated to introduce functional groups (such as hydroxyl, carboxyl, or amine groups), followed by the reaction of polymer chains with these sites under optimized conditions, applicable to both crosslinking and covalent bonding methods. However, covalent binding can form enzyme brushes with specific amino acid residues on the nanofibers, ensuring robust attachment, minimal leaching, and high tolerance.[89,90] Enzyme brushes maintain high enzymatic activity by minimizing spatial constraints, allowing more efficient catalysis. This method enables precise control over the enzyme density and distribution on the surface, optimizing catalytic performance and reducing interference between enzyme molecules. A widely used method for enzyme immobilization involves the application of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide/*N*-hydroxysuccinimide (EDC/NHS).[91] EDC activates the carboxyl groups of nanofibers, and NHS stabilizes the intermediate to form a reactive NHS ester, which reacts with amino groups on the enzyme to form a stable covalent amide bond. Golshaei *et al.*[92] constructed a novel biosensor using Au/poly [anthranilic acid-*co*-3-carboxy-*N*-(2-thenylidene)aniline/PVAc] electrospun nanofibers for the covalent immobilization of GO_x. The biosensor demonstrated good sensitivities in *N*,*N*-dimethylformamide (DMF) (7.24×10^6 Ω mM^{-1} cm^{-2}) and acetone (6.67×10^3 Ω mM^{-1} cm^{-2}) with impedance measurement.

Another method to introduce functional groups involves Haure *et al.*,[90] who endowed epoxide moieties on the surface of polyurethane nanofibers (Avalon 65 DB) to react with amino groups of HRP to form enzyme brushes. They found that immobilizing HRP at 40 °C resulted in the highest enzyme density (0.30 ± 0.02 mg cm^{-2}) but with inactivated catalytic activity, while matrices obtained at 20 °C exhibited the highest catalytic activity. Moreover, the incorporation of additives can significantly improve the effectiveness of immobilization. Liu *et al.*[93] utilized biocompatible feather polypeptide to stabilize enzyme conformation during covalent binding. The resulting poly(glycidyl methacrylate-*co*-methylacrylate)/feather polypeptide-lipase showed broad pH tolerance, high thermal stability, good reusability, and organic solvent stability. It retained about 40% activity after 3 hours at 70 °C, 62% after 7 reuses, and nearly 75% after 12 hours in methanol, outperforming previous results.

Although complex, covalent binding is well-suited for long-term use, providing enhanced stability and high performance. Shah *et al.*[94] designed functional cellulose nanofiber films for solar fuel production from CO_2 by immobilizing dendrimer and porphyrin derivatives and loading electron donors and enzymes (formate, aldehyde, and alcohol dehydrogenases). This setup facilitated the stepwise conversion of CO_2 to methanol, demonstrating high conversion efficiencies for intermediate steps. In addition to chemical production, uniform enzyme distribution, and stable bonds enhance biosensor performance. Odaci *et al.*[47] designed a biosensor for monosodium glutamate (MSG) detection using glutamate oxidase ($GluO_x$)-induced EIEN, achieving linear ranges from 0.025 to 0.25 mM with a detection limit of 1.045 μM. This biosensor effectively analyzed MSG content in tomato soup with a recovery percentage of 103.125% (Fig. 2c). In addition, the process could be treated without toxic reagents, so, the EIEN are also used in biomedical fields. Urbanek *et al.*[35] fabricated poly(lactide-*co*-glycolide) (PLGA)/chitosan-based EIEN with Auresine*Plus* by EDC/NHS treated, resulting in an efficient enzymatic attachment. This material exhibited strong antibacterial activity against antibiotic-resistant strains of *Staphylococcus aureus*. Similarly, Bösiger *et al.*[95] designed chitosan-PEO mats with GO_x (5.4 m^2 g^{-1}) for *in situ* hydrogen peroxide (H_2O_2) generation as an antibacterial system. The mats, functionalized *via* EDC/NHS covalent binding, produced a higher steady-state H_2O_2 concentration (~60 μM cm^{-2}) than cross-linking with EIEN (~50 μM), which significantly inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*.

Covalent binding provides uniform enzyme distribution and low-toxicity covalent binding enzyme nanofibers. These enzyme nanofibers offer strong binding, preventing enzyme loss, ensuring stable immobilization, long-term stability, and directional immobilization, keeping the active sites of the enzymes exposed correctly. Despite its complex process, irreversible enzyme immobilization, and potential reduction in enzyme activity, covalent binding is suitable for high value-added applications, such as biomedical fields, high-accuracy biosensors, and emerging fields like biofuels and carbon conversion, requiring high performance.

2.2 Enzymes immobilization into electrospun nanofibers

Enzyme immobilization into electrospun nanofibers involves entrapping enzymes within a nanofiber network, where the enzymes are mixed with a polymer solution or as the core of nanofibers by coaxial spinning technology without toxic reagents and complex process.[96,97] Compared to interfacial immobilization, encapsulation could make nanofibers as the

armor of enzymes to protect them from harsh external conditions (such as extreme pH, temperature fluctuations, and inhibitors), and controlled release.[98] PVA, a water-swollen polymer, is ideal for immobilizing enzymes, as it helps retain enzyme activity during the electrospinning process.[99] Duru Kamaci *et al.*[100] used CS/PVA blend polymer-based EIEN with phytase, resulting in fibers with an average diameter of about 65.3 ± 26.0 nm. The immobilized enzymes exhibited over 60% relative activity across a wide pH range (1–9) and temperatures (20–100 °C). Similarly, Wong *et al.*[101] utilized polyethylene oxide (PEO) nanofibers with Pluronic F-127 (F127) to immobilize β-galactosidase from *Aspergillus oryzae*, retaining up to 44% of enzyme activity after 4 weeks of storage. Moreover, the inactivation temperature of phytase in the encapsulated fibers was increased from 80 °C to 170 °C.[102]

However, many polymers to be applied in EIEN are water-resistant and can be toxic to enzymes.[103] To address this challenge, two main methods are used. The first method is coaxial electrospinning-encapsulation, which separates the enzymes and organic solution by physical space.[104] Ogawa *et al.*[15] designed a shell solution using polycaprolactone (PCL) in chloroform/DMF and a core solution using PVA EG-40P and lysozyme in distilled water. This preserved the enzyme's conformation during *in situ* encapsulation, resulting in high-activity enzyme nanofibers. Ji *et al.*[106] evaluated various α-chymotrypsin (CT) immobilization methods, including direct adsorption, cross-linking, encapsulation by mixture, and coaxial electrospinning-encapsulation. They found that coaxial electrospinning exhibited negligible mass transfer resistance, the lowest K_m, and the highest k_{cat} for both aqueous hydrolytic and nonaqueous esterification activities. The hollow structures provided unique stabilization, surpassing other methods in storage stability, thermostability (50 °C), and organic stability (methanol). The second method involves selecting enzyme protectors to retain enzyme activity in organic electrospinning solutions. Koplányi *et al.*[107] used surfactants with varying hydrophilic–lipophilic balance (HLB) values (Tween 80, Tween 85, Brij 30, Span 60, Span 40, Span 20) to enhance fiber formation and maintain the biocatalytic activity of *Petroselinum crispum* phenylalanine ammonia lyase (PcPAL). Tween 85 (HLB 11.0), Brij 30 (HLB 9.7), and Span 20 (HLB 8.6) positively influenced PcPAL's specific enzyme activity. Notably, Brij 30 led to a more than 6-fold increase in enzyme activity at higher loadings. Additionally, Cyclodextrins (CDs) have been proposed to manufacture laccase-loaded PCL-based nanofibers without activation loss or enzyme denaturation.[108,109] Protectors help enzymes withstand harsh environments, creating excellent enzyme-loaded nanofibers.

Due to high mass transfer resistance and low toxicity, encapsulation is suitable for sustained reaction conditions, such as drug delivery, long-time pollution treatment, and sustained food industrial.[106,110,111] Balogh-Weiser *et al.*[14] manufactured face masks based on lipase-loaded electrospun (polylactic acid (PLA) and polyvinylpyrrolidone (PVP)-based) nanofibers to treat acne vulgaris (Fig. 3a). The controllable release of lipase aids the penetration of active ingredients by mitigating increased sebum production on the patient's skin. Moreover, immobilizing lipase within nanofibers resulted in significantly higher enzyme activity compared to surface immobilization on nanofibers, with a 65% increase in activity.[112] This approach demonstrated outstanding performance, showing an 83% improvement under standard conditions and a 5% increase in specific activity compared to Mezym® forte 10 000, highlighting its potential as a novel alternative for enzyme-based oral therapies.

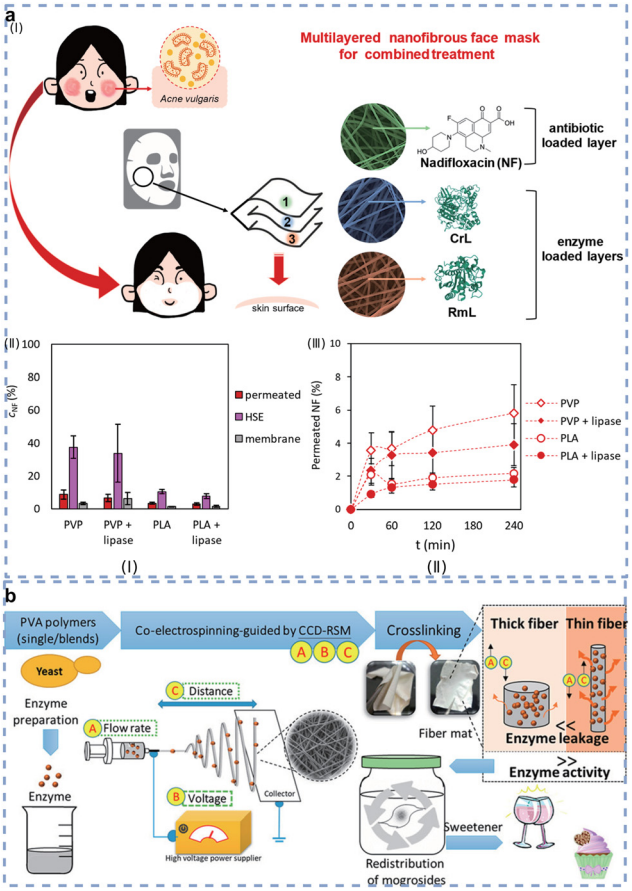


Fig. 3 (a) The composition of a multilayered nanofibrous face mask for combined treatment of acne vulgaris, and investigation of its penetration into human heat-separated epidermis (HSE) applying a topical and transdermal diffusion cell system.[14] Copyright © (2023 and MDPI). (b) Encapsulation of β-glucosidase within PVA fibers to produce specific mogroside sweetener.[105] Copyright © (2020 and ACS).

Furthermore, Duru Kamaci *et al.*[43] created PVA/sodium alginate (SA)-based nanofibers to immobilize phytate by encapsulation without the morphology influence of fibers. It exhibited a higher affinity (K_m = 4.66 mM) of substrates than that of free enzyme (K_m = 0.46 mM), showing potential for food industry applications. Virly *et al.*[105] explored the encapsulation of β-glucosidase within PVA fibers to produce specific mogroside sweeteners (Fig. 3b). The study demonstrated enhanced pH stability and increased tolerance to sodium dodecyl sulfate in the immobilized enzymes. In the batch process, the average production rate of siamenoside I was 118 ± 0.08 mg per L per

h per gram of fiber. Additionally, fructosyltransferase-EIEN showed significantly improved enzyme loading (68.1 mg g^{-1}) and activity (5.5 U mg^{-1}) compared to Sepabeads®, making it an up-and-coming candidate for the selective synthesis of various rare saccharides on an industrial scale from sucrose.[113] Furthermore, in a plug-flow reactor, the fibers demonstrated exceptional operational stability, maintaining 5% of the initial substrate conversion even after more than 2000 cycles. For sustained pollutant removal, encapsulated laccase demonstrated superior performance compared to direct adsorption and covalent binding, achieving over 99% biotransformation of 2,4,6-trinitrotoluene after 7 days.[114]

Encapsulation *via* direct electrospinning-embedment is a promising technique for enzyme immobilization, offering retention of enzyme activity, protection from harsh conditions, reusability, and controlled release. Using water-swollen polymers, enzyme protectors, and coaxial electrospinning techniques can improve enzyme stability. However, the participation of organic solutions limits the selection of polymers for enzyme encapsulation. Additionally, because most enzyme molecules are confined inside nanofibers, substrate accessibility to the enzyme can be inhibited.

As summarized and detailed in Table 1, this discussion covers the immobilization of nanofibers using non-specific

Table 1 Representative cases of enzyme-induced electrospun nanofibers (EIEN) with non-specific immobilization technique

Techniques	Application	Enzyme species	Enzyme supports	Performance	Ref.
Direct absorption	Food production	Lysozyme (*Micrococcus lysodetkticus*)	Bacterial cellulose	Retaining over 70% activity after 9 catalytic cycles.	16
Direct absorption	Food production	Lactose	Nnitrocellulose	With 59% lactose hydrolyzed in cow milk and 87% in goat milk using.	45
Direct absorption	Tea packages	Bromelain	PLA/SA	Improvement of hydrophilicity and water permeability	120
Cross-linking	Estrogens degradation	Laccase (*Trametes versicolor*)	PAN/polyethersulfone	92% degradation of 17β-estradiol and 100% degradation of 17α-ethynylestradiol within 24 hours; enzymatic conversion up to a reduction of estrogenic activities by around 99% for 17β-estradiol and 87% for 17α-ethynylestradiol.	121
Cross-linking	Biomedical	Jack bean urease (type III)	PVA/chitosan	Retaining 85% activity after 10 catalytic cycles and 45% after 20 catalytic cycles. Urea removal rates of artificial blood serum were 100% in the 1st cycle, 95% in the 2nd–4th, 85% in the 5th, 76% in the 6th, and 65% in the last three cycles.	122
Cross-linking	Blood vessels	Apyrase and 5′-nucleotidase	Hyaluronic acid-collagen/PCL	Improving antithrombotic performance; maintaining catalytic performance, reducing platelet adhesion and aggregation, and ensuring higher patency after 1 month *in situ* transplantation.	34
Covalent binding	Blood vessels	Extracted soluble proteins from aorta	Polyhedral oligomeric silsesquioxane-poly (carbonate-urea)-urethane	Enhancing cell viability and proliferation, antioxidant capacity, and hemocompatibility.	91
Covalent binding	CO_2 conversion	Alcohol dehydrogenase (*Saccharomyces cerevisiae*)	PS/copolymer of DL-lactide and glycolide (PDLG)	100% conversion for formaldehyde and ABTS at pH 6.5.	18
Covalent binding	Industrial removal of dyes.	Laccase (*T. versicolor*)	Nylon 6	Exhibiting 77% Reactive Blue 4 and 63% Reactive Black 5 removal rates after 24 hours; retaining over 70% activity after 10 catalytic cycles.	123
Covalent binding	Wastewater treatment	Laccase (*T. versicolor*)	PAA	Retaining 80% activity after 35 days; highly degradation rates of bisphenol A, 17α-ethinylestradiol, triclosan, and diclofenac, in wastewater over 14 days.	40
Covalent binding	Biofuel	Alcohol dehydrogenase (*Saccharomyces cerevisiae*)	PVP	Retaining 42% activity after 12 days of storage and repeated reaction cycles.	50
Encapsulation	Clinical diagnosis	GO_x and laccase	Cellulose acetate	Highly flexible self-powered glucose biosensor; excellent long-term stability in continuous works up to 15 h.	124
Encapsulation	Bioreactor	β-Galactosidase (*S. cerevisiae*)	PVA	An average siamenoside I production rate of 118 ± 0.08 mg per L per h per gram of fiber.	105
Encapsulation	Bioreactor	Laccase (*Trametes versicolor*)	PLA	Achieving a 79% isolated yield of the aldehyde during the oxidation of benzylamines.	125
Encapsulation	Drug delivery	Lysozyme	PVA	Exhibiting efficient entrapment with a drug content of 50%; rapid release within 30 minutes.	15

methods, specifically interfacial immobilization (direct adsorption, cross-linking, and covalent binding) and encapsulation methods. When applying EIEN in practical applications, several factors are typically considered, including enzyme bioactivity, immobilization efficiency, enzyme leaching rates, toxicity, and process complexity. For interfacial immobilization: (1) direct adsorption is suitable for instances where cost reduction is critical, such as in industrial production, as well as in food and biomedical industries where toxicity is a major concern. (2) Cross-linking is ideal for applications requiring low enzyme leakage, particularly biosensors, due to its high enzyme immobilization efficiency, enzyme structure retention, and compatibility. (3) Covalent binding is appropriate for applications needing long-term usages and high value-added products, such as in difficult waste treatment, biofuel production, and biomedical applications. For encapsulation methods, these are suitable for low-cost industrial applications and biomedical applications where controlled reaction rates are important. Thus, the optimal method for enzyme immobilization can be selected based on factors such as cost, toxicity, usage cycles, and the specific reaction pathway.

3. Based on specific immobilization techniques

3.1 Coordination immobilization

Coordination immobilization binds enzymes to a solid support through coordination bonds with metal ions (*e.g.*, Zn^{2+}, Ni^{2+}, or Cu^{2+}), utilizing the functional groups (such as carboxyl, hydroxyl, and thiol groups) on the certain enzymes (aminotransferases, proteases, dehydrogenases, and transaminases).[115,116] Compared to other immobilization techniques, coordination immobilization strikes a balance between the strong, permanent bonds seen in covalent immobilization and the weaker, reversible interactions typical of physical adsorption. The reversibility of coordination bonds allows for easy enzyme recovery and supports material reuse, offering a stable yet flexible approach ideal for applications where enzyme reuse and activity preservation are crucial. Teke *et al.*[117] designed PVA/Zn^{2+} EIEN with porcine pancreas lipase immobilization, maintaining 90% activity at 70 °C after 40 minutes and retaining 50% of its activity after 18 reuses. Additionally, they applied this method to immobilize AChE, which retained approximately 75% of its activity after 8 reuses and decreased below 50% activity after 12 reuses.[118] Park *et al.*[119] developed dual-functionalized PVA/PAA electrospun nanofibers incorporating α-chymotrypsin and copper ions (Cu(II)) to degrade extracellular polymeric substances (EPS) of bacteria. The results demonstrated a significant degradation of EPS proteins, reaching up to 0.26 mg mL^{-1} over 300 minutes, effectively reducing the number of planktonic and sessile *Pseudomonas aeruginosa* cells to enhance EIEN's anti-biofouling activity. Furthermore, $FeCl_3$ is added to enhance enzyme immobilization by ion coordination to carry laccase. Wu *et al.*[126] demonstrated an EIEN consisting of blend nanofibers of PU, amidoxime polyacrylonitrile (AOPAN), and β-CD for laccase immobilization with 186.34 mg g^{-1} immobilization efficiency. This setup exhibited significantly improved resistance to temperature and pH variations compared to free laccase. Li *et al.*[127] applied Fe(III)-PU/RC-poly(2-hydroxyethyl methacrylate)-laccase nanofibers to remove bisphenol A, with removal rates ranging from 87.3% to 75.4% over five cycles (Fig. 4a). Moreover, Šketa *et al.*[128] developed an EIEN for hexahistidine (His6)-tagged amine transaminases (ATAs), enhancing acetophenone production. The Cu^{2+} coated nanofibers (Tiss®-IMAC-Cu) achieved up to a 95.3% immobilization yield for the N-His6-ATA-wt enzyme using a one-step immobilization process from *Escherichia coli* lysate, resulting in enzyme loads of up to 1088 U mL^{-1}. In a continuous microreactor, this led to an 80% acetophenone yield from 40 mM (*S*)-α-methylbenzylamine in under 4 minutes. The system main-

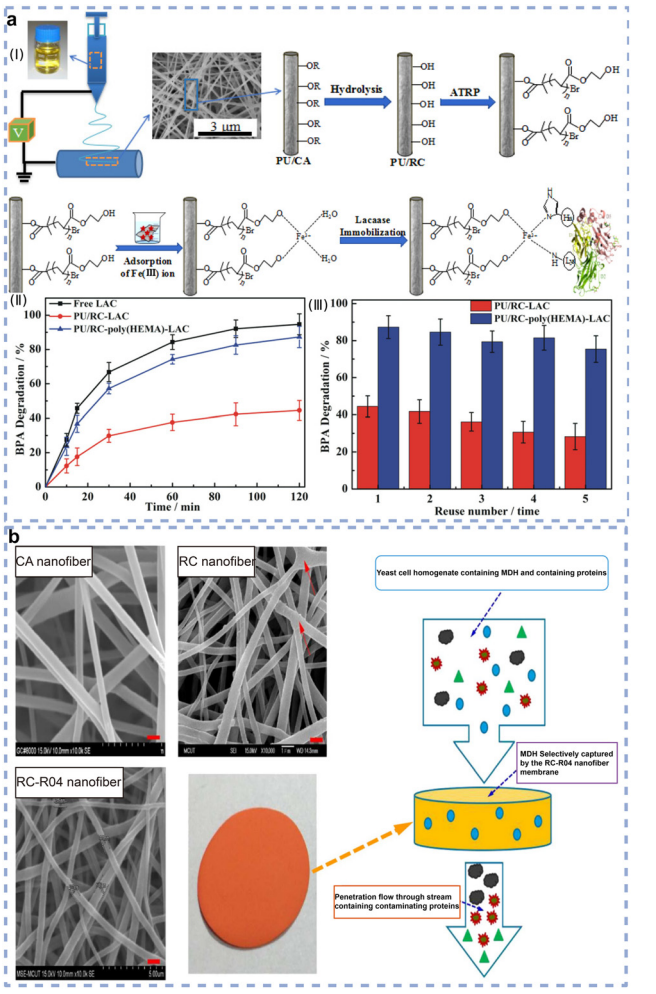


Fig. 4 (a) Schematic depicting the immobilization of laccase on PU/RC-poly(HEMA) nanofiber membrane and its removal efficiency of BPA.[127] Copyright © (2019 and Wiley). (b) Cellulose-based nanofiber membrane functionalized with dye affinity ligand for purification of malate dehydrogenase from *Saccharomyces cerevisiae*.[132] Copyright © (2022 and Springer).

tained 81% activity over 5 days, with the highest turnover number of 7.23×10^6, demonstrating industrial potential.

In conclusion, coordination immobilization offers a versatile and efficient method for the specific immobilization of enzymes with carboxyl, hydroxyl, and thiol groups, combining the benefits of specificity, stability, and reusability. As research continues to explore and refine these methods, coordination immobilization is poised to become an increasingly vital tool in enzyme technology.

3.2 Affinity immobilization

Affinity binding utilizes specific interactions between enzymes and affinity tags or ligands on the support material.[129] For instance, the high affinity of lysozyme with dye can be utilized to enhance the absorption process. The electrospun PAN nanofibers grafted with EDA and/or CS coupled with Reactive Blue 49 dye demonstrated that after 5 cycles of adsorption–desorption, there was no significant loss in lysozyme adsorption capacity.[130] Similarly, Hsu *et al.*[131] used Reactive Green 19 dye to enhance lysozyme immobilization efficiency. Under optimal conditions, the recovery yield and purification factor of lysozyme achieved from the one-step adsorption process were 98.52% and 143-fold, respectively. The dye-affinity nanofiber membrane also did not show any significant loss in binding capacity and purification performance after 5 consecutive uses. Given its excellent adsorption efficiency and durability, this approach holds promise for enhancing the applications of lysozyme in the food and pharmaceutical industries. Jian *et al.*[132] developed a RC nanofiber membrane functionalized with Reactive Orange 4 (RO4) dye for the one-step purification of malate dehydrogenase (MDH) from baker's yeast (Fig. 4b). The membrane exhibited optimal MDH adsorption at pH 7.5, with a dye density of 520 mg g^{-1} and a high binding capacity of 3985.65 U g^{-1}. Elution at pH 5 resulted in an 89% recovery and a 78-fold purification, highlighting this method for enzyme specific immobilization. The process was also scalable and reusable, maintaining efficiency with larger membrane contactors, showcasing its potential for large-scale applications. In addition to polymer modification, advancements in biotechnology have made it easier to modify enzymes to enhance their affinity for specific ligands. Jang *et al.*[133] utilized the strong interactions between polyhydroxybutyrate (PHB) nanofibers and phasins (PhaP) to design LipM7-phasins (from *Aeromonas hydrophila*) for fabricating EIEN. The EIEN loaded with LipM7-PhaP exhibited 3- to 10-fold higher lipase activity compared to Duolite A568 and Sipernat D17, retaining over 74% of its initial activity after 50 cycles. Additionally, approximately 70% of octanoic acid was converted to methyl octanoate after 60 hours in the transesterification of octanoic acid to methyl octanoate. These results highlight the strong PhaP-PHB affinity, paving the way for sustainable, reusable PHB nanofibers in enzymatic processes.

Briefly, while the affinity method is still being explored in various applications, it simplifies the enzyme separation process and enables high-purity enzyme immobilization, making it suitable for complex enzyme purification and high value-added industrial applications with stringent purity requirements. However, the specificity of enzymes and nanofibers may limit its wide applications.

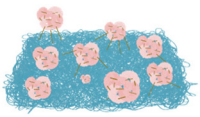
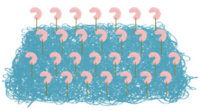
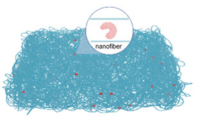
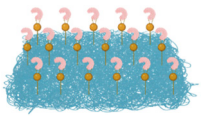
4. Future perspectives and conclusions

EIEN, developed over the past 20 years, remain a promising area for exploration. As mentioned above, various methods have been developed to immobilize enzymes on/in electrospun nanofibers, demonstrating excellent stability across a wide range of pH and temperatures, good mechanical properties, and reusability.[7] As summarized in Table 2, the main EIEN fabrication techniques and their related applications are detailed below.

While we have summarized these methods and their applications, increasingly diverse combinations are being explored to create superior EIEN. These combined approaches allow for controlled enzyme release and enhance reusability, reducing operational costs. Without crosslinking, PVA lacks the mechanical strength and stability required for many industrial and biomedical uses.[134,135] Sengor *et al.*[97] produced gelatin-containing nanofibers *in situ* cross-linked with microbial transglutaminase (mTG) by encapsulation and cross-linking, resulting in nanofibers with a uniform, bead-free morphology. Maryskova *et al.*[136] developed a method for immobilizing laccase onto PA6 nanofibers using adsorption and cross-linking. This method significantly degraded a 50 μM EDC mixture, removing BPA (93%), 17α-ethinylestradiol (98%), and triclosan (70%). This hybrid method also enhances biocompatibility, often using mild conditions and low-toxicity cross-linkers, making it suitable for various biotechnological applications. Enzymes in a polymer nanofiber matrix can improve activity retention and dry storage stability compared to traditional immobilization methods and enable rapid dissolution for ease of use. Additionally, a novel fabricated concept of EIEN is also proposed. Ye *et al.*[137] designed hierarchical biocatalytic membranes embedded with trypsin–inorganic hybrid nanoflowers to hydrolyze β-lactoglobulin. The ethylene vinyl alcohol copolymer (EVAL)-based 3D scaffolding, leading 282.1 μg cm^{-2} trypsin loading efficiency and 98.1% hydrolysis rate for β-lactoglobulin. Badoei-dalfard *et al.*[138] combined metal–organic framework fabrication and EIEN techniques to design zirconium-MOF/PVP nanofibrous composites to immobilize MG10 lipase for biodiesel production. The highest biodiesel yield (27%) from *Ricinus communis* oil was achieved after 18 hours of incubation and produced about 83% biodiesel in just 12 hours.

Building on the development of enzymes and electrospun nanofibers, EIEN's versatile properties make it suitable for a wide range of applications. These include environmental, energy, and medical fields, as well as textiles, wearables, agriculture, and advanced materials in the future.[27,51,52] Future research should focus on industrialization to reduce costs, improve the activity of enzyme-induced nanofibers, and enable

Table 2 Comparison of main EIEN fabrication techniques

Classification	Diagram	Construction	Advantages	Limitations	Potential applications	Ref.
Non-specific immobilization (direct adsorption)		Weak interactions (electrostatic attraction, hydrophobic forces, and van der Waals forces) between enzymes and nanofibers.	Simple process, low cost, low toxicity.	High reversibility, weak adsorption force, limited adsorption capacity, and sensitivity to reaction conditions	Disposable packages, food production, pollutant removal, and large-scale industrial applications.	7, 54 and 69–71
Non-specific immobilization (cross-linking)		Covalent bonds between CLEAs and nanofibers.	Reduction in enzyme leakage, enhancement of stability, reproducibility, and anti-interference capabilities	Potential toxicity, uneven enzyme distribution on the carrier, and enzyme activity loss due to improper cross-linking conditions.	Industrial production, pollutant removal, biosensors, and biomedical fields.	79, 81, 82 and 85
Non-specific immobilization (covalent binding)		Covalent bonds between enzymes and nanofibers, forming enzyme brushes.	Uniform enzyme distribution, low toxicity, prevention of enzyme loss, long-term stability, and directional immobilization with correctly exposed active sites.	Complex process, irreversible enzyme immobilization, potential reduction in enzyme activity.	High value-added applications, such as biomedical fields, high-accuracy biosensors, and emerging fields like biofuels and carbon conversion.	35, 40, 44 and 92
Non-specific immobilization (encapsulation)		Entrapping enzymes within a nanofiber network.	Protection of enzymes from harsh conditions, controlled release, enhanced enzyme stability and reusability, and low toxicity.	High mass transfer resistance, potential changes in enzyme conformation, limited polymer selection, and a complex preparation process.	Sustained reaction and toxicity-limiting conditions, such as drug delivery, long-term pollution treatment, and extended food processing.	43, 101, 103 and 106
Specific immobilization (coordination immobilization)		Coordination bonds with metal ions (*e.g.*, Zn^{2+}, Ni^{2+}, or Cu^{2+}), and certain enzymes (aminotransferases, proteases, dehydrogenases, and transaminases) with functional groups (such as carboxyl, hydroxyl, and thiol groups).	High specificity, enhanced stability, controlled orientation, and facilitated electron transfer.	Requirement for precise control of the coordination environment, potential toxicity of metal ions, and limited suitability for certain enzyme types.	Protein purification, biosensor, and bioreactor.	117–119
Specific immobilization (affinity immobilization)		Affinity bonds with enzymes and affinity tags or ligands.	High-purity enzyme immobilization, and potential for smart recognition.	Complex process, nascent research, and limitation of enzymes.	Protein purification, diagnostic assays, and high value-added therapeutic applications.	130–132

mass production of nanofibers. The main current limitations are as follows: (1) limitation of polymers. Most polymers lack functional groups to immobilize enzymes, limiting immobilization efficiency. Therefore, novel polymers or simple nanofiber modification methods should be explored to immobilize enzymes through interfacial immobilization. (2) Limitation of enzymes. Although bio-enzymes exhibit unique activity, they are prone to losing their activity during the EIEN process. Particularly during encapsulation, enhancing the enzymes' ability to withstand organic solvents would broaden the selection of polymers and simplify the process.[139] Modifying and exploring enzymes[140–142] or producing nano-enzymes[143–145] could address these issues. (3) Limitation of electrospun nanofibers technique. Currently, most applications are at the laboratory scale. Despite the development of devices such as Nanospider™ (Elmarco), NS Lab™ (Inovenso), and Nano

Spider M™ (Kato Tech), challenges remain in large-scale production, consistency, scalability, controllable fiber characteristics, environmental issues, and cost-effectiveness. Despite these challenges, EIEN offer a versatile method for wide-ranging applications by combining the benefits of enzymes and nanofibers.

In conclusion, we summarize recent advances in enzyme immobilization methods and applications to discuss potential development trends. We aim to analyse the relationship between immobilization techniques and applications to provide guidance for selecting the optimal approach effectively.

Author contributions

Writing – original draft preparation L. F.; writing – review and editing, L. F., X. M., Y. H., W. Z.; supervision, W. D., P. W., M. J. All the authors discussed and commented on the manuscript.

Data availability

No primary research results, software, or code have been included and no new data was generated or analysed as part of this review.

Conflicts of interest

There are no conflicts to declare.

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References

1 S. Arana-Pena, D. Carballares, R. Morellon-Sterlling, A. Berenguer-Murcia, A. R. Alcantara, R. C. Rodrigues and R. Fernandez-Lafuente, *Biotechnol. Adv.*, 2021, **51**, 107584.
2 Z. Ashkan, R. Hemmati, A. Homaei, A. Dinari, M. Jamlidoost and A. Tashakor, *Int. J. Biol. Macromol.*, 2021, **168**, 708–721.
3 R. C. Rodrigues, A. Berenguer-Murcia, D. Carballares, R. Morellon-Sterling and R. Fernandez-Lafuente, *Biotechnol. Adv.*, 2021, **52**, 107821.
4 H. Y. Chen, Y. Ting, H. C. Kuo, C. W. Hsieh, H. Y. Hsu, C. N. Wu and K. C. Cheng, *Int. J. Biol. Macromol.*, 2021, **172**, 270–280.
5 M. Ma, Y. Yang, Z. Huang, F. Huang, Q. Li and H. Liu, *Nanoscale*, 2024, **16**, 1600–1632.
6 W. Wang, Z. Ma, Q. Shao, J. Wang, L. Wu, X. Huang, Z. Hu, N. Jiang, J. Dai and L. He, *Nanoscale*, 2024, **16**, 12586–12598.
7 R. S. Khan, A. H. Rather, T. U. Wani, S. U. Rather, T. Amna, M. S. Hassan and F. A. Sheikh, *Biotechnol. Bioeng.*, 2023, **120**, 22–40.
8 G. Chen, S. Huang, X. Ma, R. He and G. Ouyang, *Nat. Protoc.*, 2023, **18**, 2032–2050.
9 T. Rasheed and K. Rizwan, *Biosens. Bioelectron.*, 2022, **199**, 113867.
10 M. Bilal, M. Asgher, H. Cheng, Y. Yan and H. M. N. Iqbal, *Crit. Rev. Biotechnol.*, 2019, **39**, 202–219.
11 X. Peng, C. Pei, E. W. Qian, Y. Du and J. J. Li, *Nanoscale*, 2024, **16**, 14932–14939.
12 Y. Du, X. Jia, L. Zhong, Y. Jiao, Z. Zhang, Z. Wang, Y. Feng, M. Bilal, J. Cui and S. Jia, *Coord. Chem. Rev.*, 2022, **454**, 214327.
13 X. Li, Z. Wu, X. Tao, R. Li, D. Tian and X. Liu, *J. Hazard. Mater.*, 2022, **438**, 129525.
14 D. Balogh-Weiser, A. Molnar, G. D. Toth, G. Koplanyi, J. Szemes, B. Decsi, G. Katona, M. Salamah, F. Ender, A. Kovacs, S. Berko, M. Budai-Szucs and G. T. Balogh, *Pharmaceutics*, 2023, **15**, 1174.
15 R. Ogawa, K. Hara, A. Kobayashi, N. Yoshimura, Y. Taniguchi, E. Yamazoe, T. Ito and K. Tahara, *Chem. Pharm. Bull.*, 2024, **72**, 324–329.
16 P. Bayazidi, H. Almasi and A. K. Asl, *Int. J. Biol. Macromol.*, 2018, **107**, 2544–2551.
17 F. Zhang, M. Lian, A. Alhadhrami, M. Huang, B. Li, G. A. M. Mersal, M. M. Ibrahim and M. Xu, *Adv. Compos. Hybrid Mater.*, 2022, **5**, 1852–1864.
18 K. Jankowska, Z. Su, S. B. Sigurdardóttir, M. Staszak, M. Pinelo, J. Zdarta and T. Jesionowski, *Bioorg. Chem.*, 2021, **114**, 105036.
19 R. Singh, Z. Wang, C. Marques, R. Min, B. Zhang and S. Kumar, *Biosens. Bioelectron.: X*, 2023, **236**, 115424.
20 T. D. Ros, A. Ostric, F. Andreola, M. Pietrogrande, F. Corsolini, M. Stroppiano, S. Bruni, A. Serafino and S. Fiorito, *Nanoscale*, 2018, **10**, 657–665.
21 S. Gupta and N.-H. Tai, *J. Taiwan Inst. Chem. Eng.*, 2024, **154**, 104957.
22 E. Parandi, M. Safaripour, N. Mosleh, M. Saidi, H. Rashidi Nodeh, B. Oryani and S. Rezania, *Biomass Bioenergy*, 2023, **173**, 106794.
23 B. Shao, Z. Liu, G. Zeng, Y. Liu, X. Yang, C. Zhou, M. Chen, Y. Liu, Y. Jiang and M. Yan, *J. Hazard. Mater.*, 2019, **362**, 318–326.
24 M. Bilal, Y. Zhao, T. Rasheed and H. M. N. Iqbal, *Int. J. Biol. Macromol.*, 2018, **120**, 2530–2544.
25 L. Zhou, J. Han, J. Xiao, X. Yang and S. Chen, *Small*, 2023, **19**, 2301310.

26 H. Qiu, X. Qu, Y. Zhang, S. Chen and Y. Shen, *Adv. Mater.*, 2023, **35**, 2302326.
27 D. Ji, Y. Lin, X. Guo, B. Ramasubramanian, R. Wang, N. Radacsi, R. Jose, X. Qin and S. Ramakrishna, *Nat. Rev. Methods Primers*, 2024, **4**, 1.
28 A. J. Robinson, A. Pérez-Nava, S. C. Ali, J. B. González-Campos, J. L. Holloway and E. M. Cosgriff-Hernandez, *Matter*, 2021, **4**, 821–844.
29 C. Işik, *ACS Omega*, 2024, **9**, 31313–31327.
30 K. Puttananjegowda, A. Takshi and S. Thomas, *Biosens. Bioelectron.: X*, 2021, **186**, 113285.
31 J. Zhang, M. Yu and S. Tao, *Nano Res.*, 2024, **17**, 7077–7116.
32 Electrospinning of nanofibres, *Nat. Rev. Methods Primers*, 2024, **4**, 2, DOI: **10.1038/s43586-023-00289-w**.
33 H. Jia, G. Zhu, B. Vugrinovich, W. Kataphinan, D. H. Reneker and P. Wang, *Biotechnol. Prog.*, 2002, **18**, 1027–1032.
34 W. Jia, L. Liu, M. Li, Y. Zhou, H. Zhou, H. Weng, G. Gu, M. Xiao and Z. Chen, *Acta Biomater.*, 2022, **153**, 287–298.
35 O. Urbanek, A. Wysocka, P. Nakielski, F. Pierini, E. Jagielska and I. Sabała, *Pharmaceutics*, 2021, **13**, 711.
36 K. Furuno, K. Suzuki and S. Sakai, *Biomolecules*, 2022, **12**, 1638.
37 E. S. Ribeiro, B. S. Farias, T. R. S. C. Junior, L. A. de A. Pinto and P. S. Diaz, *Int. J. Biol. Macromol.*, 2021, **183**, 1959–1970.
38 L. Kuang, Q. Zhang, J. Li and H. Tian, *J. Agric. Food Chem.*, 2020, **68**, 8362–8369.
39 A. Chenafa, A. A. A. Abdo, A. A. Mahdi, Q. Zhang, C. Chen, Y. Zhu, J. Li, G. Fan and J. Liu, *Int. J. Biol. Macromol.*, 2024, **270**, 132312.
40 M. Maryskova, M. Vrsanska, A. Sevcu, V. Novotny, A. Blahutova and S. Voberkova, *Environ. Technol. Innovation*, 2022, **26**, 102316.
41 J. Zdarta, K. Jankowska, K. Bachosz, E. Kijeńska-Gawrońska, A. Zgoła-Grześkowiak, E. Kaczorek and T. Jesionowski, *Catal. Today*, 2020, **348**, 127–136.
42 D. Zhao, M. Louise Leth, M. Abou Hachem, I. Aziz, N. Jančič, T. Luxbacher, C. Hélix-Nielsen and W. Zhang, *Chem. Eng. J.*, 2023, **451**, 138902.
43 U. Duru Kamaci and A. Peksel, *Catal. Lett.*, 2020, **151**, 1–831.
44 A. C. Sass and H. J. Jordening, *Appl. Biochem. Biotechnol.*, 2020, **191**, 1155–1170.
45 S. Yılmaz-Karaoğlu, B. Gürel-Gökmen and T. Tunali-Akbay, *Food Biosci.*, 2022, **49**, 101828.
46 S. Alim, A. K. M. Kafi, J. Rajan and M. M. Yusoff, *Int. J. Biol. Macromol.*, 2019, **123**, 1028–1034.
47 H. Atilgan, B. Unal, E. E. Yalcinkaya, G. Evren, G. Atik, F. Ozturk Kirbay, N. M. Kilic and D. Odaci, *Biosensors*, 2023, **13**, 430.
48 P. Bosiger, G. Tegl, I. M. T. Richard, L. Le Gat, L. Huber, V. Stagl, A. Mensah, G. M. Guebitz, R. M. Rossi and G. Fortunato, *Carbohydr. Polym.*, 2018, **181**, 551–559.
49 S. S. W. Effendi, C. Y. Chiu, Y. K. Chang and I. S. Ng, *Int. J. Biol. Macromol.*, 2020, **152**, 930–938.
50 I. Aziz, S. B. Sigurdardottir, J. Lehmann, A. Nambi, W. Zhang, M. Pinelo and A. Kaiser, *Nanotechnology*, 2022, **33**, 435601.
51 A. M. Al-Dhahebi, J. Ling, S. G. Krishnan, M. Yousefzadeh, N. K. Elumalai, M. S. M. Saheed, S. Ramakrishna and R. Jose, *Appl. Phys. Rev.*, 2022, **9**, 011319.
52 L. A. Mercante, R. S. Andre, M. H. M. Facure, D. S. Correa and L. H. C. Mattoso, *Chem. Eng. J.*, 2023, **465**, 142847.
53 Y. Zhang, X. Rui and B. K. Simpson, *Curr. Opin. Food Sci.*, 2021, **37**, 10–16.
54 D. M. Liu, J. Chen and Y.-P. Shi, *TrAC, Trends Anal. Chem.*, 2018, **102**, 332–342.
55 S. Haghju, M. R. Bari and M. A. Khaled-Abad, *Carbohydr. Polym.*, 2018, **200**, 137–143.
56 A. Luraghi, F. Peri and L. Moroni, *J. Controlled Release*, 2021, **334**, 463–484.
57 W. Zhou, Y. Rao, W. Zhuang, L. Ge, R. Lin, T. Tang, J. Wu, M. Li, P. Yang, C. Zhu, H. Wang and H. Ying, *Composites, Part B*, 2021, **216**, 108788.
58 S. Qiao, X. Zheng, Y. Ou, T. Li, X. Zhao, J. Quan, X. Zhao and Q. Li, *Colloids Surf., B*, 2024, **236**, 113818.
59 S. Guo, S. Liu, C. Liu, Y. Wang, D. Gu, J. Tian and Y. Yang, *Int. J. Biol. Macromol.*, 2024, **268**, 131697.
60 Q. Ma, B. He, G. Tang, R. Xie and P. Zheng, *Molecules*, 2023, **28**, 379.
61 M. S. Mohd Syukri, Z. Mohamad, R. A. Rahman, R. M. Illias and H. Tokuyama, *J. Chem. Technol. Biotechnol.*, 2021, **97**, 140–146.
62 C. Coscolín, A. Beloqui, M. Martínez-Martínez, R. Bargiela, G. Santiago, R. M. Blanco, G. Delaittre, C. Márquez-Álvarez and M. Ferrer, *Appl. Catal., A*, 2018, **565**, 59–67.
63 X. Huang, S. Sang, Z. Yuan, Q. Duan, X. Guo, H. Zhang and C. Zhao, *ACS Sens.*, 2021, **6**, 3933–3939.
64 S. Osali, Y. Ghiyasi, H. Esfahani, R. Jose and S. Ramakrishna, *Mater. Today*, 2023, **67**, 151–177.
65 S. Smith, K. Goodge, M. Delaney, A. Struzyk, N. Tansey and M. Frey, *Nanomaterials*, 2020, **10**, 2142.
66 R. C. Rodrigues, J. J. Virgen-Ortíz, J. C. S. dos Santos, Á. Berenguer-Murcia, A. R. Alcantara, O. Barbosa, C. Ortiz and R. Fernandez-Lafuente, *Biotechnol. Adv.*, 2019, **37**, 746–770.
67 R. C. Rodrigues, Á. Berenguer-Murcia, D. Carballares, R. Morellon-Sterling and R. Fernandez-Lafuente, *Biotechnol. Adv.*, 2021, **52**, 107821.
68 L. Xu, J. Wang, A. Zhang, Y. Pang, D. Yang, H. Lou and X. Qiu, *J. Colloid Interface Sci.*, 2023, **647**, 318–330.
69 M. Bilal and H. M. N. Iqbal, *Coord. Chem. Rev.*, 2019, **388**, 1–23.
70 J. Zdarta, K. Jankowska, M. Wyszowska, E. Kijeńska-Gawrońska, A. Zgoła-Grześkowiak, M. Pinelo, A. S. Meyer, D. Moszyński and T. Jesionowski, *Mater. Sci. Eng., C*, 2019, **103**, 109789.
71 S. Zeng, J. Shi, A. Feng and Z. Wang, *Polymers*, 2021, **13**, 182.

72 F. Rafiee and M. Rezaee, *Int. J. Biol. Macromol.*, 2021, **179**, 170–195.
73 H. A. Christ, N. P. Daniel, J. Solarczek, L. S. Fresenborg, A. Schallmey and H. Menzel, *Appl. Microbiol. Biotechnol.*, 2023, **107**, 7071–7087.
74 Z. Zhang, X. Wang, K. C. Tam and G. Sèbe, *Carbohydr. Polym.*, 2019, **205**, 322–329.
75 A. L. Medina-Castillo, L. Ruzic, B. Nidetzky and J. M. Bolivar, *ACS Appl. Polym. Mater.*, 2022, **4**, 6054–6066.
76 G. G. Sivas, İ. Ünal, B. Gürel-Gökmen, E. Emekli-Alturfan and T. Tunalı Akbay, *Food Chem. Toxicol.*, 2024, **191**, 114871.
77 S. Chen, M. A. Carlson, Y. S. Zhang, Y. Hu and J. Xie, *Biomaterials*, 2018, **179**, 46–59.
78 A. Zarei, F. Alihosseini, D. Parida, R. Nazir and S. Gaan, *ACS Appl. Mater. Interfaces*, 2021, **13**, 49816–49827.
79 A. N. Ademakinwa, *J. Hazard. Mater.*, 2021, **419**, 126480.
80 N. Chen, B. Chang, N. Shi, W. Yan, F. Lu and F. Liu, *Crit. Rev. Biotechnol.*, 2023, **43**, 369–383.
81 I. O. Costa, J. R. F. Morais, J. M. M. Dantas, L. R. B. Gonçalves, E. S. Santos and N. S. Rios, *Enzyme Microb. Technol.*, 2023, **170**, 110300.
82 J. M. Park, M. Kim, H. S. Park, A. Jang, J. Min and Y. H. Kim, *Int. J. Biol. Macromol.*, 2013, **54**, 37–43.
83 M. de Melo Brites, A. A. Cerón, S. M. Costa, R. C. Oliveira, H. G. Ferraz, L. H. Catalani and S. A. Costa, *Enzyme Microb. Technol.*, 2020, **132**, 109384.
84 P. Y. Hong, Y. H. Huang, G. C. W. Lim, Y. P. Chen, C. J. Hsiao, L. H. Chen, J. Y. Ciou and L. S. Hsieh, *Int. J. Mol. Sci.*, 2021, **22**, 11184.
85 B. Cakiroglu, A. B. Cigil, A. Ogan, M. V. Kahraman and S. Demir, *Eng. Life Sci.*, 2018, **18**, 254–262.
86 M. S. Mohd Syukri, R. A. Rahman, Z. Mohamad, R. Md Illias, N. A. Nik Mahmoo and N. R. Jaafar, *Int. J. Biol. Macromol.*, 2021, **166**, 876–883.
87 Z. Su, K. Jankowska, S. Björk Sigurdardóttir, W. A. Zhang, A. Kaiser, J. Luo and M. Pinelo, *Chem. Eng. Sci.*, 2024, **283**, 119367.
88 K. Jankowska, J. Zdarta, A. Grzywaczyk, O. Degórska, E. Kijeńska-Gawrońska, M. Pinelo and T. Jesionowski, *Process Biochem.*, 2021, **102**, 10–21.
89 T. Marcelino, M. A. R. Docampo, X. Qian, C. Ade, E. Brodszkij, M. Ceccato, M. Foss, M. Dulchavsky, J. C. A. Bardwell and B. Städler, *Macromol. Biosci.*, 2023, **8**, 2200528.
90 D. A. Morales Urrea, P. C. Caracciolo, P. M. Haure and E. M. Contreras, *Polym. Adv. Technol.*, 2021, **32**, 4902–4914.
91 Z. Zakeri, R. Salehi, R. Rahbarghazi, Y. D. Taghipour, M. Mahkam and E. Sokullu, *Int. J. Biol. Macromol.*, 2024, **277**, 134064.
92 R. Golshaei, T. Karazehir, S. M. Ghoreishi, M. Ates and A. S. Sarac, *Polym. Bull.*, 2016, **74**, 1493–1517.
93 X. Liu, Y. Fang, X. Yang, Y. Li and C. Wang, *Colloids Surf., B*, 2018, **166**, 277–285.
94 K. J. Shah and T. Imae, *J. Mater. Chem. A*, 2017, **5**, 9691–9701.
95 P. Bösiger, G. Tegl, I. M. T. Richard, L. Le Gat, L. Huber, V. Stagl, A. Mensah, G. M. Guebitz, R. M. Rossi and G. Fortunato, *Carbohydr. Polym.*, 2018, **181**, 551–559.
96 M. Sengor, A. Ozgun, O. Gunduz and S. Altintas, *Mater. Lett.*, 2020, **263**, 127233.
97 M. M. Aldhahri, Y. Q. Almulaiky, R. M. El-Shishtawy, W. Al-Shawafi, A. Alngadh and R. Maghrabi, *ACS Omega*, 2018, **3**, 6346–6350.
98 A. H. Rather, R. S. Khan, T. U. Wani, M. A. Beigh and F. A. Sheikh, *Biotechnol. Bioeng.*, 2022, **119**, 9–33.
99 N. Guajardo, *Polymers*, 2023, **15**, 2073–4360.
100 U. Duru Kamaci and A. Peksel, *Int. J. Biol. Macromol.*, 2020, **164**, 3315–3322.
101 D. E. Wong, M. Dai, J. N. Talbert, S. R. Nugen and J. M. Goddard, *J. Mol. Catal. B: Enzym.*, 2014, **110**, 16–22.
102 U. A. Rathnayake, T. Senapathi, C. Sandaruwan, S. Gunawardene, V. Karunaratne and N. Kottegoda, *Chem. Cent. J.*, 2018, **12**, 28.
103 M. Heredero and A. Beloqui, *ChemBioChem*, 2023, **24**, e202200611.
104 Y. Tanikawa, Y. Ido, R. Ando, A. Obata, K. Nagata, T. Kasuga and T. Mizuno, *Bull. Chem. Soc. Jpn.*, 2020, **93**, 1155–1163.
105 Virly, C.-H. Chiu, T.-Y. Tsai, Y.-C. Yeh and R. Wang, *J. Agric. Food Chem.*, 2020, **68**, 11790–11801.
106 X. Ji, Z. Su, C. Liu, P. Wang and S. Zhang, *Biochem. Eng. J.*, 2017, **121**, 147–155.
107 G. Koplányi, E. Sánta-Bell, Z. Molnár, G. D. Tóth, M. Józó, A. Szilágyi, F. Ender, B. Pukánszky, B. G. Vértessy, L. Poppe and D. Balogh-Weiser, *Catalysts*, 2021, **11**, 1149.
108 M. F. Canbolat, H. B. Savas and F. Gultekin, *Anal. Biochem.*, 2017, **528**, 13–18.
109 M. F. Canbolat, H. B. Savas and F. Gultekin, *J. Appl. Polym. Sci.*, 2017, **134**, 44404.
110 M. C. Mazzocato and J. Jacquier, *Foods*, 2024, **13**, 2127.
111 K. Sharma, P. Tewatia, M. Kaur, D. Pathania, F. Banat, G. Rattan, S. Singhal and A. Kaushik, *Sci. Total Environ.*, 2023, **864**, 161137.
112 G. D. Tóth, N. Kállai-Szabó, M. Lengyel, K. Süvegh, F. Ender, G. Katona, A. Kazsoki, R. Zelkó, I. Antal, G. T. Balogh and D. Balogh-Weiser, *J. Mol. Liq.*, 2023, **389**, 122819.
113 J. Gabrielczyk, T. Duensing, S. Buchholz, A. Schwinges and H.-J. Jördening, *Appl. Biochem. Biotechnol.*, 2018, **185**, 847–862.
114 L. Alvarado-Ramírez, S. E. Rodríguez-De Luna, J. Rodríguez-Rodríguez, M. d. J. Rostro-Alanis and R. Parra-Saldívar, *Int. Biodeterior. Biodegrad.*, 2024, **190**, 105771.
115 K. Çetin, *J. Appl. Polym. Sci.*, 2023, **140**, e54404.
116 J. Dong, G. Liu, Y. V. Petrov, Y. Feng, D. Jia, V. E. Baulin, A. Y. Tsivadze, Y. Zhou and B. Li, *ACS Mater. Lett.*, 2024, **6**, 1112–1119.

117 C. Isik, G. Arabaci, Y. Ispirli Dogac, I. Deveci and M. Teke, *Mater. Sci. Eng., C*, 2019, **99**, 1226–1235.
118 C. Işık, Y. İ. Doğaç, İ. Deveci and M. Teke, *ChemistrySelect*, 2020, **5**, 14380–14386.
119 J.-A. Park, S.-C. Lee and S.-B. Kim, *J. Mater. Sci.*, 2019, **54**, 9969–9982.
120 Y. Zhang, J. Guo, J. Sun, F. Guan, J. Tao, X. Song and Z. Li, *Int. J. Biol. Macromol.*, 2024, **260**, 129449.
121 J. Zdarta, K. Jankowska, U. Strybel, Ł. Marczak, L. N. Nguyen, P. Oleskowicz-Popiel and T. Jesionowski, *J. Hazard. Mater.*, 2022, **432**, 128688.
122 N. Kutlu, Y. I. Doğaç, I. Deveci and M. Teke, *Prep. Biochem. Biotechnol.*, 2020, **50**, 425–437.
123 K. Jankowska, A. Grzywaczyk, A. Piasecki, E. Kijeńska-Gawrońska, L. N. Nguyen, J. Zdarta, L. D. Nghiem, M. Pinelo and T. Jesionowski, *Environ. Technol. Innovation*, 2021, **21**, 101332.
124 X. Li, Q. Feng, K. Lu, J. Huang, Y. Zhang, Y. Hou, H. Qiao, D. Li and Q. Wei, *Biosens. Bioelectron.*, 2021, **171**, 112690.
125 V. Giraldi, M. L. Focarete and D. Giacomini, *J. Funct. Biomater.*, 2023, **14**, 25.
126 D. Wu, Q. Feng, T. Xu, A. Wei and H. Fong, *Chem. Eng. J.*, 2018, **331**, 517–526.
127 X. Li, D. Li, P. Lv, J. Hu, Q. Feng and Q. Wei, *Eng. Life Sci.*, 2019, **19**, 815–824.
128 B. Šketa, J. L. Galman, N. J. Turner and P. Žnidaršič-Plazl, *New Biotechnol.*, 2024, **83**, 46–55.
129 N. C. Dubey and B. P. Tripathi, *ACS Appl. Bio Mater.*, 2021, **4**, 1077–1114.
130 P. L. Show, C. W. Ooi, X. J. Lee, C. L. Yang, B. L. Liu and Y. K. Chang, *Int. J. Biol. Macromol.*, 2020, **162**, 1711–1724.
131 J. L. Hsu, S. S. Wang, C. W. Ooi, X. E. C. Thew, Y. R. Lai, C. Y. Chiu, M. Hsu, K. H. Chen and Y. K. Chang, *Food Chem.*, 2023, **406**, 135028.
132 S. J. Jian, S. S. S. Wang, C. W. Ooi, B. C. Hoe, Y. R. Lai, C. Y. Chiu, M. Hsu, K. H. Chen and Y. K. Chang, *Cellulose*, 2022, **29**, 9251–9281.
133 S. A. Jang, J. H. Park, H. J. Lim, J. Y. Oh, H. B. Ki, K. J. Lee, J. K. Song and D. M. Kim, *Enzyme Microb. Technol.*, 2021, **145**, 109749.
134 B. C. Filiz, Y. B. Elalmis, I. S. Bektaş and A. K. Figen, *Int. J. Biol. Macromol.*, 2021, **192**, 999–1012.
135 A. Amani, S. T. Kalajahi, F. Yazdian, S. Mirzababaei, H. Rashedi, M. A. Faramarzi and M. Vahidi, *Biotechnol. Prog.*, 2022, **38**, e3282.
136 M. Maryskova, M. Rysova, V. Novotny and A. Sevcu, *Polymers*, 2019, **11**, 1560.
137 H. Ye, J. Zhang, G. Yang, R. Jiao, S. Han, J. Lv, Y. Zhang, L. Zhao, Q. Xin, L. Lin, X. Ding and H. Li, *Colloids Surf., A*, 2024, **699**, 134706.
138 A. Badoei-dalfard, A. Shahba, F. Zaare, G. Sargazi, B. Seyedalipour and Z. Karami, *Int. J. Biol. Macromol.*, 2021, **192**, 1292–1303.
139 A. S. Kowsarkhizi, S. Abedi, E. Karimi, B. Ghorani and E. Oskoueian, *J. Funct. Foods*, 2024, **120**, 106334.
140 I. Bernhardsgrütter, K. Schell, D. M. Peter, F. Borjian, D. A. Saez, E. Vöhringer-Martinez and T. J. Erb, *J. Am. Chem. Soc.*, 2019, **141**, 9778–9782.
141 R. Xue, C. Qiu, X. Zhou, Y. Cheng, Z. Zhang, Y. Zhang, U. Schröder, U. T. Bornscheuer, W. Dong, R. Wei and M. Jiang, *Angew. Chem., Int. Ed.*, 2024, **63**, e202313633.
142 J. Liu, Q. Zeng, H. Lei, K. Xin, A. Xu, R. Wei, D. Li, J. Zhou, W. Dong and M. Jiang, *J. Hazard. Mater.*, 2023, **448**, 130776.
143 X. Ren, D. Chen, Y. Wang, H. Li, Y. Zhang, H. Chen, X. Li and M. Huo, *J. Nanobiotechnol.*, 2022, **20**, 92.
144 Z. Wang, R. Zhang, X. Yan and K. Fan, *Mater. Today*, 2020, **41**, 81–119.
145 R. Zhang, X. Yan and K. Fan, *Acc. Mater. Res.*, 2021, **2**, 534–547.