Green Chemistry

CRITICAL REVIEW

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Biomass-derived functional materials as carriers for enzymes: towards sustainable and robust biocatalysts

Meena Bisht,*†[a,b] Sarath Kumar Thayallath,†[c] Pranav Bharadwaj,[c] Gregory Franklin [a] and Dibyendu Mondal *[a,c]

The unique catalytic properties of enzymes have led to the production of useful medicinal intermediates, foods, and biofuels from sustainable sources. However, the instability of soluble/free enzymes under several challenging conditions (*e.g.*, pH, proteolysis, temperature, ionic potential, chemical denaturants) restricts the use of enzyme-based biocatalysts. Encapsulation of enzymes on suitable carriers would mitigate the instability issues faced in robust biocatalysis. An "ideal" carrier material employed for protein immobilization should be nontoxic, scalable, biocompatible, and should not compromise the biological activity and structure of proteins/enzymes. Thus, biodegradable and renewable biomass-derived functional materials (BDFMs) are envisaged as promising carriers for enzymes. BDFMs have in-built chemical functionalities and desirable physicochemical properties that enable their use in enzyme catalysis at the industrial scale. Numerous BDFMs have been used as immobilization matrices to improve the biocatalytic activity and stability of various enzymes. These solid materials are renewable and environmentally friendly compared with synthetic polymers. This review highlights the advancements, challenges and prospects in the emerging field of BDFMs (cellulose, silk protein, chitin, chitosan, lignocellulose, and a combination of biopolymers such as chitin/lignin and chitosan/alginate) for immobilization of enzymes (*e.g.*, α-chymotrypsin, cytochrome c, carbonic anhydrase, glucose oxidase, ribonuclease, cholesterol oxidase, alkaline phosphatase, β-glucosidase, lipase, horseradish peroxidase, catalase, tyrosinase, acetylcholinesterase, amylase, invertase, protease, laccase, β-galactosidase, and several others) for biocatalytic processes. This review also describes the relationship between the structural properties and functionality of several enzymes immobilized in BDFMs, and profiles the impact of pH, temperature, reusability, stability of storage, and the activity of these enzymes. Future perspectives in this promising field, as well as potential difficulties, are discussed. This review will help in refining biocatalysis technologies whereby biomass-derived, environmentally friendly materials are employed as enzyme supports.

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1. Introduction

Society has high dependence on non-renewable resources, which is unsustainable in the long run. Increases in population sizes and environmental pollution have prompted strategies for "green" chemistry, sustainable resources for fuels, chemicals, and materials, and reducing waste.[1,2] In this scenario, the development of flexible and integrated "biorefineries" to produce biofuels and bioproducts from renewable biomass sources can aid transition to a novel "bioeconomy" for more efficient and sustainable global development. The "circular economy" concept is taking a central role in sustainable development and, for this reason, deserves attention.[3–6] Renewable denotes sustainable and abundantly available feedstocks for the production of biofuel and biochemicals *via* suitable bioconversion. "Biocatalysis" is a charming technology used in various industrial applications. An enzyme or biocatalyst is essentially a non-hazardous, non-toxic material that is easily obtained from widely available renewable resources.[7] Enzymes are Nature's sustainable catalysts which drive a variety of reactions. In accordance with the theory of green chemistry, biocatalytic processes are more sustainable, environmentally friendly, and cost-effective than traditional chemical processes. In the context of green chemistry and sustainable development, the utilization of enzymes as efficient catalysts offers

[a]*Institute of Plant Genetics (IPG), Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland. E-mail: meenabisht56@gmail.com, meena@svc.ac.in, dmtapu@gmail.com, dmon@igr.poznan.pl*
[b]*Department of Chemistry, Sri Venkateswara College, Delhi University, Dhaula Kuan, New Delhi, 110021, India*
[c]*Centre for Nano and Material Sciences, JAIN (Deemed-to-be University), Jain Global Campus, Bangalore 562112, India*
†This authors have contributed equally.

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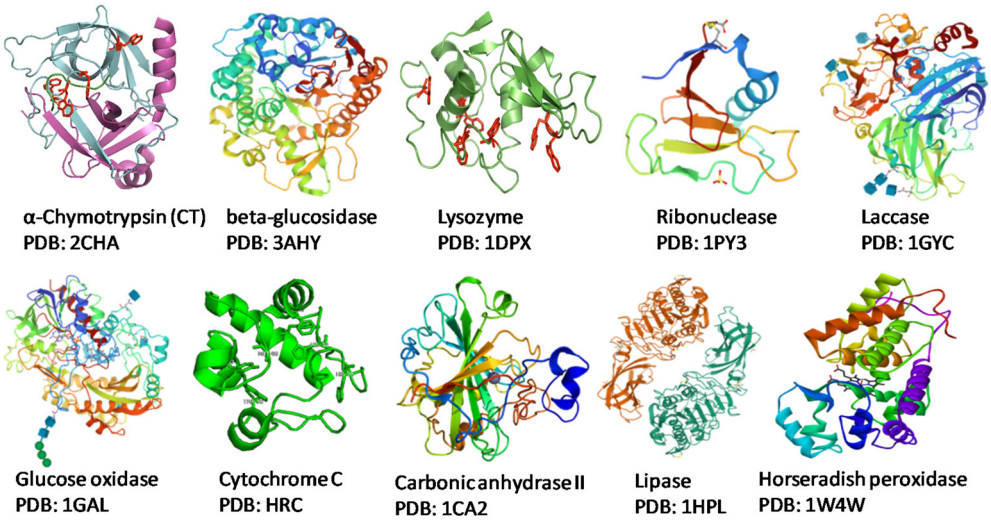

Fig. 1 Structures of commonly used enzymes. Source: Protein Data Bank.

several interesting characteristics.[1,2] Enzymes are biodegradable, reusable biocatalysts which do not create by-products. Enzymes are made up of several amino acids that are linked together by a peptide bond (–CONH–).[8,9] The native state of a protein is a result of complex interactions (*e.g.*, hydrogen bonds, van der Waals interactions, ionic interactions, hydrophobic interactions) which ultimately provide stability to proteins and prevent ruinous conformational changes.[10] Each enzyme exhibits a great degree of chemo-, regio- and stereospecificity towards a substrate because of the binding properties at the active site. The native or tertiary structure of some common enzymes that have been modified with biomass-derived functional materials (BDFMs) is shown in Fig. 1.

In the synthesis of complex pharmaceuticals, enzymatic biocatalytic approaches are more environmentally friendly and sustainable than chemical methods. Due to their capacity to catalyse reactions with high efficiency and specificity, they have emerged as preferred tools in green chemistry and are being used more frequently in industrial processes.[11] Fig. 2 shows various factors that have an impact on the stability and activity of enzymes. In recent years, biocatalysis has emerged as an imperative technology for meeting the growing demands for green and sustainable chemical manufacturing. However, enzymes are moderately stable in terms of their structural and chemical stabilities, and enzymatic processes are conducted under physiological conditions in a buffer, with high rates and selectivities.[1] Harsh processing conditions, such as the presence

Meena Bisht

Dr Meena Bisht received a doctorate in chemistry from Department of Chemistry, University of Delhi (Delhi, India). She is an Assistant Professor in the Department of Chemistry at Sri Venkateswara College (University of Delhi). She was a postdoctoral fellow at CICECO (University of Aveiro, Aveiro, Portugal) and Institute of Plant Genetics (Poznan, Poland). She has carried out substantial and innovative research in protein stabilization using a wide variety of "green" solvents (e.g., ionic liquids and deep eutectic solvents). She has developed a green, innovative, and cost-effective technology for the enhanced dissolution and extraction of bioactive compounds from sustainable biomass.

Sarath Kumar Thayallath

Mr Sarath Kumar T. is working as a Research Associate in a Research and Development department at Syngene International Limited (Bangalore, India). He completed a postgraduate degree in chemistry in 2020 from the Centre for Nano Materials and Sciences (CNMS) in Jain University (Bangalore, India). Immediately after completing a Master's degree, he worked for 1 year as a Junior Research Fellow at CNMS. His research interests are the synthesis, characterization, and purification of oligonucleotides.

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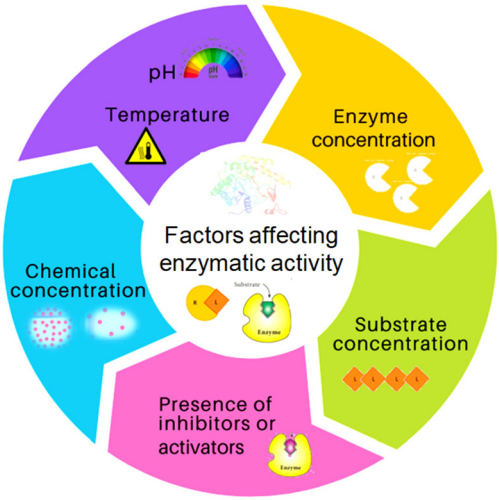

Fig. 2 Various factors that influence the activity and stability of enzymes/proteins.

of volatile organic solvents, extreme pH, and temperature, are the main barriers to the effective use of enzymes in therapeutics and as biocatalysts. Moreover, a lack of long-term activity, stability, reusability, and a challenging recovery rate can hinder the industrial application of enzymes.[1,2] To overcome these problems, numerous solutions have been postulated by researchers, such as methods for protein engineering, chemical modifications, excipient addition, and immobilization.[12]

The progress of effective immobilization strategies has paved the way for enhancing the recovery, recycling, operational stability, and storage of enzymes.[13] Immobilization is also very important for effective biocatalysis. Enzyme immobilization is the stable fixation of the protein within a solid support/matrix by physical or chemical interactions (or both) so that the enzyme remains fully functional (or at least retains most of its catalytic activity). There are various methods for enzyme immobilization: adsorption, entrapment, covalent binding, and crosslinking.[13–16] During immobilization, the integrity of the structure and the function of an enzyme are at great risk, and the biocatalytic performance may be compromised. In addition, the co-immobilization of two or more enzymes can afford multifunctional solid biocatalysts capable of catalysing processes in biocatalytic cascades.[14] Therefore, immobilization of enzymes is an essential method that has a significant effect on the stability and effectiveness of enzymes. Several strategies for enzyme immobilization have been categorized: adsorption binding, entrapment, covalent binding, and crosslinking with/without a support.[15,16] Immobilized proteins have been widely use in industrial applications (*e.g.*, analytical, pharmaceutical, commodity chemicals, food, and cosmetic industries) as well as energy production and biomedicine.[15,16] Therefore, immobilization technologies must be improved and diversified to support the development of fresh formats, better economies, and higher performance.[13,14] Supports can be made of hybrid materials, inorganic materials, metal–organic frameworks (MOFs), nanoflowers, hydrogels, polymers, or biomass-based materials.[15,17,18–21] BDFMs are exceptional supports for immobilising enzymes to enhance the activity and selectivity of enzymes.[21,22] BDFMs have attracted much interest due to their abundance, environmental friendliness, sustainability, and unique structures.[1] Biomass-based matrices have great microbiological resistance, biocompatibility, robust mechanical properties, acceptable stability, and controllable biodegradability, which might offer a favourable microenvironment for enzyme stability. BDFMs with superior properties and precise morphology for improving facile biocatalysis is a research "hotspot" (Fig. 3). BDFMs are created by transforming the

Pranav Bharadwaj

Mr Pranav Bharadwaj holds a Master's degree in chemistry from the Centre for Nano Materials and Sciences (CNMS) in Jain University (Bangalore, India), which he earned in 2022 after completing a Bachelor's degree in chemistry in 2020 from Karnataka University (Dharwad, India). His interests lie in protein packaging and green chemistry. He works as a Junior Research Fellow at CNMS with a focus on developing robust solvent-manipulation strategies for biocatalytic applications. Pranav has received numerous accolades, including a Gold Medal for his excellent academic performance during his Master's degree and a Young Achiever Award in 2020 for his overall accomplishments.

Gregory Franklin

Dr Gregory Franklin is a professor at the Institute of Plant Genetics within the Polish Academy of Sciences (Poznan, Poland). He received a doctorate in plant biotechnology from the University of Madras (Chennai, India) in 2001. He held various research positions in the USA, Saudi Arabia, and Portugal before moving to Poland in 2015. He is an interdisciplinary scientist whose research focuses on plant nanotechnology and materials science. Understanding the fundamental mechanisms of green chemistry and the interaction between plants and nanomaterials is the main focus of his research team.

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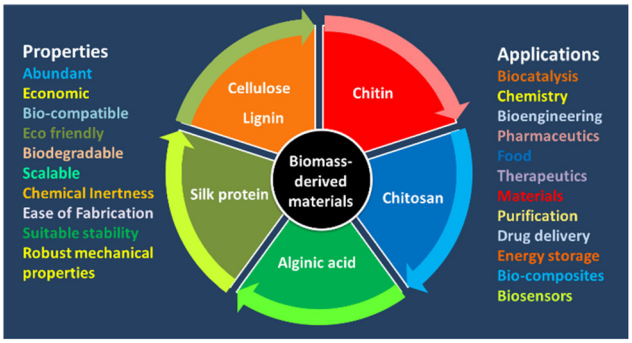

Fig. 3 Potential applications of BDFMs in biocatalytic processes.

natural resources (plants, food, animals, microorganisms) and their wastes into high-performance materials using physical or chemical processes.[2] BDFMs promote the stabilization of entrained molecules, such as therapeutic proteins or enzymes, without compromising their activity.[23] Furthermore, because BDFMs have been approved by the US Food and Drug Administration, these supports/matrices are ingestible and may have useful applications *in vivo*.[23] The immobilization of industrial enzymes would enable the reuse of expensive enzymes for enhanced utility in industrial processes, whereas the immobilization of bioactive enzymes might be incorporated into diagnostic and therapeutic applications.[24] In this review, we evaluate available data regarding the potential of BDFMs as sustainable carriers for enzyme stabilization.

Precursors for BDFMs are readily available, inexpensive, and primarily produced from plants (cellulose, lignin, alginate), animals (silk, chitin, chitosan), and microorganisms (bacterial cellulose). BDFMs possess numerous desirable inherent qualities that point to their potential use as an enzyme-stabilisation matrices to protect them from different stresses. This review focuses on the main categories of biomass-based support materials for different enzymes, or biocatalyst designs/functionalization for improving the stability and activity of enzymes and maximizing reaction efficiencies.

Fig. 4 depicts an overlay analysis which focuses on evaluating the qualities and academic importance describing BDFM use to increase enzymatic efficiency. An extensive evaluation of the academic and scientific literature accessible in this field of research was carried out using the Web of Science (WOS). In the current review, VOS viewer was used to display the co-occurrence of keywords, and an overlay map was developed. The circles in the overlay map depict the occurrence of the keywords in the chosen dataset (Fig. 4). The keyword co-occurrence map was created by choosing keywords that appeared ≥10 times. As a result, 315 out of 7211 keywords met the criteria and were further classified into eight major clusters. As shown in Fig. 4, since 2000, there have been sporadic reports on enzyme immobilizations using biomass-derived materials. In the years between 2008 and 2018, biomass-related research has experienced explosive growth. The important discoveries made possible by the use of biomass-derived materials as carriers for long-term stability and enhancing the catalytic activity of enzymes and proteins are summarised in this overview. Recent biocatalytic contributions of biomass-based supports are discussed in the next sections.

Dibyendu Mondal

Dr Dibyendu Mondal is the ERA chair holder in the NANOPLANT project at the Institute of Plant Genetics within the Polish Academy of Sciences (Poznan, Poland) since September 2021. He received a doctorate in chemical science from the Central Salt And Marine Chemical Research Institute (Bhavnagar, India) in 2015. From 2015 to 2017 he was a post-doctoral research assistant at CICECO within the University of Aveiro (Aveiro, Portugal) and then he joined as an Assistant Professor at the Centre for Nano Materials and Sciences in Jain University (Bangalore, India) in 2017. His main research areas focus on (i) value addition of bioresources using neoteric solvents; (ii) protein engineering and biocatalysis; (iii) bio-inspired and active nano-constructs; (iv) nanotechnology for sustainable agriculture.

2. Lignocellulosic-based supports (carriers) for enzyme immobilization

Lignocellulose and cellulose-based materials are attractive support materials for enzyme immobilization because they have high porosity, strong mechanical properties, are highly resistant to microorganisms, as well as having tunable biodegradability, excellent stability, and dense network of hydrogen bond crosslinks.[25–27] Being the most abundant polysaccharide, cellulose is made up of linear chains of D-glucose units connected through β(1 → 4)-fashioned glycosidic linkages, as well as intra and intermolecular H-bonding (Fig. 5). This results in the formation of cellulose microfibrils that are firm and strong. To create β(1 → 4) glycosidic bonds, every alternate glucose molecule in cellulose is inverted. Cellulose-functionalized materials can be made from cellulosic sources such as cotton and bacterial cellulose wherein the hydrolysis of cellulose generates nano-sized crystalline material.[26–28] Several such forms of cellulose, such as cellulose hydrogel microspheres (CHMs), cellulose nanocrystal (CNCs), cellulose nanowhiskers (CNWs), and nanofibrillated cellulose (NFC) have been studied as possible supports for enzyme immobilization. They are characterized by high enzyme loading, enhanced stability, improved activity, and increased mass transfer rate.[29–47] Various immobilization techniques can be applied to load enzymes onto the matrices of lignocellulosic biomass/modified cellulose-based materials for the conjugation and stabilization of enzymes and proteins. Recently, Ren *et al.*[45]

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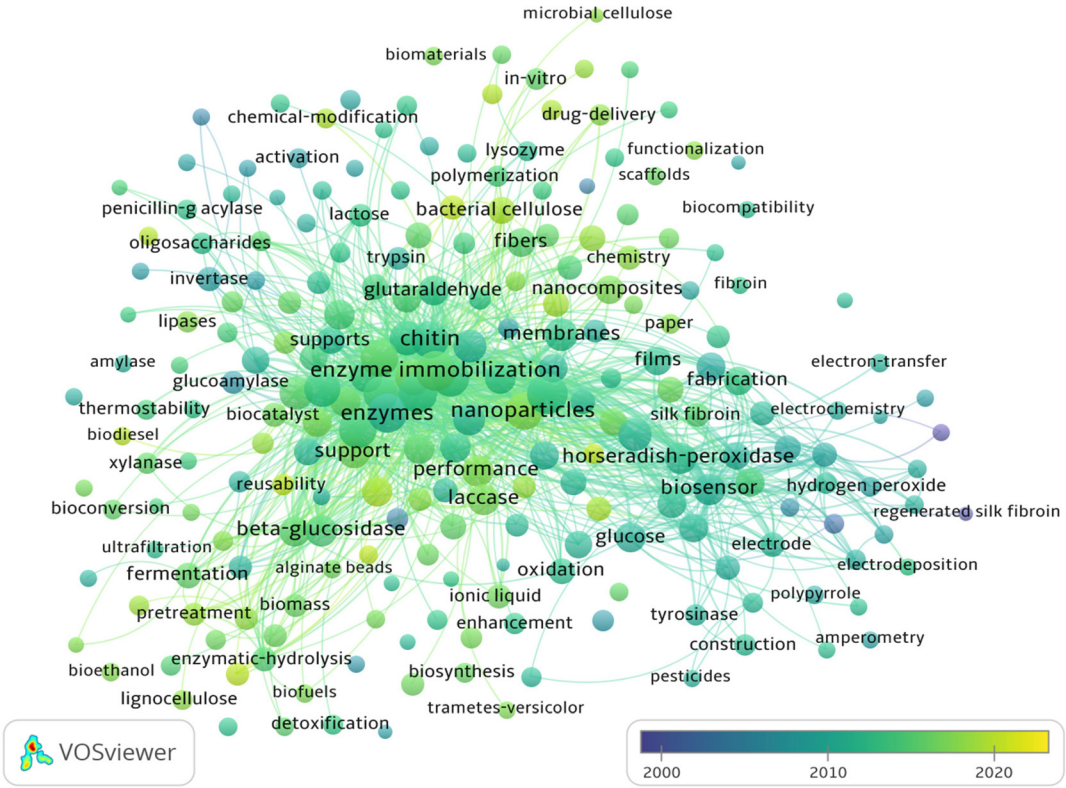

Fig. 4 Overlay map of co-occurrence of keywords concerning BDFMs as carriers for enzymes.

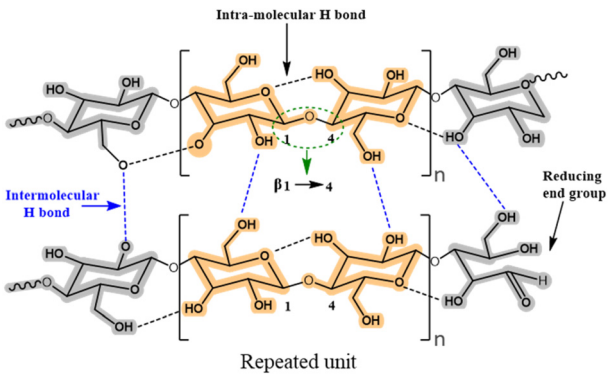

Fig. 5 Intra- and inter-molecular hydrogen bonding and possible reaction sites in cellulose.

reported the superior potential of delignified bamboo over the immobilization of β-glucuronidase (BGU) with a significantly higher loading capacity (~8 mg $m^{-2}\cdot g^{-1}$), recyclability (13 cycles), and longer shelf life (≤7 weeks). Cellulose matrices were employed to enhance the robustness of the enzyme in analytical and synthetic applications for hierarchical flow-based bioreactors.

Uth and co-workers[47] used a modular approach for site-directed, biorthogonal protein immobilization. A general two-step method was created that took advantage of extremely effective oxime ligation along with enzyme-mediated protein coupling onto the surface of peptide-modified crystalline nanocellulose. Conjugate analyses revealed that, after being grafted onto CNC, the immobilized glucose oxidase (GOx) maintained its activity (Fig. 6). To improve the salt and thermal tolerance of cellulase enzyme, Montamedi and colleagues[46] immobilized its cloned form (PersiCeL3) over a carboxymethyl cellulose (CMC) hydrogel matrix. With halotolerant behaviour >65% and almost two-folds higher activity than the native enzyme, immobilized PersiCel3 showed superb performance which could be used in lignocellulosic biomass industries to boost the production of value-added products in hot and salty environments.

Authors transformed the primary alcohols on the surface of CNC to the corresponding aldehyde functionality, which enabled immobilization of bioactive modules *via* extremely effective oxime ligation. Immobilization of the enzyme on the CMC-hydrogel matrix could be used to improve the robustness of enzymes with strong resistance towards high temperatures and heavy salt concentrations.[7,46] Suo *et al.*[7] prepared ionic liquid (IL)-modified magnetic CMC nanoparticles on which porcine pancreatic lipase (PPL) was immobilized covalently (Fig. 7). The immobilized lipase (PPL-IL-MCMC) demonstrated specific activities that were 1.43- and 2.81-fold greater than those of free PPL and PPL-MCMC, respectively, with simple and efficient recoverability.

Extending the work further, the authors studied the effect of urea (Fig. 8a) and thermal stresses (Fig. 8b) over the activity

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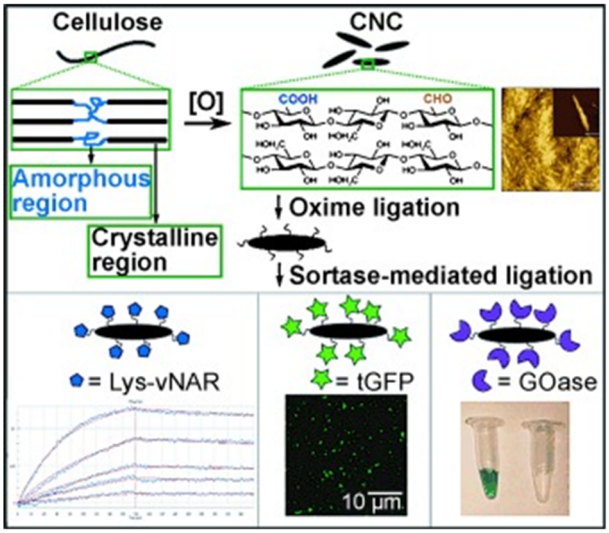

Fig. 6 Postulated chemoenzymatic approach to protein immobilization onto crystalline cellulose nanoscaffolds (schematic). Reproduced from ref. 47 with permission from Wiley-VCH, copyright 2014.

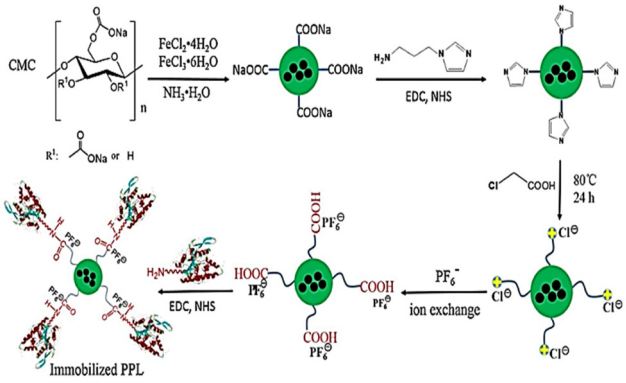

Fig. 7 A common method for preparation of IL-modified BDFMs and enzyme immobilization upon it. Reproduced from ref. 7 with permission from Elsevier, copyright 2020.

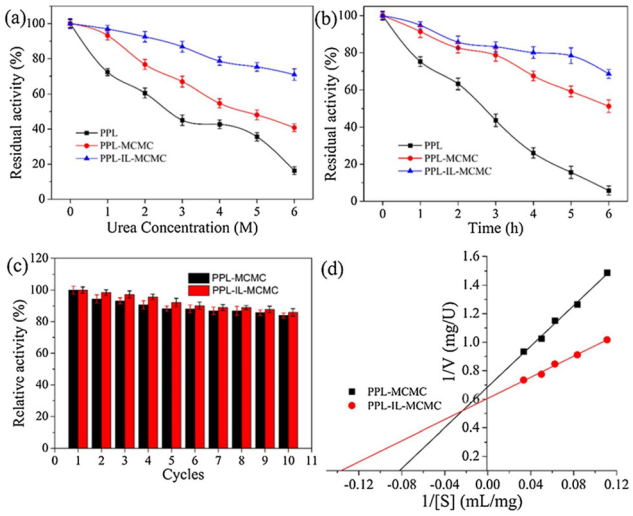

Fig. 8 Effect of the CMC-hydrogel matrix on the stability (a–c) and kinetic parameters (d) of lipases. Reproduced from ref. 7 with permission from Elsevier, copyright 2020.

and stability of PPL. The conjugate retained >68% activity but the native enzyme could barely maintain its functions. The immobilized enzymes PPL-MCMC and PLL-IL-MCMC could maintain 40.8% and 70.9% of their original activity, respectively, at a urea concentration of 6 mol L^{-1}, whereas the native enzymes retained only 15% of their activity. A similar trend was reported for thermal stress for a long time (Fig. 8b). The advantage of combining two stabilizing systems can be clearly envisioned by recyclability and Michaelis–Menten parameters (Fig. 8c and d). This is due to ion interactions, covalent bonds, H-bonds, and π–π stacking between the carrier and enzyme. This could also support maintenance of the stiffness and integrity of the enzyme structure under harsh conditions, thereby stopping the enzymes from becoming partially inactivated.[7] Utilizing halloysite nanotubes (HNTs) as a template, Sillu *et al.*,[48] described the synthesis of a nanobiocatalyst that immobilized the enzyme that catalysed the breakdown of cellulose into glucose: cellulase.

As compared with free enzyme, immobilized cellulase showed greater stability at high temperatures (≥60 °C) and storage capacity and activity. Luo and co-workers[49] created porous magnetic cellulose microspheres (MCMs) activated by epoxy chloropropane to boost the covalent immobilization of penicillin G acylase (PGA) to increase the effectiveness of catalyst recovery. The immobilized PGA displayed strong catalytic activity, improved pH tolerance, and enhanced thermal stability. The catalysts were recovered by separating the enzyme-loaded matrix from the reaction solution. In the quest of producing protein-friendly biomaterial for biocatalysis and biotechnological applications, our research team[50] synthesized tendril-like functional carbon helices (TLFCHs) from lignocellulosic biomass *via* a "green" solvothermal technique utilising deep eutectic solvent (DES) as a soft template and catalyst. Exploiting the benefits of helicity and in-built chemical functionalities, we immobilized cytochrome c (Cyt c) on the surface of TLFCHs (Fig. 9). At optimized conditions, the specific activity, pH stability, and peroxidase activity of Cyt c were increased without impacting the structural integrity of the protein. Furthermore, lignocellulose-based hydrogels possess the advantages of low cost, biocompatibility, biodegradability, hydrophilicity, non-toxicity and, most importantly, controllable properties.[17,51–62] Hydrogels based on lignocellulose have revealed promising applications in biocatalytic, biomedical, and bioelectronic areas.[51,52] Jo *et al.*[51] generated cellulose hydrogel microspheres by sol–gel transition employing a 1-ethyl-3-methylimidazolium acetate ([Emim][Ac])-in-oil emulsion for immobilization of lipase. Immobilized lipase showed greater efficiency as compared with the native enzyme. In contrast to lipase immobilized on MCC or mm-sized hydrogel beads, lipase immobilized on cellulose microspheres demon-

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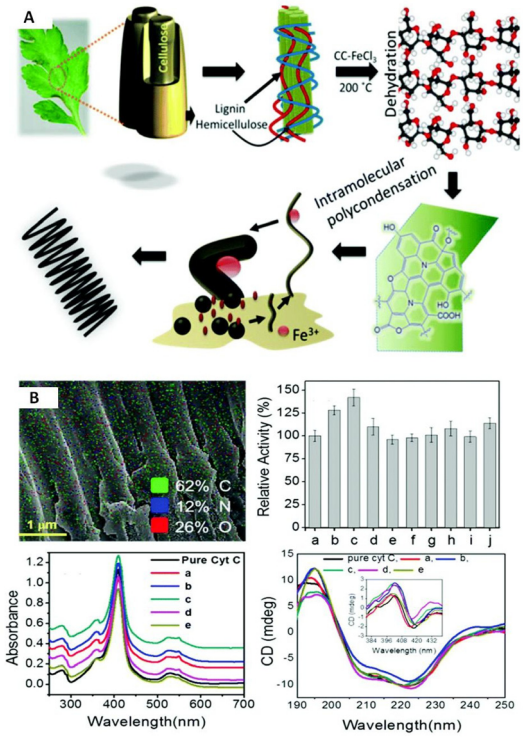

Fig. 9 Schematic depictions of (A) a plausible mechanism of the sequential growth of TLFCHs from *Parthenium* biomass during a solvothermal process in the presence of a DES (CC : $FeCl_3$, 1 : 2 mol ratio) (B) Cyt C immobilized on TLECH and showing activity and stability results (ref. 50).

strated a much greater loading efficiency, immobilization yield, and specificity constant.[51] In another study,[52] cellulose and lignin were co-dissolved in an IL to produce cellulose/lignin composite hydrogel beads. The activity, protein loading, and specific activity of the lipase immobilized on the cellulose/lignin beads were 2.6-, 2.2-, and 1.2-times higher than those of the lipase immobilized on cellulose beads, respectively.[52] Kim *et al.*[53] extended this work by conjugating cellulose with other bio-polymers, such as chitosan, carrageenan, agarose and agar. *Candida rugose* lipase (CRL) immobilized over these hydrogels in the presence of [Emim][Ac] demonstrated greater immobilization yield compared with that of cellulose beads.[53] In addition to the cellulose materials mentioned above, chemically altered nanoscale cellulose materials make excellent matrices for enzyme immobilization. Arola and co-workers[17] immobilized proteins over NFC through amine, epoxy, and carboxylic-acid functionalization.[17] The NFC structure is beneficial for the stability and catalytic activity of proteins.[17] Organophosphorus hydrolase (OPH) from Flavobacterium ATCC 27551 was immobilized on powdered plant cellulose treated with epoxy (Fig. 10) by Sharifi *et al.*[54] The immobilized OPH demonstrated better storage, temperature, pH, and reusability properties than the free enzyme. Apart from epoxy groups, introducing aldehyde and carboxyl groups on cellulose matrices is an effective approach to attach them with the amino groups of enzymes.[56,57]

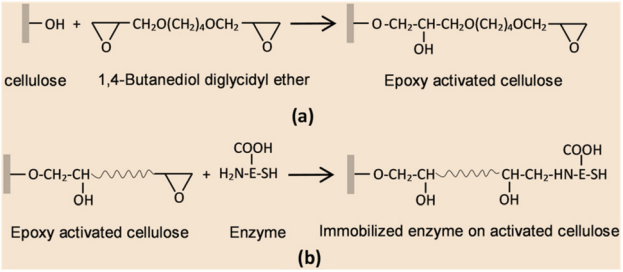

Fig. 10 Plausible reaction mechanism of enzyme immobilization through the epoxy method. (a) Surface reaction of a cellulose matrix with 1,4-butanediol diglycidyl ether (BTDE). (b) Covalent coupling of enzyme onto the surface of activated cellulose. Reproduced from ref. 54 with permission from Elsevier, copyright 2018.

Different chemical processes are used to oxidise the hydroxyl groups in cellulose to produce aldehyde or carboxyl groups. For example, Hao *et al.*[56] used sodium periodate ($NaIO_4$) for oxidizing cotton fibres to develop aldehyde groups in them.[56] A similar study was reported for oxidation of cotton yarn by $NaIO_4$ to introduce aldehyde groups and further used for immobilization of trypsin.[57] The highest concentration of immobilized trypsin was 6.1 mg g^{-1} of dry cotton yarn. Over 60 days of storage, the activity of immobilized trypsin increased, and revealed >90% and >70% of the initial activity at 4 °C and 25 °C, respectively.[57]

In another study by the Gong group,[58] $NaIO_4$ first oxidised the cellulose in a loofah sponge to produce aldehyde groups. Then, the oxidised loofah sponge was used as a carrier for the covalent immobilization of lipase. The immobilized lipase provided better thermal stability, storage stability, and reusability. Besides periodate, 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) was used by Qian and co-authors to control the distribution of carboxylic groups over CNF macrogels, over which phospholipase from *Thermomyces lanuginosus* was immobilized and showed great reusability.[43]

To cutdown the time frame of purification and immobilization processes, Gennari *et al.*,[59] engineered a single-step methodology by altering the surface of MCC with CBD. β-Galactosidase from *Kluveromyces* sp. was simultaneously purified and immobilized over this carrier and showed high stability at up to 40 cycles for the hydrolysis of milk lactose. Various other types of cellulose matrices have been used as carriers for enzyme immobilization (Table 1).[63–85] To improve the tolerability of enzymes in enzymatic membrane bioreactors (EMBRs), it is crucial to immobilize enzymes on supports while retaining their structure and activity. In a recent study, Liu[60] described a unique technique utilizing dicarboxylic acid halides as spacers for surface-initiated, covalent immobilization of enzymes onto cellulose microporous membranes. Sebacoyl chlorides, dicarboxylic acid halides, exhibit excellent reactivity with hydroxyl groups and amino groups, which are easily introduced onto membrane surfaces (Fig. 11). Trypsin and lipase were immobilized on the membrane surface while maintaining their molecular structure and activity substan-

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Table 1 Influence of lignocellulosic (or cellulose)-based support materials on the stability and activity of proteins

Biomass	Biomass-derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Cellulose	Spin coating of protein NFC conjugates	Alkaline phosphatase	Conjugation	No loss in activity at 21 °C and >20% remaining activity after a week of incubation at 37 °C	17
Lignocellulosic	Tendril-like carbon helices	Cytochrome c		Enhanced structural stability and >150% higher activity than native Cyt c	50
Cellulose	CHM	Lipase	Physical adsorption	1.4 times higher specific activity, 41 folds half-life (at 45 °C), enhanced thermal and pH stabilities	51
Lignocellulosic	Cellulose/lignin hydrogel beads	Lipase	Physical adsorption	2.6, 1.2 folds higher relative and specific activity than lipase-cellulose bead system. >3 times higher thermal stability and against lower pH (*i.e.* 3)	52
Wood mimetic	Cellulose–biopolymer composite hydrogel beads	Lipase	Entrapment	Improved reusability with >80% activity retention	53
Cellulose	Cellulose based membranes entrapping enzymes over the electrode	GOx	Entrapment	The enzyme electrode was stable at for least 6 months. Protected the leakage of enzyme. Glucose detection up to 10 μM with response time of ~10 s	55
Cotton knit fabrics (cellulose)	Cotton fabrics oxidized by periodate	Cellulase		(1) Enhanced binding capacity of cellulase (>10.3 mg g^{-1}). (2) Enhanced cotton hydrolysis and cellulase potential for adsorption	56
Cotton yarn (cellulose)	Cotton yarn oxidised with sodium periodate	Trypsin	Covalent binding	Retained the initial activity after 60 days of storage in physiological solution	57
Cellulose	Cellulose-CBD	β-Galactosidase		Reusability for milk sugar hydrolysis till 40 cycles with 64% activity retention	59
Paper wastes (PW)	Fe_3O_4 and chitosan functionalised αCFs	Laccase	Covalent bonding	Great loading capacity of >73 mg g^{-1}; ~92% activity recovery and excellent reusability capability with ~74% activity retention at the end of 11th cycle	61
Waste newspaper	Dialdehyde-modified cellulose nanocrystals (DMC)	Laccase	Covalent bonding	64.94% yield, excellent stability, and reusability	62
Cellulose	Magnetic dialdehyde cellulose nanoparticles (MDC)	Rhizopus lipase	Cross linking	Enhanced long term stability with recovery rate >50% after 30 days	63
Sugarcane bagasse	CNC	Lipase	Covalent bonding	With optimum pH shifted to 8.25, found best suited to catalyse a triglyceride lipolysis reaction from palm oil	64
Nanocellulose from almond shell	Nanocellulose (NC) extracted using *p*-toluenesulfonic acid (PTSA) and sulfuric acid (ASS) with sugar-based natural deep eutectic solvent (NADES1a) as a biocatalyst system	CRL		Significant improvement in half-life *i.e.*, 14 days greater than native enzyme	65
Cellulose	Cellulose beads	Glucose oxidase (GOx)		Effective antimicrobial action against *Pseudomonas aeruginosa*, *Escherichia coli* and two of the methicillin-resistant *Staphylococcus* species	66
Cellulose	Nonwoven cotton fabric	Lysozyme	Cross linking	Enhanced antimicrobial activity and storage stability	67
Cellulose	CMC-silver nanoparticle (AgNp)-silica hybrids	Amylase	Adsorption	Activity increased and maintained the activity at room temperature (40 °C) for 15 days	68
Cellulose	Cellulose powder, cotton buds, disc make-up remover pads, cotton and linen tissues	Lipase	Covalent bonding	Increased effectiveness in removing aged linseed oil layers in 45 min at pH 6 and 40 °C	69

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Table 1 *(Contd.)*

Biomass	Biomass-derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Cellulose from coffee filter	Cellulose nanofibers (CNFs)	α-Chymotrypsin (α-CT)	Enzyme precipitate coating (EPC)	Magnetic separation technique proved to be the best with >38 times higher activity than other methods. For long term, precipitate coating method proved to be superior with >70% activity retention even after 1 month of incubation	70
Cellulose	TEMPO-oxidized cellulose fibres used to build cellulose-based microspheres	Phospholipase	Covalent bonding	Showed a greater thermal stability and resistance to pH, as well as easy recovery and reusability	71
Cellulose	Dialdehyde cellulose	α-Amylase	Covalent binding	Lys142 has been found to be involved in α-amylase immobilization to dialdehyde cellulose	72
Cellulose	Magnetic dialdehyde cellulose (MDAC)	Bacterial laccase	Cross linking	(1) With >210 mg g^{-1} loading capacity and >34 emu g^{-1} of magnetization, the material offers 10 cycles of reusability with >70%. (2) Efficient against crystal violet decolouration	73
Cellulose	Microcrystalline cellulose (MCC) magnetic support	β-Galactosidase		>90% immobilization efficiency, 1.2 folds higher substrate affinity, 7 times higher thermal stability and recyclability up to 15 cycles	74
Nanocellulose (NC) from an agro-waste of quinoa husks (QS)	Nanocellulose nano-carrier	Laccase enzyme (PersiLac1)	Adsorption	Having ~98% and ~60% dye degradation capacity pertaining to Malachite green and Congo red respectively, biocatalyst can be reused with >83% initial activity after 18 h	75
Cellulose from Balsa wood	Balsa wood-derived cellulose scaffold crosslinked with glutaraldehyde	Horseradish peroxidase (HRP), glucose oxidase (GOD), and catalase (CAT)	Adsorption	>90% phenol degradation rate and 95% sodium gluconate yield were achieved in the reactor	76

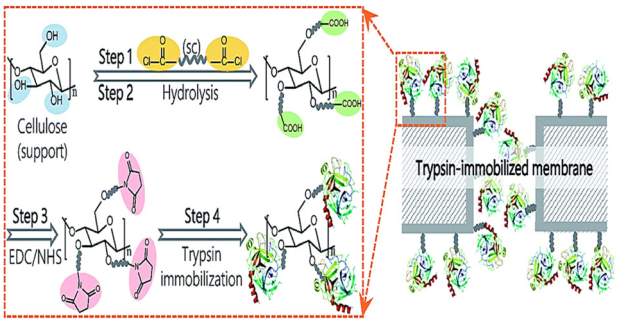

Fig. 11 Proposed pathways for trypsin immobilization on regenerated cellulose membranes. Reproduced from ref. 60 with permission from RSC, copyright 2017.

tially. The corresponding membrane demonstrated outstanding specific activity with strong activity retention (>25%) in a soaking mode at the optimal surface density. Moreover, the immobilized enzyme exhibited outstanding enhancement in reusability, thermal resistance, and continuous-operation capability.

Gajanan *et al.*[61] used paper wastes (PWs) to extract multifunctional α-cellulose fibers (αCFs), which were further tuned with magnetic Fe_3O_4 and chitosan. This system was successfully employed for immobilization of laccase (Lac) with loading capacity of >70 mg g^{-1} and >90% of activity recovery. Due to excellent pH, storage and temperature stabilities, authors claimed that the catalytic system could be applied for the degradation of DR28 (xenobiotic benzidine-based azo dye).[61] Overall, the unique microstructure of cellulose helps in improving the long-term stability and prevents back-diffusion of enzymes, which makes it an appropriate immobilization matrix for enzymes (Table 1).

3. Lignin-based supports (carriers) for enzyme immobilization

Lignin is a valuable and less explored natural resource. It is an intriguing class of material that has become a popular alternative in the search for more affordable and environmentally acceptable materials for enzyme immobilization. It possesses high strength and stability at extremes of pH, temperature, or

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Table 2 Influence of lignin-based support materials on the stability and activity of proteins

Biomass	Biomass-derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Dendrocalamus Latiforu	BSS lignin as a support material	α-Amylase	Adsorption	With 19 mg g^{-1} loading capacity, immobilized enzyme showed >2-folds higher activity with >53% activity retention after 14 recycles	86
Bio-waste of kraft pulp lignin	Amino-modified microspheres (A-LMS)	β-Galactosidase	Electrostatic interaction	1.5 times higher rate of galacto-oligosaccharide production and efficient in degradation of pesticide – lindane	87
Green coconut fiber (GCF)	Lignin/Fe_3O_4 nanoparticles synthesized by organosolv pre-treatment	β-Glucosidase	Covalent bonding	(1) Improved digestibility performance (>21 g L^{-1} of cellulose and >6 g L^{-1} of green coconut fibre) and stability. (2) Good synergism with cellulases during enzymatic hydrolysis. (3) High adsorption capacity of nanoparticles towards Methylene blue (203.66 mg g^{-1}), Cibacron blue (112.36 mg g^{-1}) and Remazol red (96.46 mg g^{-1}) dyes	88
Kraft lignin	Novel hybrid support made of titanium and lignin	α-Amylase from *Aspergillus* oryzae	Covalent bonding	Improved pH range of 3–7 and best suited for low temperature catalytic applications (5–10 °C)	89
Lignin	Lignin nanocapsules	Tyrosinase and laccase		Used for the synthesis of bioinks with different shades. The nanocarriers found to prevent alkaline and UV degradation of synthesized melanin	90
Organosolv lignin (OL)	LNPs	Enzymatic cascade of lipase-tyrosinase	Layer-by-layer method	Successfully synthesised high yield lipophilic hydroxytyrosol esters	91
Kraft lignin	Chitosan-coated LNPs	Glucose oxidase (GOx)	Adsorption	Enhanced the enzyme activity up to 70° C and display of self-scavenger property towards H_2O_2	92
Lignin	Lignin-based spherical particle	Lipase	Electrostatic interaction	Retained 75% and 81% relative activity at higher temperature (60 °C) and after 10 reuses respectively	94

pressures (Table 2).[86–94] Weihua *et al.*[86] demonstrated the use of lignin particles from bamboo shoot shells (BSSs) as novel carriers for immobilization of α-amylase from *Bacillus subtilis* with a maximum protein load of 19.0 mg g^{-1} in 20 min.[86] The immobilized enzyme was superior compared with the free enzyme in terms of overall catalytic efficiency, storage stability, and recovery. Bebić and co-workers[87] revealed amino-modified microspheres of kraft-lignin as potential supports for the immobilization of β-galactosidase and Lac enzymes. The former was used for the selective synthesis of galacto-oligosaccharides, whereas the latter was employed for the degradation of a pesticide (lindane). Lac immobilized onto modified kraft-lignin showed 3-fold greater efficiency in degrading the pesticide than amino-modified silica nanoparticles.[87] Furthermore, multifunctional lignin/Fe_3O_4 nanoparticles were synthesized using green coconut fibres, which were used to remove colours from the effluents of the textile industry and to achieve a high stability for β-glucosidase.[88] Immobilized β-glucosidase revealed significant adsorption capacity for the elimination of methylene blue (203.66 mg g^{-1}), remazol red (96.46 mg g^{-1}), and cibacron blue (112.36 mg g^{-1}). It also showed excellent digestion performance towards crystalline cellulose compared with that of the native enzyme.[88] Therefore, the proposed approach proved successful in obtaining lignins isolated from lignocellulosic residues.[88] Łukasz *et al.*[89] designed a hybrid TiO_2/lignin support for the covalent immobilization of *Aspergillus oryzae* α-amylase. The immobilized enzyme displayed improvement in chemical and thermal stabilities with 10 cycles of reusability, and after 1 month of storage its initial activity was retained at >80%. Considering sustainable and green synthetic protocols, Capecchi and colleagues[90] described a method of preparing sustainable bio-ink ingredient from poly(diallyldimethylammonium chloride) (PDDA) or chitosan-functionalized organosolv lignin nanocapsules. Pigments were catalysed by Lac and tyrosinase immobilized over functionalized lignin nanocapsules *via* a layer-by-layer (LBL) immobilization strategy.

The lignin nanocapsules were employed as multifunctional devices acting as scaffolds, activators for enzymes to catalyse pigment synthesis and, finally, being the vehicle or binder for pigments. L-Tyrosine, epicatechin, 3,4-dihydroxyphenyl-L-alanine (L-DOPA), and several other derivatives of phenol were used as reactants giving out melanin pigment with high alkaline and UV tolerance due to interaction with nanocapsules. Similarly, Tomaino *et al.*[91] synthesized one-pot esterification of lipophilic hydroxytyrosol esters by a lignin nanoparticles (LNPs)-supported enzymatic cascade. Tyrosinase and lipase M were immobilized on LNPs by an LBL method using chitosan as a positive-charged polyelectrolyte and concanavalin A acting as molecular spacer to regulate the optimal separation between the active sites of the two enzymes. Interestingly, compared with the native counterparts, both immobilized enzymes on LNPs showed greater kinetic parameters (maximum reaction speed (V_{max}) and Michaelis constant (K_m)). By further

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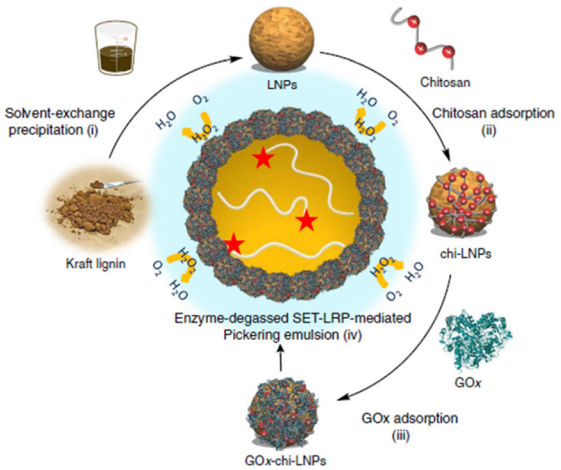

Fig. 12 Preparation of GOx-chi-LNPs and application in enzyme-degassed SET-LRP-mediated Pickering emulsion (Schematic). (i) Preparation of LNPs *via* solvent-exchange precipitation. (ii) Adsorption of chitosan on LNPs to yield chi-LNPs. (iii) Adsorption of GOx on chi-LNPs to yield Gox-chi-LNPs. (iv) Application of GOx-chi-LNPs as biocatalytic degassing stabilizers for SET-LRP in Pickering emulsion media. Reproduced from ref. 92 with permission from Nature Portfolio, copyright 2020.

extending the LBL strategy, Moreno and colleagues[92] prepared a multifunctional particulate emulsifier where GOx was immobilized over chitosan-coated LNPs (Fig. 12).

This approach proved to be efficient in stabilizing the Pickering emulsions and *in situ* enzymatic degassing of single electron transfer-living radical polymerization (SET-LRP). Because of the auto-degradation of H_2O_2, GOx is protected against the oxidative stress caused *in situ*, leading it to withstand temperatures higher than the optimum. This rational design of the process is a breakthrough in avoiding the use of additives for SET-LRP and in opening new avenues for the green synthesis of block co-polymers having complex architectures. For the first time, applications of functionalized lignin as a matrix for immobilizing a wide range of enzymes (transaminase, carboxylase, dehydrogenase) using various binding chemistries were carried out by Benítez and co-workers.[93] As a proof of concept, ω-transaminase was incorporated into a packed bead reactor and reversibly immobilized on polyethyleneimine-lignin, and its endurance was investigated in continuous-flow deamination reactions maintaining the same conversion for 100 cycles. All of these results demonstrated that fully closed-loop sustainable flow-biocatalytic systems based on bio-waste and natural lignin material to be effective substitutes for typical immobilization supports.

4. Silk fibroin-based supports (carriers) for enzyme immobilization

Silk fibroin (SF) is a well-known and widely used natural protein procured from silkworm cocoons. Large numbers of

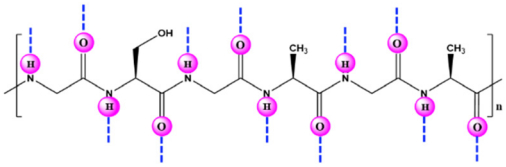

Fig. 13 Chemical structure and illustration of the possible hydrogen bonding (– –) sites in silk fibroin.

serine, glycine and alanine residues are found in this macromolecular protein (Fig. 13),[24,95,96] along with readily reacting chemical groups (*e.g.*, imidazole, tyrosyl/phenol and sulfhydryl).[97] Due of its low cost, abundance, nontoxicity, and biodegradability, SF makes an appealing material for enzyme entrapment. Its peculiar amino-acid sequence and structure result in the formation of discrete nanoscale pockets and ensure that a sufficient number of water molecules is retained for protein interaction and stabilization, which make it a promising substrate for enzyme immobilization.[16] The distinctive structure of SF protein (α-helices, β-sheets and random coils) synchronize to create a propitious space for stabilizing entrapped enzymes. SF can be transformed into fibres, powdery nanoparticles or microspheres, membranes, gels, films, hydrogels, or scaffolds under mild, ambient, or aqueous conditions.[90] Numerous studies have shown that SF can serve as a matrix for immobilization, and thereby to impart stability to a variety of enzymes.[87–101] Recently, various works have been published addressing the use of silk biomaterials as excellent supports for the covalent and noncovalent immobilization of enzymes with high retention of catalytic activity and stability against various stresses (Table 3).[100–114] Silk nanofiber (SNF) is an excellent candidate for enzyme immobilization due to superior stiffness, larger specific surface area, higher interconnectivity and porosity with high capacity for loading enzymes. In this regard, Lee *et al.*[91] prepared SF nanofibers by electrospinning and applied it for immobilization of α-CT.

The activity of immobilized α-CT on SF nanofibers was shown to be eight-times more active than that of bulk material, and the activity was indirectly proportional to fibre diameter.[99] Many reaction sites (*e.g.*, $-NH_2$, –COOH, imidazole and phenol) are provided by the diverse amino-acid constituents of silk fiber. Lipase was immobilized onto SF using a chemical method.[100] This lipase was then used for the hydrolysis of *Helianthus annuus* (sunflower) oil for producing fatty acids.[100] Chen and co-workers[101] designed an inexpensive woven fabric support made from SF for the immobilization of *Canadida* sp. lipase. The stability and activity of immobilized lipases on two types of silk fabrics were examined, and it was shown that the lipase immobilized over the hydrophobic fabric had 2-fold higher esterification and hydrolysis activity than the lipase immobilized on native silk fibres. In comparison with free lipase, lipase after immobilization demonstrated stability over a larger pH range and a change in its optimum temperature. The method described in this work indicates that woven SF might be an ideal material for use as an immobilization

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Table 3 Influence of silk-based support materials on the stability and activity of proteins

Biomass	Biomass-derived support material	Used enzyme	Immobilization method	Result/performance study	Ref.
Bombyx mori silkworm cocoons	Silk fibroin film	GOx	Entrapment	Activity maintained more than 10 months of preservation up to 37 °C of temperature	23
Bombyx mori silkworm cocoons	Silk fibroin film	HRP	Entrapment	Even 2 months later, 90% of activity retained at room temperature	23
Silkworm cocoons	Silk Fibroin nanofibers	α-CT		High loading capacity of 5.6% with 8 times higher activity than the one immobilized on silk fiber and >90% activity retention after 24 h incubation	99
Woven Muga Silk	Glutaraldehyde – crosslinked silk fibers	Lipase	Cross linking	12% lower activity than native enzyme but significant conversion rates in the emulsion phase with 3 cycles of recycling	100
Bombyx mori silkworm cocoons	Hydrophobic silk fiber treated with polydimethylsiloxane (PDMS)	Lipase	Adsorption	2-Fold increase in the activity and improved thermal stability	101
Bombyx mori silk	Silk fibroin crosslinked with tyrosinase using glutaraldehyde	Tyrosinase	Cross linking	(1) A higher degree of stability. (2) 80% of initial activity retained even after multiple times reuses	102
Woven Muga silk	Silk mat using *N*-ethyl-*N*′-(3-dimethylaminopropyl carbodiimide) EDC-NHS (*N*-hydroxysuccinimide) linkage	ChOx	Covalent bonding	Greater catalytic activity and structural stability sustaining for 13 months and 25 individual assays	103
Bombyx mori silk	Silk fibroin-based hydrogel	CA		(1) ~100% immobilization efficiency. (2) Stable against pH (pH-3) denaturation	104
Bombyx mori cocoon	Silk film by entrapment	β-Glucosidase	Entrapment	Enhanced stability against various stresses such as heat, electrodialysis, and protease	105
Bombyx mori cocoons	Silk film entrapment	OPH	Entrapment	(1) Excellent long term stability with >60% activity even after 17 weeks of incubation. (2) Higher stability against UV radiation and extreme temperature	106
Bombyx mori silk cocoons	Silk powder by entrapment	Invertase	Entrapment	Enhanced thermal stability	107
Bombyx mori silk cocoons	Powder of Silk-ASNase bioconjugates by glutaraldehyde	ASNase	Bioconjugation	Improved half life (63 h), 6 times higher substrate affinity, resistance to trypsin digestion and good thermostability	108
Bombyx mori silk cocoon and lignin	Hydrogels composite made of silk fibroin and alkaline lignin (SF-AL)	Laccase (Lac)	Cross-linking	Enhanced working range of pH from 4.5 to 9	109
Bombyx mori silk cocoons	SF nano-cocoons	GOx and HRP	Encapsulation	Improved the stability and efficiency of the catalytic reaction, as well as the temperature stability and storage stability	111
Bombyx mori silk cocoons	Silk fibroin hydrogel	Microalgae	Entrapment	Good mechanical strength and stability of material with 6 times higher oxygen generation compared to controlled algal culture	112
Bombyx mori silk cocoons	SF	GOx	Encapsulation	Biocompatible and biodegradable sensor with improved current response of 0.2 μA mM^{-1}	113
Bombyx mori silk cocoons	SF	Protease		Good extraction efficiency	114
Silk	A film made of sericin, polyvinyl alcohol, and cassava starch	*Botryosphaeria ribis* EC-01 lipase	Adsorption	>9.8 folds higher activity, better yield, and recyclability up to 7 cycles	115
Bombyx mori silkworm cocoons	Sensor-based on regenerated silk fibroin film	HRP	Entrapment	Improved detection limit of 100 nM with <40 s response time	116
Bombyx mori silkworm cocoons	Porous membranes of Bombyx mori silk fibroin	GOx	Entrapment	Improved catalytic activity and thermal stability with >20 times increased permeability of NaCl and glucose	117

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Table 3 *(Contd.)*

Biomass	Biomass-derived support material	Used enzyme	Immobilization method	Result/performance study	Ref.
Bombyx mori and *Philosamia cynthia ricini* silk	SF membrane	GOx	Entrapment	Improved shelf life with lower extent of leakage (0.05%), and improved thermal stability at 60 °C	118
Woven silk	Immobilization on silk Fibre using the diazo method	Ribonuclease	Adsorption	63% of activity retained for 7.2 months	119
Bombyx mori silk cocoons	Silk powder by entrapment	Phenylalanine ammonia-lyase	Entrapment	Improved resistance against chymotrypsin and trypsin	120
Bombyx mori silk cocoons	Entrapment with silk blend membrane and polyvinyl alcohol (PVA)	GOx	Entrapment	Improved catalytic activity	121
Bombyx mori silk cocoons	Graphene silk fiber nanocomposite decorated by platinum nanosphere	GOx	Cross linking	Stable conductivity of ~57 S m^{-1} with an improved sensitivity of 150 μA mM^{-1} cm^{-2} and detection limit of 1 μM	122

matrix in industrial preparations.[101] Similar to silk fibers, silk films offer handy and extremely efficient supports for the long-standing stability of enzymes that are confined. Lu and co-workers[23] investigated the stabilization of horseradish peroxidase (HRP), lipase, and GOx entrapped in self-assembled SF films with higher enzyme loading. Among the three enzymes studied, GOx retained significant activity over 10 months of shelf storage at a wider temperatures range until 37 °C. The extent of molecular interaction between enzyme molecules and SF chains, the susceptibility of the enzyme to undergo oxidation, and hydrophobic–hydrophilic interfaces have been postulated to influence the relative stabilizing outcomes among the three enzymes used in that study.[23] Acharya *et al.*[102] explored an alternative and efficient method for the biosynthesis of L-DOPA from tyrosine using tyrosinase immobilized onto a novel silk protein: fibroin.[102] Saxena and co-workers[103] further demonstrated silk mat produced by weaving silk fibres of *Antheraea assamensis* as a promising biocompatible matrix for cholesterol oxidase (ChOx).[103] The porous and fibrous structural morphology of the silk mat provided an ambient microenvironment for immobilizing ChOx with loading efficiency of >70%, which resulted in a good analytical display with higher sensitivity, stability, reproducibility and much greater selectivity for the substrate (cholesterol).[103] On the other hand, protecting enzymes and proteins from undergoing inactivation induced by pH is a significantly difficult task. Taking up the challenge, Han and co-authors demonstrated that bombyx mori SF-based hydrogel with Ru(II)-mediated photo-chemical crosslinking served as an efficacious carrier for protecting an immobilized enzyme, carbonic anhydrase (CA), from pH denaturation.[104] The free enzyme lost its activity entirely at pH 3, whereas the immobilized CA maintained >20% of its initial activity. These results implied that CA was stabilized to a greater extent in SF-based hydrogels than the non-immobilized counterpart. Furthermore, the authors applied the same method for xylanase and lysozyme. They found that the immobilized enzymes achieved comparatively higher activity even at an unfavourable basic pH of 9.[104] Silk membranes have also been found to impart stability to enzymes against heat. Miyairi and colleagues[105] used a fibroin membrane as a support to immobilize β-glucosidase. Apart from heat tolerance, the immobilized biocatalyst could counter the effects of electrodialysis and protease treatments as well.[105] In addition, Dennis *et al.* demonstrated the value of SF entrapment for the preservation of OPH activity under a wide range of environmental circumstances, including high temperature, exposure to ultra violet light and to denaturing conditions imposed by organic reagents.[106] An enhancement in thermal stability similar to this work has also been reported by Yoshimizu[107] for SF powder over the enzyme invertase. In parallel, for treating acute lymphoblastic leukaemia, L-asparaginase (ASNase) is employed as a drug. However, to overcome its shortcomings in triggering an allergic response and a very short half-life, SF has been found to be suitable candidate for bioconjugation.[108] In this regard, to lower the immunogenic responses and the innate property of antigenicity of the enzyme, Zhang and colleagues[108] bioconjugated ASNase with polar functionalities of fibroin protein. The manipulated enzyme not only increased its stability against protease digestion (by trypsin), heat and storage, it also increased its half-life from 33 h to 63 h under laboratory conditions.[108] SF protein also offers a promising strategy to exploit the tremendous capabilities of Lac.[109] The adoption of gentle, aqueous processing techniques that were made possible by the specific chemistry, structure, and assembling of silk into nanodomains with low water content may be responsible for preservation of bioactivity during the manufacture of silk devices (Fig. 14).[110]

5. Chitin-based supports (carriers) for enzyme immobilization

Chitin is a biopolymer with potential applications in the biomedical sector.[123,124] After cellulose, chitin is the second most abundant natural polysaccharide in the world.[124–126] It is made up of β-(1–4) connected repeating units of *N*-acetyl-D-glucosamine (Fig. 15a) that can be found in the outer skeleton

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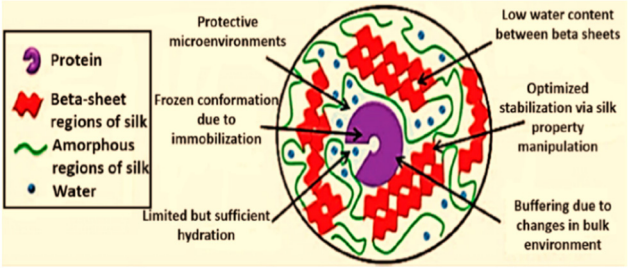

Fig. 14 Summary of the effects of stabilization of silk fibroin on immobilized compounds. Reproduced from ref. 110 with permission from Wiley-VCH, copyright 2012.

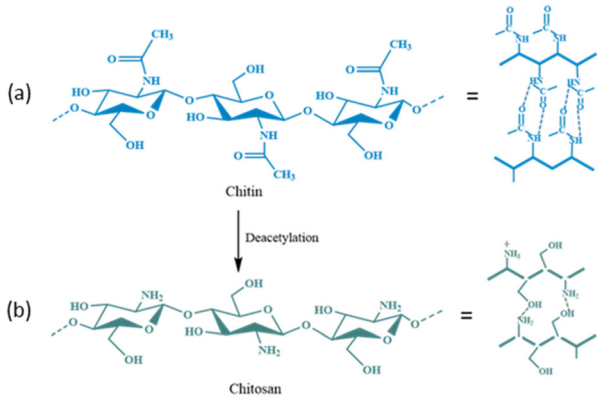

Fig. 15 Chemical structure and possible intermolecular hydrogen bonding (– –) in (a) chitin and (b) chitosan.

of arthropods, insects, marine invertebrates, and cell walls of some fungi.[125–127] This biopolymer has many potential applications in medicine, food, cosmetic, agriculture, and immobilization processes due to its low toxicity and bioactivity, as well as high biocompatibility, biodegradability, and antibacterial properties.[128–136] The supports based on chitin and its derivatives provide enzymes with good stability and affordable bioprocessing, and they can be used in various applications, ranging from agriculture to drug delivery. The enzymes bound to the chitin matrix also have greater thermal activity than that of free enzymes. Table 4[130–150] displays examples of recent research that used chitin-based supports to enhance the stability and activity of enzymes.

Important factors for choosing an appropriate enzyme support include the degree of chitin deproteinization, the availability of amino groups, the presence of minerals, mesh size, surface structure, and its conformation.[131,132] The activity of invertase and amyloglucosidase has been improved by immobilizing them on chitin supports.[131] Xavier *et al.*[132] compared the characteristics of glucoamylase and α-amylase on two distinct types of support: ceramic and chitin. The initial activities of the glucoamylase complexes immobilized on chitin were five-times higher than those on ceramics, and the proportion of activity loss was two-times lower.[132] The physical properties of chitin are also dependent upon its source. For example, due to the β-type crystalline structure, chitin from squid pens possesses superior physical qualities than chitin from crabs.[133] Chitin from squid pens was converted into a thin membrane that was immobilized with GOx. The membrane had the potential to entrap enzyme molecules as well as sufficient glucose and oxygen permeabilities to be beneficial for biosensors. The immobilized GOx on the chitin membrane was combined with an oxygen electrode to create a glucose sensor that had good stability and a wide linearity range (0.125–20 mM).[133] Immobilization methods can improved the properties of chitin further. To bind CRL, Gomes and coworkers[135] functionalized chitin with hexamethylenediamine followed by activation of glutaraldehyde. The activity profile as a function of pH, temperature, and thermal stability was determined for free and immobilized lipases. Authors revealed that the immobilized CRL had higher thermal stability than free CRL.[135] Leissy *et al.*[136] described a novel technique for immobilizing enzymes that relied on creation of polyelectrolyte complexes with supports coated in polymers with opposite charge. They used chitosan to chemically modify *Saccharomyces cerevisiae* invertase, which was immobilized further on a chitin support coated with sodium alginate. The protein-immobilized yield and activity was 85% and 97%, respectively. After immobilization, the optimal temperature and thermostability of invertase were increased by about 9–10 °C. The immobilized enzyme was four-times more thermally resistant than its native counterpart at 65 °C and stable against incubation in solutions of high ionic strength.[136] The authors suggested that the electrostatic immobilization technique they described might enhance the operational and functional features of the enzyme.[136] In another study, the same authors[137] described the immobilization of chitosan–invertase derivative on hyaluronic-acid-coated chitin. The immobilized enzyme was six-times more robust to thermal treatment at 65 °C than the native enzyme, and was stable against incubation in a solution of high ionic strength. Eighty percent of the initial activity of invertase was retained in the immobilized enzyme. The optimum temperature and thermal stability improved upon immobilization. The prepared biocatalyst showed exceptional temperature, storage, and operational-stability characteristics.[137] The reusability and recovery of enzymes can be enhanced further by using magnetic-chitin (MCT) matrices,[138] wherein magnetic granules were synthesized using chitin as a protective and dispersive matrix. The easily synthesised MCT particles with an average size of ~1.5 μm were modified with dopamine to provide a useful matrix for immobilizing enzymes. Dopamine was self-polymerized and coated onto MCT to provide an enzyme-adhesive surface. An enzyme involved in starch hydrolysis, α-amylase, was immobilized on the polydopamine-coated MCT (DMCT). The effectiveness of enzyme immobilization on DMCT particles was boosted by glutaraldehyde treatment.[138] Over a longer range of pH and temperature, the surface-immobilized α-amylase showed comparatively greater activity than that of the free enzyme. Also, the glutaraldehyde-treated polydopamine functionalized MCT microparticles with immobilized α-amylase had excellent dura-

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Table 4 Influence of chitin-based support materials on the stability and activity of proteins

Chitin source	Chitin derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Chitin	Glutaraldehyde-activated chitin flakes	Amylase	Covalent bonding and crosslinking	Superior operational stability with ~90% activity after 10 recycles and shelf life of >30 days with no loss in activity	130
Chitin from Krill	Krill chitin	Invertase and amyloglucosidase	Cross linking	Improved stability and activity	131
Chitin from squid pen	β-Crystalline chitin membrane	GOx	Entrapment	The enzyme-immobilized membrane is used for biosensors with wider linearity range of 0.125–2 mM	133
Chitin from crab shells	Hexamethylenediamine functionalized the chitin, and activated it with glutaraldehyde	*CRL*	Covalent bonding	Improved half-life (>425 h), excellent thermal stability, great activity yield and protein retention	135
Chitin from lobster shells	Sodium alginate-coated chitin support	*Saccharomyces cerevisiae* invertase	Electrostatic interactions	97% initial activity retained and good thermal stability	136
Chitin from lobster shells	Hyaluronic-acid-coated chitin support	Baker yeast invertase	Polyelectrostatic interactions	Enhanced thermal stability, storage, and operational stability. 69% of enzyme activity retained at 37 °C after 50 days of storage	137
Chitin	Poly dopamine coated magnetic chitin (DMCT) particles with glutaraldehyde	α-Amylase	Cross linking	Immobilized α-amylase showed higher durability, magnetic recovery with 6 times reusability with >70% activity retained	138
Chitin	Immobilization of lysine decarboxylase (CadA) through fusion of a chitin-binding domain (ChBD)	Lysine decarboxylase (CadA)	Fusion of a chitin-binding domain	Better pH stability with >73% activity at pH8 and high molar yield of cadaverine (~97%)	139
Chitin from lobster shells	Chitosan-coated chitin support with glutaraldehyde	Pectinase	Adsorption	Greater stability and after nine consecutive uses activity retained 100%	140
shells of shrimps and crabs	CHNW	Lysozyme	Adsorption	(1) Enhanced antimicrobial activity against *Escherichia*, *Staphylococcus* and *Bacillus species*. (2) Improved enzymatic activity (>1.5 folds)	141
Chitin	Chitin and starch linked as a support	Lipase and protease from sunflower seeds	Covalent binding	Enhanced the efficiency of detergents stain removal	142
Chitin powder	AuNPs immobilized on CNFs	GOx	Not mentioned	Enhanced colorimetric glucose detection with an LOD of 94.5 nM	143
Chitin	Aerogel beads made of chitin/graphene oxide (Ch/GO) composite crosslinked with glutaraldehyde	CRL	Adsorption followed by crosslinking	With immobilization capacity of >140 mg g^{-1}, the biocatalyst can be reused for over 5 cycles with retention of 90% initial activity	144
Shrimp shells chitin	Chitin	Dextranase	Adsorption and covalent binding	A maximum of 88% immobilization yield achieved with superior 22 folds higher stability at 80 °C	145
Chitin	Magnetic chitin nanofiber composite (MCNC)	Chymotrypsin (CT)	Cross linking	Improved the loading capacity by 6.3 times after cross-linking, increased thermal stability and retained initial activity	146
Chitin	Chitin powder	α-Amylase from *Bacillus subtilis* ITBCCB148	Adsorption	Immobilized α-amylase can be used up to five times and has an optimum temperature of 75 °C, increased the residual activity	147
Chitin	3D fibrous chitinous scaffolds and silica nanopowder	HRP	Adsorption	Showed reusability of nano-SiO_2 (HRP)-chitin (HRP) scaffolds and high efficiency towards removal of 17-α-ethinylestradiol (EE2)	148
Chitin	Chitin powder	*Aspergillus* Fumigatus α-Amylase	Adsorption	Improved thermal stability with >39% activity retained at 80 °C and 1.5 times higher activity at ambient conditions	149
Chitin	Chitin-Bentonite Hybrid matrix	α-Amylase from *Aspergillus* fumigatus	Physical adsorption	Enhanced stability with ~4 folds higher $t_{1/2}$ compared to native enzyme	150

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bility, magnetic recovery and reusability.[138] Zhou *et al.*[139] used chitin as the carrier support for efficient immobilization of CadA through fusion with a chitin-binding domain (ChBD) for the synthesis of cadaverine from L-lysine.[139] Furthermore, compared with wild CadA, the fusion protein (ChBD-CadA) had greater pH stability and maintained >73% activity at pH 8.[139] In a batch conversion, the immobilized ChBD-CadA (I-ChBD-CadA) converted L-lysine (200.0 g L^{-1}) to cadaverine (135.6 g L^{-1}), obtaining a 97% molar yield of the substrate (L-lysine). Furthermore, I-ChBD-CadA was reusable at high L-lysine concentrations and retained >57% of its initial activity after four cycles of usage without the addition of acid to maintain pH (Fig. 16). These results indicate that immobilizing CadA with a chitin-binding domain could be used for the industrial synthesis of cadaverine.[141] Ramirez and co-authors[140] developed a novel bioconjugate for immobilization of pectinase on a chitosan-coated chitin support. As a result of immobilization, heat and temperature resistance were improved and the enzyme remained stable with 100% of activity retained after nine consecutive uses, and ~70% of initial activity after 15 cycles of reuse.[140]

Chitin-derived supports can also be used to enhance the antibacterial activity of various enzymes. Suisui *et al.*[141] investigated how chitin nanowhiskers (CHNWs) could enhance the antimicrobial and enzymatic activities of lysozyme adsorbed on CHNWs. Enzyme assays displayed that lysozyme-CHNW had 1.5-times higher enzymatic activity and greater antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* compared with those of the native lysozyme. These results revealed that lysozyme-CHNW could be used as a powerful antibacterial agent in food and medical fields.[141] Mehdi and co-workers[142] developed a unique method for immobilizing protease and lipase on chitin-starch material as an enzyme biocatalyst matrix. Compared with native enzymes, immobilized enzymes had better pH, thermal, reusability, and storage stability.[142] Enzyme-immobilized chitin-coated nanoparticles are effective biocatalysts due to their surface area and mechanical strength.[143,144] Yao *et al.*[143] presented a simple green method for the heterogeneous synthesis of stable, size-controllable gold nanoparticles (AuNPs) immobilized on biocompatible chitin nanofibrils (CNFs). CNF-AuNPs showed peroxidase-mimic behaviour and catalysed the oxidation reaction of 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2. When coupled with GOx, with a limit of 94.5 nM, CNF-AuNPs could detect glucose sensitively. This method had excellent specificity and efficiency, was affordable, and suffered negligible contamination, which showed its importance for the diagnosis of diabetes mellitus.[143] Chen *et al.*[144] fabricated chitin/graphene oxide (Ch/GO) composite aerogel beads for lipase immobilization using chitin as a great blending material. The synthesised beads had a three-dimensional porous structure, and GO was attached tightly to chitin with enhanced mechanical strength and surface area. Under ideal conditions, CRL was immobilized on Ch/GO aerogel beads. The immobilized CRL demonstrated excellent thermal stability compared with that of the native enzyme and, even after recycling five times, the immobilized lipase maintained initial activity of >90%.[144] Shahid *et al.*[145] improved the functionality of dextranase (isolated from a thermophilic bacteria *Bacillus megaterium* KIBGE-IB31) with the help of its immobilization on chitin by various protocols. Adsorption and covalent binding strategies were used to immobilize the isolated dextranase on chitin. Compared with the enzyme immobilized by an adsorption approach, dextranase immobilized by covalent cross-linking demonstrated maximum stability at high temperatures combined with improved recycling efficiency. Thus, chitin appears to be an inexpensive and convenient matrix for immobilizing a variety of enzymes to increase the stability and reusability of enzymes at an industrial scale.

Fig. 16 Immobilization of lysine decarboxylase for converting L-lysine to cadaverine *via* fusion of chitin-binding domains. Reproduced from ref. 139 with permission from Frontiers, copyright 2020.

6. Chitosan-based supports (carriers) for enzyme immobilization

Chitosan is a linear polysaccharide made up of *N*-acetyl-D-glucosamine (acetylated unit) and randomly arranged β-(1 → 4)-linked D-glucosamine (deacetylated unit) (Fig. 15b).[151,152] Chitosan can be used in medical applications due to its antibacterial, wound-healing, biodegradable, biocompatible, and non-toxic features.[153–155] The chitosan matrix can be researched as a support for enzyme immobilization. The storage, thermal, operational, and pH stabilities can be greatly improved for enzymes that are immobilized on the surface of chitosan (Table 5).[156–214] Chitosan-based materials can be produced in different geometrical configurations (*e.g.*, microcapsules, membranes, beads/microspheres, coatings, fibers, gels, and sponges), which have shown good storage, thermal/operational stability, and reusability properties. Owing to these flexible features, their capacity as carriers for a wide range of enzymes could be applied practically. Immobilizing xylanase in xanthan/chitosan matrices has revealed higher thermal stability and activity than those of native enzymes.[164] Çetinus *et al.*[167] demonstrated that catalase (CAT) immobilized into chitosan beads had enhanced resistance against denaturation due to pH or heat. Immobilized CAT had a K_m value that was two-times greater than that of free CAT and its V_{max} decreased

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Table 5 Influence of chitosan-based support materials on the stability and activity of proteins

Biomass	Biomass derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Chitosan	Magnetic gel microspheres made of chitosan, cellulose, and Fe_3O_4	GOx	Cross linking	Higher conversion yield (>91%) and great recyclability with >84% activity retention even after 15 cycles	79
Chitosan	Mesoporous silica/titania with a chitosan coating	β-D-Galactosidase	Covalent binding	High activity (1223 U g^{-1}), excellent efficiency (74%) and >90% activity retained after 15 cycles of reuses	153
Chitosan	Chitosan beads activated with glutaraldehyde	Lipase	Not mentioned	46.5% of initial activity was improved	155
Chitosan	Glutaraldehyde cross-linked chitosan beads	*Pleurotus nebrodensis* WC 850 laccase	Cross linking	Great decolourization efficiencies for various reactive and disperse dyes (>83–90%) and >84% retained activity after 8 cycles of reuse	156
Chitosan	Manganese-ferrite nanoparticles coated with chitosan	Laccase	Covalent bonding	Best suited for pharmaceutical waste degradation with 80% removal rate for diclofenac	157
Chitosan	Genipin-activated chitosan support	β-Galactosidase	Cross linking	Increased thermal and storage stabilities with potential application in food industries harbouring lactose hydrolysis	158
Chitosan	Chitosan matrix	Bromelain, Papain and Cysteine proteases	Adsorption	(1) Intact optimum pH and temperatures. (2) ~5.8 times and ~7.6 enhanced stability for Bromelain and Papain respectively	159
Chitosan	Chitosan/organic rectorite composites	Polyphenol oxidase (PPO)	Physical adsorption and covalent binding	(1) Higher enzyme activity ~8920–16370 U g^{-1}. (2) 89–93% phenol derivative removal within 2 h with 10 cycles of recyclability	161
Chitosan	Chitosan nanoparticles	L-ASNase	Encapsulation	Significant decrease in the minimum inhibitory concentration (MIC) against microbes (5.26 mg mL^{-1})	162
Chitosan	Chitosan microbeads by microencapsulation	α-Amylase and invertase	Microencapsulation	Enhanced the stability and activity	163
Chitosan	Chitosan-xanthan hydrogels	Endo-1,4-β-xylanase	Noncovalent link	Enzymes show 60–70% higher activity than free enzymes	164
Chitosan	Copolymer of chitosan and poly-glycidyl methacrylate (GMA)	Urease	Covalent bonding	Enhanced enzyme specific activity, temperature stability, pH stability, and storage stability	165
Chitosan	flakes and porous chitosan beads (PCB)	Lipase	Physical adsorption	Immobilized enzyme showed better thermal stability at pH-6 and 40 °C	166
Chitosan	chitosan beads crosslinked with glutaraldehyde	Bovine liver catalase (CAT)	Cross linking	Improved enzyme thermal, operational, and storage stabilities	167
Chitosan	The chitosan and TMSO were coupled using the sol–gel method to create porous gels of chitosan-SiO_2	GOx	Entrapment	Enhanced stability	168
Chitosan from dried uttlebone cartilage	Chitosan and glutaraldehyde-crosslinked activated clay beads in equal weights	α-Amylase, glucoamylase and β-amylase	Cross linking	Superior operational stability with 81% retention of activity after 50 cycles of reuse	169
Chitosan	Magnetic chitosan microspheres cross-linked with glutaraldehyde	Laccase	Adsorption and cross-linking	Increased pH and temperature stability	170
Chitosan	Chitosan beads activated with glutaraldehyde	PGA	Covalent attachment	Good thermal and alkaline pH stabilities	171
Chitosan powder	Chitosan microspheres and sponges	Cellulase	Covalent method	Higher protein loading (~145 mg g^{-1}), better storage stability and reusability	172
Chitosan	Nanoparticles of chitosan made by the ionisation gelation process	Neutral proteinase	Adsorption	Enhanced operational, storage, and thermal stability	173
Chitosan	Nanoparticles of chitosan made by the ionisation gelation process	Neutral lipase	Adsorption	Increased the enzyme's efficiency by 13.17% compared to free lipase	174

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Table 5 *(Contd.)*

Biomass	Biomass derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Chitosan	Chitosan beads (Ch-bead) were attached to cibacron blue F3GA dye (CB-Ch-bead)	CAT	Adsorption	Better thermal, operational and storage stabilities	175
Chitosan	Chitosan beads	Pepsin	Cross linking	Increased thermal stability with shift in optimum temperature from 40 to 50 °C	176
Chitosan	Polyvinyl alcohol (PVA) coated chitosan beads	Cellulase	Adsorption	Immobilized cellulase showed better yield, pH stability (at pH 7), good storage and operational stability	177
Chitosan	Chitosan nanofibrous membrane	Lipase	Entrapment	Improved storage stability with ~92% activity after 48 days of incubation	178
Antarctic krill shells	Glutaraldehyde-pretreated chitosan membranes	Urease	Entrapment	Improved thermal stability with raise in optimum temperature from 65 to 75 °C	179
Cuttlebone chitosan	Chitosan–clay composite bead cross-linked with glutaraldehyde	β-Glucosidase	Cross linking	Enhanced storage stability and activity in dried composite	180
Powdered chitosan	Chitosan beads	Chymotrypsin	Covalent multipoint attachment	At 65 °C, the immobilised enzyme's half-life increased from 0.57 hours at 55 °C to 7.8 hours	181
Powdered chitosan	Chitosan and agarose with glutaraldehyde	*Candida Antarctica* lipase type B (CALB)	Multipoint covalent attachment	Enhanced the thermal stability and activity	183
Chitosan	Nanocapsules made of CKGM-CS	L-ASNase	Entrapment	Prevent the leaking. Better stability and activity for broad pH range	184
Chitosan	Chitosan crosslinked with glutaraldehyde used for adsorption of Cu(II)	CAT	Cross linking	Though Cu-Ch-CAT had 3 folds lesser substrate affinity, but imparted greater thermal and storage stabilities compared to Ch-CAT	186
Chitosan	Magnetic chitosan nanoparticles	Laccase	Entrapment	Increased storage stability and operation stability	187
Pink shrimp	Modified chitosan beads	HRP	Covalent method	Enhanced the thermal stability and operational stability	188
Chitosan	Chitosan beads	CA	Physical adsorption	Half-life increased by 24 h and 48 h compared to native enzyme at −20 °C and R.T. respectively	189
Chitosan from crab shells	Chitosan-coated magnetite nanoparticles (Fe_3O_4-CS)	Laccase	Adsorption	Enhanced storage stability with >70% activity after 30 batches of use	190
Chitosan from shrimp shells	Chitosan macroparticles and nanoparticles	β-Galactosidase	Adsorption	75–83% activity was retained even after 50 cycles of reuse	191
Chitosan	Glutaraldehyde crosslinked chitosan-clay composite beads	Tyrosinase	Cross linking	Optimum temperature, loading effectiveness, and activity were all increased	192
Chitosan powder	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) are utilised as coupling agents to covalently immobilise chitosan-coated Fe_3O_4 nanoparticles	Lipase	Covalent method	The stability remains same after 13 days of storage at 25 °C, the immobilised lipase exhibits enhanced operational stability, including wider temperature and pH ranges	193
Chitosan from shrimp shells	Chitosan macrobeads by covalent crosslinking using glutaraldehyde	Trypsin	Covalent cross-linking	Improved pH and temperature stability	194
Chitosan	Chitosan–halloysite hybrid-nanotubes cross-linking with glutaraldehyde	HRP	Cross-linking	The immobilised HRP did not lose any activity after 35 days of storage	195
Chitosan	Magnetic chitosan carriers	Lipase and β galactosidase	Entrapment	Demonstrated excellent long-term stability without enzyme leaking from the support	196
Chitosan	Glutaraldehyde-hardened alginate-chitosan beads	Inulinase	Cross linking	At 50 °C gel beads showed excellent activity. In six days at room temperature, the immobilised enzyme retained 76% of its activity	197

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Table 5 *(Contd.)*

Biomass	Biomass derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Chitosan	Fe_3O_4 magnetic nanoparticles with chitosan coating and glutaraldehyde coupling agent	Cellulase	Covalent method	Greater operating stability throughout a wider range of temperatures and pHs, as well as good activity and reusability during magnetic separation recovery	198
Chitosan	Hydrogel beads made of a bacterial cellulose (BC) and chitosan composite	Lipase	Physical adsorption and covalent cross-linking	BC-chitosan hydrogel beads immobilized showed higher catalytic activity (1.9 times), and greater enzyme stability than microcrystalline cellulose MCC-chitosan hydrogel beads (22.7 times higher $t_{1/2}$)	199
Chitosan	Dextran polyaldehyde is used as a macromolecular cross-linking agent to create chitosan magnetic nanoparticles (CMNPs)	Pectinase	Cross-linking	2 folds improved thermal stability (55–75 °C) excellent stability and durability reusability	200
Chitosan	Chitosan-blended cellulose monoacetate nanofibers	Protease	Physical adsorption	Higher operational stability, activity and reusability	201
Chitosan	Chitosan-montmorillonite nanocomposite beads with glutaraldehyde	α-Amylase	Covalent bonding	95% activity retained after 40 days of incubation at 4 °C (~2.6 times higher than native enzyme)	202
Chitosan	Chitosan nanoparticles (CS-NPs)	GOx	Covalent attachment, cross linking	Improved the stability and activity with ~26 folds higher activity (with precipitation method) compared to covalent attachment	203
Shellfish derived chitosan (CS) powder	Clay/chitosan biocomposite systems	Protease	Covalent method	Increased the immobilization yield	204
Chitosan	Magnetic chitosan nanoparticles (CS-Fe_3O_4) modified with imidazole based ionic liquid (IL-CS-Fe_3O_4)	Porcine pancreatic lipase (PPL)	Physical adsorption	Having 1.93 folds higher specific activity with 382% activity recovery, immobilized PPL showed 84% remaining activity after 10 cycles of reuse	205
Chitosan	Chitosan-based nanoparticles	Glucoamylase	Cross linking	Greatly increased loading capacity and storage stability	206
Chitosan	Silica/chitosan composite	Laccase	Covalent method	Good residual activity after 7 months of storage	207
Chitosan from crab shell	biocompatible glutaraldehyde (GA) cross-linked chitosan beads as a matrix	Chitinase	Cross linking	Immobilized enzyme was found to be highly stable up to 1 month of storage and exhibited wider range of pH tolerance	208
Cuttle-fish waste	Highly swollen chitosan beads cross-linked with glutaraldehyde	Acid phosphatase and β-glucosidase	Cross linking	Enzymes were stable for long term	209
Chitosan	Fe_2O_3/chitosan coated superpara-magnetic nanoparticles was cross-linked with glutaraldehyde	Lipase	Cross linking	(1) Broader working temperature range from 27–85 °C and pH tolerance in between 5.5–9.5. (2) Achieved 70.2% of microwave assisted biodiesel recovery	210
Chitosan	Chitosan functionalized with Concanavalin A (ConA)	α-Galactosidase	Adsorption, cross linking	Enhanced reusability and activity yield	211
Chitosan	Chitosan matrix	Papain	Adsorption	Enhanced stability against UV irradiation (up to 6040 J m^{-2})	212
Chitosan	Chitosan coated polylactic acid (PLA) based nanofiber (CCN) matrix	α-Amylase		Higher substrate conversion ratio of 0.85–0.99 obtained with 40 min of residence time and lower rate of dilution	213
Chitosan	Chitosan gel activated with glutaraldehyde	Cellulases	Cross linking	Better hydrolysis performance with Avicel and sugarcane bagasse leading to glucose concentrations of 10–13 g L^{-1} in the hydrolysate	214

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from 32 000 μmol to 122 μmol (min mg protein)$^{-1}$. However, immobilization enhanced the thermal, operational, and storage stabilities of CAT.[167] Yang *et al.*[168] immobilized GOx on a novel porous chitosan-SiO_2 gel that was synthesized by coupling chitosan with tetramethoxysilane (TMOS) using a sol–gel method. With a profound immobilization yield of 97% and GOD activity of 1585 U g^{-1}, even after 10 days and 15 days, the immobilized enzyme remained stable and functional, with activity of 86% and 56% at 30 °C, respectively, whereas most of the free enzyme became inactive after 5 days in a similar condition.[168] Mao and co-workers developed chitosan microspheres and sponges in smaller sizes to immobilize cellulase.[172] With rapid adsorption of cellulase over microspheres (<25 min), the immobilized cellulase displayed higher stability with respect to pH, higher K_m, thermal stability, reuse, and storage stability than those of the native enzyme. Hence, this system could be employed in various industries demanding an enzyme with good performance at extreme physiochemical conditions. Dinçer *et al.*[177] enhanced the stability of acid cellulase at neutral pH. Chitosan beads were coated with modified polyanionic PVA, and cellulase was immobilized on modified PVA-coated chitosan beads. Compared with the free enzyme, cellulase immobilized on chitosan beads demonstrated improved pH stability. The optimal pH of the enzyme moved from 4.0 to 7.0, signifying its ability for catalysis over a wide range of pH conditions, and also displayed excellent storage and operational stabilities.[169]

To further explore the ability of a chitosan matrix to stabilize enzymes at different pH values, Tang *et al.*[166] prepared chitosan nanoparticles *via* ionization gelation, which yielded spherical nanoparticles of diameter 100 nm. Immobilized lipase showed improved activity, thermal, storage, and operational stabilities. The immobilized neutral lipase had a higher K_m (0.37×10^2 g l^{-1}) than that of free neutral lipase (1.01×10^2 g l^{-1}). Moreover, immobilization improved the stability of neutral lipase in the acidic range. The stability features of the immobilized enzyme were enhanced noticeably with respect to temperature, reuse, and storage duration.[166]

To solve the problem of the separation and reusability of a biocatalyst, Liu and group[79] used the sol–gel transition method to develop magnetic Fe_3O_4-cellulose-chitosan hybrid gel microspheres by employing ILs as the solvent for the dissolution and regeneration of cellulose and chitosan. Glutaraldehyde was used to further immobilize GOx on hybrid gel microspheres (Fig. 17), which had a broader pH range, greater thermal stability, and enhanced storage stability than those of free GOx. The pH optimum of immobilized GOx was in the range 4–7, whereas that of free GOx exhibited maximal activity at pH 6. After 28 h of heating at 50 °C at pH 7, the immobilized enzyme exhibited 63% of its initial activity. However, under identical conditions, the free enzyme displayed only 22% of its initial activity.[79] Similar to the report stated above, Lac was immobilized on chitosan-coated magnetite (Fe_3O_4) nanoparticles. Kinetic analyses revealed that the immobilized Lac system had identical catalytic efficiencies. However, at optimal conditions, after 30 batches of use,

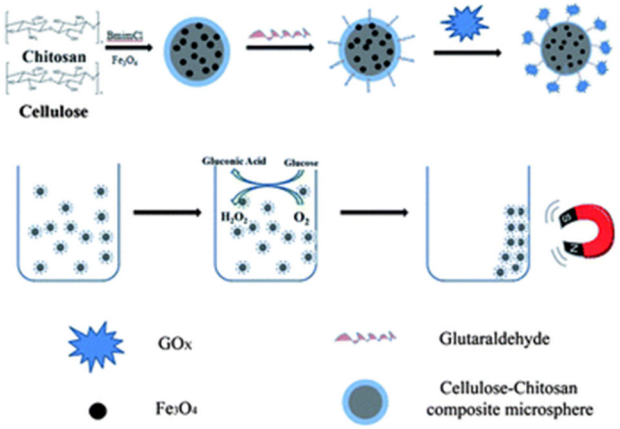

Fig. 17 Preparation of magnetic Fe_3O_4–cellulose–chitosan microspheres after enzyme immobilization onto magnetic cellulose–chitosan (schematic). Reproduced from ref. 79 with permission from RSC, copyright 2012.

immobilized systems continued to exhibit >71% of their initial activity.[190] With regard to other closure studies,[188,191–196] lipase, β-galactosidase, cellulose, pectinase, and glucoamylase were immobilized onto chitosan-coated Fe_3O_4 NPs.[191–196] These immobilized enzymes on magnetic chitosan carriers demonstrated prolonged stability with minimal leaching. They also conveyed better operational stability, including a wider thermal range, pH, reusability and storage stabilities, than those of the free enzyme. Hence, Fe_3O_4-chitosan nanoparticles could be used as efficient supports for lipase immobilization, particularly in applications involving the need for recycling.[188] Huang *et al.*[178] synthesised a chitosan nanofibrous membrane, and CRL was immobilized on the nanofibrous membrane using glutaraldehyde as a coupling reagent. Lipase loading on the nanofibrous membrane attained 63.6 mg g^{-1}, and immobilized lipase activity was 49.8%. After immobilization on the chitosan nanofibrous membrane, the stability towards temperature, pH, reuse, and storage of immobilized lipase was enhanced. With this good immobilization fraction of loading capacity and improved stability, the membrane of chitosan nanofibers is an excellent biocompatible and potential support for enzyme immobilization.[178] In the case pf porcine pepsin, immobilization onto chitosan beads shifted the optimum temperature by 10 °C, which was higher than that of the native version, revealing the resistance of the immobilized enzyme towards heat-induced denaturation along with a longer shelf-life. Observing these properties, Altun and colleagues[176] demonstrated the applicability of this system in industries based on the clotting and processing of milk. Adriano and co-workers[181] revealed a very noticeable improvement in the thermal stability of α-CT after covalent bonding of the enzyme on a chitosan-based hybrid gel. The half-life of immobilized chymotrypsin was enhanced from 0.57 h at 55 °C to 7.8 h at 65 °C.[181] To improve the enzyme-holding capacity and to prevent leakage, Wang *et al.*[184] prepared carboxymethyl konjac glucomannan–chitosan (CKGM–CS) nanocapsules as a

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new biocompatible matrix system for the immobilization L-ASNase. The semi-permeability of the matrix retained L-ASNase in the matrix and inhibited leakage while allowing the substrate and product to pass through. The immobilized enzyme system demonstrated noticeably greater stability and activity in comparison with those of free L-ASNase. These investigations could offer a brand-new material for the immobilization of pH- and temperature-sensitive enzymes.[184] Pertaining to the challenge of reusability, Flores *et al.*[182] discovered a technique to activate chitosan beads using genipin, over which β-galactosidase from *Aspergillus oryzae* was immobilized. During batch processing, the immobilized enzyme maintained 100% of relative initial activity up to 40 cycles without much compromise in thermal stability, thereby showing its potential applicability. Similarly, a system of inulin over chitosan beads showed a recycling capacity up to 14 cycles after engineering by Singh *et al.*[185] Monier and co-workers[188] developed a method to immobilize HRP on modified chitosan beads by graft copolymerization of polyethylacrylate with potassium persulphate and Mohr's salt as redox initiators (Fig. 18). Even after immobilization, the optimum temperature remained at 45 °C with higher relative activity than that of the native enzyme.[188] Wanjari *et al.*[189] immobilized CA on chitosan beads and its activity was evaluated using the *p*-nitrophenol assay (Table 5). The storage stability was profiled for up to 20 days and the half-life of immobilized CA increased up to 48 h. The authors suggested that this system could be applied for carbon-capture and higher efficiency for converting CO_2 to $CaCO_3$.[189] Zhai and colleagues[195] developed chitosan–halloysite hybrid-nanotubes (CTS-HNTs) through the assembly of chitosan onto halloysite (natural nanotubular aluminosilicate). HRP was covalently immobilized over nanotubes through crosslinking (Fig. 19). Even 35 days later, the immobilized HRP maintained its full activity whereas the free enzyme could maintain only 27% of its initial activity. The authors claimed that the CTS-HNT-immobilized HRP system could be employed for wastewater rejuvenation, particularly for the destruction of phenolic chemicals, because of its strong catalytic activity.[195]

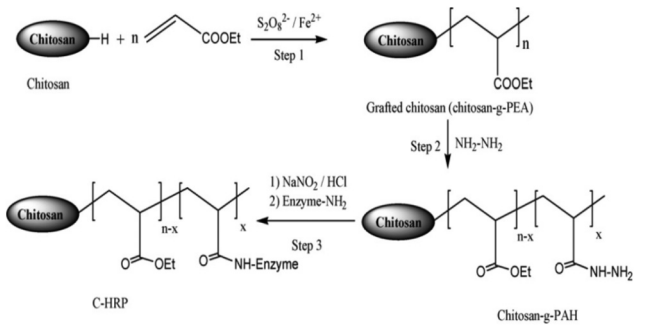

Fig. 18 HRP immobilization onto modified chitosan beads (schematic). Reproduced from ref. 188 with permission from Elsevier, copyright 2010.

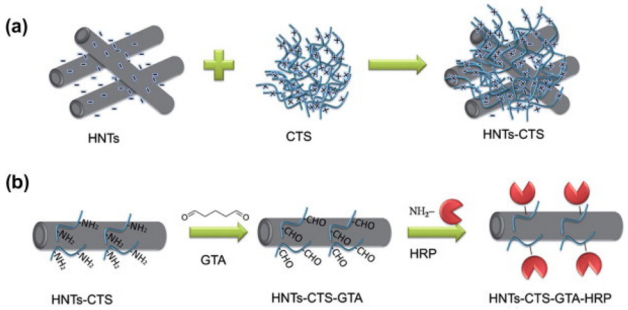

Fig. 19 (a) Stepwise preparation and (b) immobilization of HRP on a chitosan (CTS) matrix. Reproduced from ref. 195 with permission from Elsevier, copyright 2013.

7. Alginate-based supports (carriers) for enzyme immobilization

Alginic acid is a naturally occurring polysaccharide and has a negative charge. It is extracted from algal cell walls, in particular brown algae or seaweeds. Alginate is a liner copolymer that consists of two residues: β-D-mannuronate (termed "M block") and α-L-guluronate (termed "G block"). Both residues are C5-epimers (Fig. 20).[215] Depending on the source, the blocks M and G can be arranged in a consecutive manner (*e.g.*, MMMM followed by GGGG or *vice versa*), alternating patterns (MGMGMGM), or random.[215] The properties of alginate (*e.g.*, interaction with cationic metal species, solubility, and viscosity,) are directly attributed to the molecular weight of mannuronic acid/guluronic acid.[215,216] Alginate is stable, biocompatible, biodegradable, nontoxic, has chelating ability, and relatively inexpensive. Thus, this natural polysaccharide and

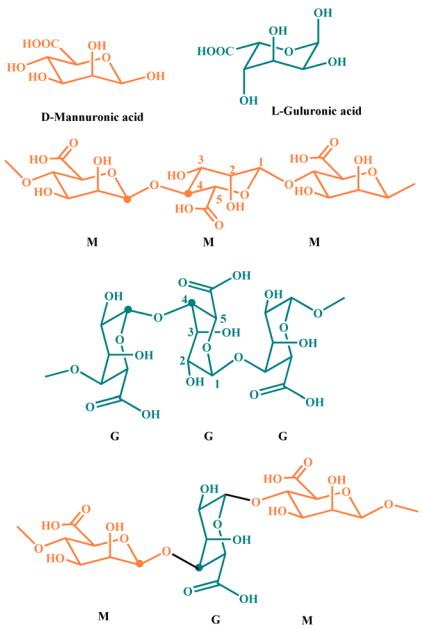

Fig. 20 Chemical structure of alginate. Reproduced from ref. 215 with permission from ACS, copyright 2005.

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its derivatives have a multitude of industrial uses.[216] Alginate-based products have been used for drug delivery, regenerative engineering, environmental clean-up, wound dressing, biosensors, and transfection of genetic material.[217,218] An attractive approach to increase the stability of enzymatic processes and economic feasibility in terms of reusability can be achieved with the use of alginate-based supports for enzyme immobilization.[219–226] The mechanical characteristics and robustness of alginate-based supports can be enhanced further using polymer blending (much like cellulose and chitosan). These alginate forms have been demonstrated to increase thermal stability, enzyme activity, and reusability.[227–236]

Palmieri *et al.*[230] developed a novel procedure for enzyme immobilization by entrapment in copper alginate gel, which was applied for immobilizing fungal phenol oxidase. The immobilized enzyme boosted the rate of oxidation of various aromatic substrates, was active over a large pH range, and had a low temperature for optimal activity. Thus, a system for detoxifying wastewater contaminated with phenolic derivatives from industrial or agricultural sources could be developed using an enzyme that has been immobilized in this way.[230] By first combining polysaccharides with Ca^{2+} and then cross-linking glutaraldehyde with the amine groups of the gelatine found in the initial mixture, Elçin and colleagues[231] created urease-containing xanthan-alginate spheres. Higher enzymatic activity was shown by encapsulated urease even with variations in pH or temperature. Even after 20-times of its use in optimal conditions, xanthan-alginate spheres continued to have 75% of the maximum urease activity.[231] More recently, a multifunctional alginate-derived carbon (AAC) material having variable oxygen functionalities was created by our research team[232] for the immobilization of Cyt c. Without affecting the structural stability if Cyt C, the enzyme activity was increased significantly in comparison with that of the native protein (Fig. 21).

For the entrapment of different enzymes, calcium alginate (Ca-ALG) use seems to be economical and biocompatible. Blandino *et al.*[233] systematically studied the activity of encapsulated GOx within Ca-ALG gel capsules. Comparison of the apparent kinetic characteristics of immobilized and free GOx revealed that the immobilized GOx had a higher K_m and more pronounced differences in V_{max}.[233] Tanriseven and co-authors[235] immobilized *Saccharomyces cerevisiae* invertase in alginate capsules, which achieved 87% relative activity along with enzyme stabilization at high pH and temperatures.[235]

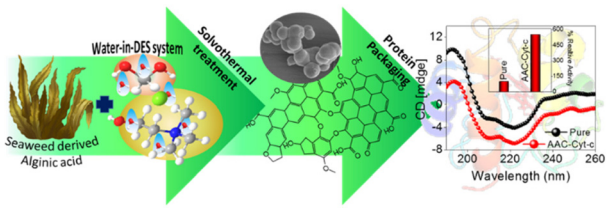

Fig. 21 Preparation of functionalised solvothermal carbon derived from alginate using "water-in-deep eutectic solvents" for enhancing enzyme activity (schematic). From ref. 232.

Alginate-based gels have also enhanced the thermal and storage stabilities of various proteins.[236] Vu and colleagues[236] investigated the biochemical characteristics of invertase entrapped within alginate gels. The K_m of immobilized invertase (139.19 mM) was higher than that of free invertase (93.19 mM), but its V_{max} was smaller. Nevertheless, the support induced greater thermal stability to invertase (as manifested by a longer half-life) and endowed significant long-term stability (up to 40 days).[236] To study the normalized effect of alginate matrices, Fadnavis *et al.*[237] employed lipase from porcine pancreas, *Pseudomonas cepacia*, and *Candida rugosa*. Hydrogels prepared by crosslinking a blend of sodium alginate (5%) and gelatin (3%) with glutaraldehyde were used to immobilize these lipases, which had excellent efficiency and greater stability and reclaimability for ~10 recycles without much loss of enzyme activity. Then, using a straightforward procedure of protein binding at pH 5 and release at pH 8.5, these functionalized beads were employed to purify crude porcine pancreatic lipase 7.4-times, showing their feasibility for biomedical applications.[237] Thanks to advances in core–shell chemistry within nanoscience, Taqieddin and co-workers[238] designed alginate-chitosan core–shell microcapsules to create biocompatible carriers for enzyme immobilization. These shells offered permeability control over substrates and end products, whereas the protein was maintained in a liquid or solid core. To create a liquid or solid core for the microcapsule, sodium alginate was crosslinked with calcium or barium ions, respectively. *o*-Nitrophenyl-β-D-galactopyranoside (ONPG) was used to assess the catalytic activity of a model enzyme, β-galactosidase, which was immobilized in the alginate core. Due to the extra layer required for the influx of the substrate and outflow of the product, V_{max} for Ca^{2+} and Ba^{2+} alginate-chitosan core–shell microcapsules was much lower than that of the free enzyme.[238] Ultrathin alginate/protamine/silica (APSi) composite membranes were constructed by Wang *et al.*[239] using a co-extrusion minifluidic and biosilicification methodology for Lac immobilization. The prepared hybrid capsules were highly monodisperse and dispensed Lac with significantly high thermal, storage, and pH stabilities. With ~100% immobilization yield, Lac displayed an activity of 61.8 mmol g^{-1} min^{-1}, along with withholding 67% activity after 20 days and residual relative activity in APSi capsules remaining at 45% after 10 cycles.[239] Alginate-based hybrid composites have become an innovative class of materials, providing a larger range of uses for enzyme encapsulation encompassing biocatalysis, biomedicine, bioseparation, and biosensing.[240–245] To immobilize Lac, Lu *et al.* created alginate-chitosan microcapsules using emulsification-internal gelation.[240] In addition to stability, a higher loading efficiency and immobilized yields were obtained under ideal immobilization conditions (0.3% chitosan, 2% sodium alginate, 2% $CaCl_2$, and a 1 : 8 ratio by volume of enzyme : alginate). However, the recyclability was only three cycles, so finding a remedy for this problem is an issue.[240] A multidimensional approach using biomass and nanomaterials to prevent leakage of yeast alcohol dehydrogenase (YADH) from *Saccharomyces*

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cerevisiae source was availed by Xu *et al.*[241] By adding silica nanotubes (SiNTs) to the alginate (ALG) gel and then cross-linking it with calcium, they designed an alginate-silica nanotube (ALG-SiNT) composite, which was used to encapsulate YADH. Enzyme leakage from the carrier was reduced significantly (~50%) compared with the alginate matrix composite only. These results showed that an ALG-SiNT carrier furnished stronger affinity with the enzyme compared with YADH immobilized over simple ALG support and led to longer storage, increased activity retention, and a stable operation.[241]

We applied a new and straightforward approach to prepare stable and versatile alginate-based white light-emitting hydrogels (WLEs) for protein packaging.[242] Using the notion of complementary colors, a WLE composite was constructed by engineering the surface of an orange light-emitting ZnS quantum dot (QD) doped with Mn^{2+} using a blue light-emitting IL-choline-tosylate. The WLE QD-IL composite was combined with an alginate bio-polymer to fabricate the WLE hydrogel. After that, Cyt c was confined within the WLE hydrogel matrix, which led to higher relative activity of ~180% compared with that of the free enzyme (Fig. 22). The authors counteracted the harsh effects of chemical denaturants (*e.g.*, urea) and extreme temperature over the stability of the metalloprotein Cyt c. Extending this concept further, Ma *et al.*[243] discovered an efficient carrier, double walled carbon nanotube-doped alginate gel (DWCNT-ALG) for immobilization of lactate dehydrogenase (LDH). Leakage of LDH from the LDH-DWCNT-ALG biocomposite was decreased significantly, and LDH that had been immobilized displayed increased activity. The authors demonstrated the practical use of this type of carrier for lactate bioconversion in industry, as well as its usage for the immobilization of additional enzymes and microorganisms (including LDH and ADH).[243] Enzyme entrapment in ALG beads has been shown to be relatively inexpensive, safe, and straightforward technique for separation of the product and enzyme at the industrial scale.[244] Bhushan *et al.*[244] prepared ALG beads in an aqueous mixture containing sodium alginate, lipase from *Arthrobacter* sp. (ABL), and $CaCl_2$ to empower its reusability and all-round enzyme stability. In comparison with the free enzyme, the entrapped enzyme was much more stable throughout greater temperature domains, pH, and storage times.[244–248] Viet and colleagues[246] immobilized cellulase in Ca-ALG beads by entrapment and its activity was assessed using CMC. With several cycles of use, the immobilized enzyme exhibited greater stability in response to changes in pH and temperature. The activity of immobilized cellulase remained 69.2% after five recycles and 20.3% after eight recycles.[246] Manganese peroxidase (MnP), generated from an indigenous variant of *Ganoderma lucidum* IBL-05, was immobilized on Ca-ALG beads by Bilal and co-workers[247] using a promising and environmentally friendly entrapment method. Ca-ALG-bound MnP was catalytically more vigorous and could be used eventually to decolorize dyes and detoxify industrial effluents more effectively.[247] Nunes and co-workers[248] developed conjugated polyvinyl alcohol-alginate (PVA-ALG) beads with excellent thermochemical and mechanical stabilities at high temperatures (>80 °C). Naringinase, an enzyme derived from *Penicillium decumbens*, was immobilized in ALG (0.2–1.0%)-PVA (10%) beads featuring three sizes (1–3 mm). At pH 4.0 and 70 °C, immobilized naringinase bestowed 80% activity and displayed >90% retention of initial activity after 6 weeks of incubation in acetate buffer. The importance of this immobilization approach for the system under consideration was illustrated by these promising outcomes, which also implied that it could be used to entrap other biocatalysts.[248] Bonine *et al.*[249] reported immobilization of a novel lipase isolated from the seeds of *Pachira aquatica* (PAL) using Ca-ALG beads and PVA. Similar results were obtained with immobilization of PAL in ALG and ALG/PVA beads, with improvement in their stability against temperature than the free enzyme.[249] Ortega *et al.*[250] covalently immobilized neutrase on ALG-glutaraldehyde beads. Under optimized conditions (pH 6.2, 2% ALG, 6.2% glutaraldehyde, and interaction for 60 min), the immobilization yield of encompassed neutrase (61.84 U mL^{-1}) was ~50% and the confined enzyme exhibited maximum activity at 10 °C higher than that of the native enzyme, which clearly showed the stability induced by the support. Immobilization caused the estimated activation energy to drop from 47.7 kJ mol^{-1} to 22.0 kJ mol^{-1}, which suggested the role of the support in favouring of the forward reaction at the thermodynamic level.[250] Eldin *et al.*[251] extended the covalent immobilization technique towards glucoamylase to form a novel affinity with *p*-benzoquinone-activated ALG beads. The covalently mounted enzyme sustained its activity after 30 successive runs and shelf space of 36 days.[251] To improve the stability and catalytic rate of GOx, Wang and co-workers[252] designed a protocol for undertaking emulsification-internal gelation followed by GOx adsorption and chitosan deposition to encapsulate the enzyme in CaALG-chitosan composite microspheres (CACMs). Before grafting with chitosan, the Ca-ALG matrix was made highly porous with the release of

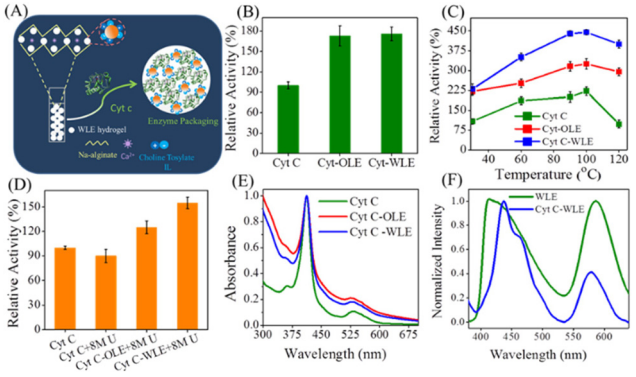

Fig. 22 Alginate-based hydrogels for protein packaging with enhanced activity and stability of Cyt c. (A) Schematic of the interaction of Cyt C with hydrogel composites. Percentage relative activity of Cyt C at room temperature (B), at various temperatures (C), and in presence of 8 M urea (D). (E) UV–vis spectra of Cyt C in presence of WLE and OLE hydrogels. (F) Fluorescence spectra of only WLE hydrogel and Cyt C + WLE hydrogels. Reproduced from ref. 242 with permission from Wiley-VCH, copyright 2020.

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CO_2, which helped to enhance the adsorption efficiency of the carrier. The concentration and molecular weight of chitosan, incubation duration, and pH seemed to have an impact on the GOx loading, encapsulation efficiency, and activity of CACM-GOx. Exhibiting the maximum enzymatic activity and encapsulation efficiency at an isoelectric point of 4, immobilized GOx retained >70% activity, which was tenfold higher than the activity retention by the free enzyme.[252] With some modification of this work, Zhao and colleagues[253] reported a unique and feasible approach for non-covalent GOx immobilization in chemically reduced graphene oxide (RGO)/ALG hybrid gel beads. The enzyme contained in the hybrid microbeads demonstrated high environmental tolerance and could maintain optimal activity over a wide range of conditions (45–60 °C and pH 4–6). To produce a continuous fixed-bed enzyme catalytic process, the microbeads could also be recycled simply through conventional filtering and reintroduced to a column.[253] Both of these works revealed the great potential to further explore CACM-GOx and ALG/RGO for developments in glucose bio-sensing. Abd El-Ghaffar *et al.*[254] used a grafting-encapsulation approach for α-CT immobilization. First, chitosan grafted with polymethyl methacrylate (PMMA-*g*-CS) through free-radical polymerization was used as a support for α-CT. Ca-ALG beads were fabricated for sheathing PMMA-*g*-CS-CT to produce composite beads. For immobilized α CT, greater retention of activity (~97.7%) was attained at pH 9 for 24 h. Immobilized α-CT retained 75% of its original activity after 60 days of storage at 25 °C and continued to work very well even after 25 reuses. Such results suggested that the recycling ability of ALG composite carriers could be used for continuous catalytic reactions in the industrial sector.[254] Muthuvelu and colleagues[255] designed a tri-enzyme biocatalyst by co-immobilizing Lac, cellulase, and β-glucosidase with ALG for evaluating bioethanol. The co-immobilized enzyme system had long shelf-life and thermal stability, offering an effective one-pot pre-treatment protocol for the production of bioethanol from lignocellulosic biomass, of which the maximum ethanol conversion was obtained with *Ipomoea carnea* (Fig. 23).

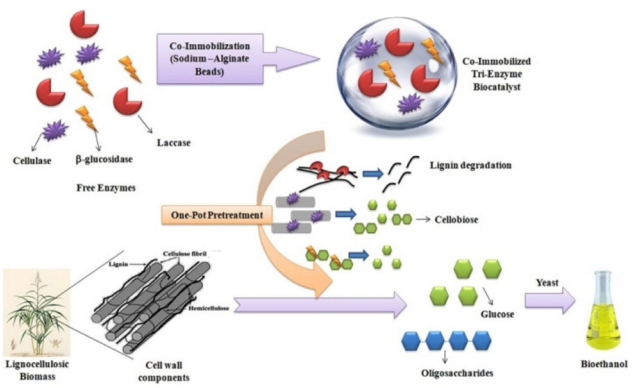

Fig. 23 Immobilization of co-immobilized laccase, cellulase, and β-glucosidase for ethanol production. Reproduced from ref. 255 with permission from Elsevier, copyright 2018.

Electrospun nanofibers comprising ALG-supporting immobilized lipase showed high enzyme loading capacity, catalytic activity, and stability. Doğaç *et al.*[256] produced PVA/ALG and polyethylene oxide/alginate (PEO/ALG) nanofibres using electrospinning, and lipase was adsorbed and cross-linked further with glutaraldehyde. Free lipase lost all of its activity at high temperatures after 40–60 min, but lipase-immobilized nanofibers sustained approximately 65–70% activity during the same timeframe. Furthermore, PVA/ALG and PEO/ALG composite nanofibers immobilized with lipase exhibited 50% of their original activity after 14 and seven cycles, respectively.[256] Zhao *et al.*[257] synthesized novel nanoflower/ALG microbeads and immobilized α-acetolactate decarboxylase (ALDC) using a facile method. First, ALDC was co-precipitated with $Ca_3(PO_4)_2$ to form enzyme-inorganic composite nanoflowers (ALDC-$Ca_3(PO_4)_2$). Later, these nanoflowers were encapsulated in ALG gel beads (ALDC-$Ca_3(PO_4)_2$ + ALG).[256] The microbeads demonstrated outstanding stability and environmental endurance in contrast to free ALDC and ALDC-$Ca_3(PO_4)_2$ nanoflowers, displaying optimal activity over a wide range of temperature (45–70 °C) and pH (3.5–7), but retained >98% of activity in comparison with unbound ALDC. Furthermore, the immobilized enzyme was employed in a 300 L beer fermenter to stop diacetyl generation. This resolved the issue of off-flavor in beer and reduced the time it takes for beer to mature, thereby demonstrating enormous scope for application in breweries.[257] Xylanase has several uses in the food industry. Kumar *et al.*[258] purified xylanase from *Bacillus licheniformis Alk-1*, and entrapped it within crosslinked Ca-ALG beads, and undertook activation through glutaraldehyde. In comparison with the free form, immobilized xylanase demonstrated improved overall chemical characteristics, recycling, and reusability efficiency. Hence, xylanase-ALG beads with higher xylanolytic activity and stability could be prepared, thereby aiding the design of efficient bioreactors for various applications (*e.g.*, formulations of poultry feeds).[258] Using citric acid as a nontoxic crosslinker, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) as activators, Bedade and co-authors[259] mounted acrylamidase from *Cupriavidus* oxa laticus ICTDB921 on chitosan-coated Ca-ALG beads. After four cycles, the immobilized acrylamidase maintained 80% of its activity while exhibiting increased pH, temperature, and shelf stability.[259] Pectic compounds cause turbidity in juices. The aesthetics and storage stability of such products can be enhanced by removing these substances with the help of pectinase.

Mohammadi and co-workers[260] immobilized *Aspergillus aculeatus*-originated pectinase on ALG-montmorillonite beads. At extremes of pH, immobilized pectinase showed higher efficacy than that of the free enzyme. After six successive cycles, immobilized pectinase exhibited >53% of its initial activity, demonstrating improved stability and renewability for pectin hydrolysis. Pineapple juice was clarified using this catalytic system, demonstrating the potential of this enzyme-carrier model for use in fruit juices.[260] Table 6 summarizes the immobilization of other enzymes on the ALG matrix due to

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Table 6 Influence of alginate-based support materials on the stability and activity of proteins

Biomass	Biomass derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Alginate	Polyacrylamide alginate cryogel (PAG) functionalized with glycidyl methacrylate (GMA)	Laccase	Covalent immobilization	>70% removal of phenolics from olive mill wastewater, >55% dye removal from textile wastewater and 93–99% decolouration of few other dyes were achieved	219
Alginate	Carrageenan-alginate beads	CAT	Covalent immobilization	Enhanced pH tolerance towards alkaline media and 6 folds higher V_{max} achieved	220
Alginate	sodium alginate (SA)-polyethylene glycol (PEG)	Cellulase	Cross linking	With only 22.68% immobilization yield, 133% of yield (reducing sugar) obtained	221
Alginate	Polypyrrole/silver nanocomposite coated with calcium alginate	Polygalacturonase (PG)		Excellent immobilization efficiency (>84%) and long term stability (83% of initial activity after 2 months)	222
Alginate	Silver-alginate nanoparticle matrix	Lipase	Conjugation	Application in high-fat meat processing is demonstrated with loss of 40% initial weight after 2 weeks	223
Alginate	Dopamine-alginate beads	Laccase from *Streptomyces cyaneus*		Amido Black 10B, Reactive Black 5, Evans Blue, and Remazol Brilliant Blue were all completely decoloured with 100% efficiency	224
Alginate	Alginate micron and submicron beads	Alcalase	Covalent immobilization	Improved performance and utilised in 7 subsequent cycles of soy protein hydrolysis with a slight decrease in activity	225
Alginate	Sodium alginate (SA)-polyethylene glycol (PEG)-Chitosan (CS)	Cellulase	Entrapment	Increased stability and recyclability with 22.68% activity higher than native enzyme after 5 cycles	226
Alginate	Oxidized tyramine-alginates micro beads	HRP	Encapsulation	With 20 mol% of tyramine-alginate, 96% of the phenol was eliminated from the solution. Increased reusability	227
Alginate	Carrageenan-alginate beads with chitosan-glutaraldehyde activation	β-D-Galactosidase	Covalent immobilization	Reusability was improved, and 79.74% of the activity from the first round maintained till the tenth reusability round	228
Alginate	Calcium alginate hydrogels with liposomes	Bovine Carbonic Anhydrase (BCA)	Entrapment	Entrapment of BCA in alginate hydrogels is stable and effectiveness for using to catalytic reactions in bioreactors	229
Alginate	Entrapment in copper-alginate beads	Fungal phenol oxidase	Entrapment	Enhanced thermal activity and stability. Wider pH ranges allowed the enzyme to remain active, and storage at 4 °C considerably enhanced	230
Alginate	The gelatin component contained in the gel solution was cross-linked with glutaraldehyde (GA) and encapsulated in xanthan-alginate (XA) spheres	Urease	Encapsulation	Increased temperature and pH stability. Even after 20 times of use, the xanthan-alginate spheres still had 75% of their initial activity	231
Alginate	Calcium alginate gel capsule encapsulation	GOx	Encapsulation	Studied gelation conditions affected on capsule factors like capsule thickness, enzyme leakage rate, and encapsulation effectiveness	233
Alginic acid (AA)	AAC	Cyt c		Enhanced enzymatic activity up to 5.5-fold and improved the thermal stability	232
Alginate	Calcium alginate gel capsule encapsulation	GOx	Encapsulation	Studied gelation conditions affected on capsule factors like capsule thickness, enzyme leakage rate, and encapsulation effectiveness	233
Alginate	Encapsulation in microcapsules alginate coated with alternating multiple layers membrane of poly *N*-vinylamine and polyacrylic acid	Cyt c	Encapsulation	Increased the encapsulation yield, activity as well as stability	234
Alginate	Ca-ALG gel capsules	*Saccharomyces cerevisiae* invertase	Entrapment	Relative activity was found 87% and active for 36 days. More stable at high pH and temperatures	235

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Table 6 *(Contd.)*

Biomass	Biomass derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Alginate from *Sargassum*	Entrapped in alginate gel	Invertase	Entrapment	3.5 times higher $t_{1/2}$ than native enzyme at 60 °C	236
Alginate	Hydrogels produced by combining glutaraldehyde-stabilized gelatin and natural polysaccharide alginate	Lipase	Cross linking	Stable and can be recycled for 10 and 20 times in aqueous and micellar media respectively without noticeably losing enzyme activity	237
Alginate	Microcapsules with an alginate-chitosan shell	β-Galactosidase	Entrapment	(1) Ca^{2+} alginate has a lesser loading efficiency than Ba^{2+} alginate. (2) Compared to liquid core Ca^{2+} alginate microcapsules and the unbound enzyme, solid core Ba^{2+} alginate microcapsules increased the stability of the enzyme at 37 °C	238
Alginate	Ultrathin alginate/protamine/silica (APSi) hybrid membranes in the core–shell capsules	Laccase	Entrapment	After 20 days, the stability of the encapsulated laccase increased by 67%. Improved storage, pH, and thermal stabilities	239
Alginate	Alginate-chitosan microcapsules made using an internal gelation and emulsification process to create alginate beads	Laccase	Not mentioned	Increased loading effectiveness and stability. In the Alizarin Red dye decolorization test, the free and immobilised laccase alone both had very low decolorization efficiency	240
Alginate gel	Alginate-silica nanotubes (ALG-SiNTs) composite was created by incorporating silica nanotubes (SiNTs) into the alginate (ALG) gel and then encapsulating them with Ca^{2+} through cross-linking	Yeast alcohol dehydrogenase (YADH)	Encapsulation	Improved storage and operational stability with ALG-SiNT composite showing ∼50% lesser leaching compared to only alginate matrix	241
Alginate	Biomaterials made from alginate gel doped with double-walled carbon nanotubes (DWCNT-ALG)	LDH	Adsorption, encapsulation and cross linking	Improved enzyme adherence with >61% reduction in leaching and 25 folds higher shelf-life than native enzyme	243
Alginate	Ca-alginate beads by entrapment	Lipase	Entrapment	40% higher activity than the free enzyme with 10 cycles of reusability without any loss of activity	244
Alginate	Calcium alginate beads	Protease	Entrapment	Activity retained for a longer period of time and reused for 3 cycles. Up to 10 days of storage stability at 4 °C was observed	245
Alginate	Calcium alginate gel by entrapment method	Cellulase	Entrapment	Increased stability with 5 °C raise in optimum temperature (55 to 60 °C) and pH tolerance in acidic regime	246
Alginate	Calcium-alginate beads made using the entrapment technique	MnP	Entrapment	(1) >83% immobilization yield; tremendous shift in the optimum temperature from 35 to 60 °C and pH (towards acidic range). (2) >82–95% decolouration capacity with Sandal-fix dyes	247
Sodium salt of alginic acid from brown algae	PVA-ALG beads	Naringinase	Entrapment	Residual activity retained 70% after 8 successive batches	248
Alginate	PVA with calcium alginate (Alg) beads	Lipase	Entrapment	Improved the thermal stability and reusability	249
Alginate	Alginate-glutaraldehyde beads made through covalent bonding	Neutrase	Covalent bonding	Immobilization yield was ∼50% and bestowed 10 °C higher thermal tolerance than free enzyme (50 °C)	250
Alginate	ρ-Benzoquinone-activated alginate beads	Glucoamylase	Entrapment	No change in optimum pH, temperature and activity over 36 days and 30 cycles of recycling	251
Alginate	Calcium alginate-chitosan microspheres (CACM)	GOx	Encapsulation and adsorption method	Increased the enzymatic activity and storage stability was increased up to 2 month and retained activity of CACM-GOX	252
Alginate	Hybrid gel beads made of chemically reduced graphene oxide (CRGO) and alginate	GOx	Non-covalent adsorption–entrapment method	Good recyclability, mechanical properties, and enhanced stability in a wide pH (4–6) and temperature (45–60 °C) range	253

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Table 6 *(Contd.)*

Biomass	Biomass derived material	Used enzyme	Immobilization method	Result/performance study	Ref.
Alginate	Polymethyl methacrylate (PMMA-*g*-CS) nanoparticles were grafted onto chitosan encapsulated calcium alginate beads	α-CT	Covalent bonding	Higher retained activity at pH 9 for 24 h	254
Alginate	polyvinyl alcohol/alginate and polyethylene oxide/alginate nanofibers by electrospinning method	Lipase	Adsorption and cross linking method	Enhanced the stability. Maintained 60% of their activities after 14 and 7 reuses and increased the stability	256
Alginate	Nanoflower/alginate microbeads	ALDC	Entrapment	Restored 98% of activity with improving stability and recyclability	257
Alginate	Calcium alginate beads with glutaraldehyde activation	Xylanase	Entrapment and cross linking	After 30 days at 4 °C, the enzyme still exhibits 80% of its initial activity and maintains recycling efficiency up to five reaction cycles with 37% retention activity	258
Alginate	Functionalized calcium alginate beads with chitosan coating	Acrylamidase	Cross linking and covalent immobilization	Enhanced pH, thermal, shelf, and mechanical stability, and maintained 80% activity after four cycles	259
Alginate	Activated alginate-montmorillonite (MMT) beads	Pectinase	Covalent binding	Showed greater activity, however the optimal pH dropped from 5.5to 5.0. The immobilised enzyme's initial activity was maintained at around 53% after 6 cycles of reuse	260
Alginate	Glutaraldehyde is utilized to harden hybrid alginate-chitosan beads	*Saccharomyces cerevisiae* alcohol dehydrogenase (SCAD)	Cross linking	Improved thermal stability with enhanced optimum temperature (from 30 to 40 °C)	261
Alginate	Boehmite/alginate hybrid beads	YADH	Encapsulation	After 67 hours of incubation, encapsulated YADH can practically approach "zero leaching" and retain 86.6% of its activity after 12 cycles	262
Alginate	Entrapped BG crosslinked glutaraldehyde in calcium alginate particles	β-Glucosidase	Entrapment and crosslinking	Under the optimum conditions, more than 60% of enzyme activity restored and cross linkage of glutaraldehyde reduced leakage of BG from the calcium alginate particles	263
Alginate	Polyvinyl alcohol-sodium alginate (PVA-SA) nanofibers by electrospun nanofiber	Phytase	Cross linking	Enhanced the catalytic activity with optimum pH shifting towards higher pH (from 5 to 6) and temperature from 45 to 55 °C	264
Alginate	Entrapment of alginate beads	Lipase	Entrapment	High lipase activity in the acidic pH of 3 and 40 °C temperature	265
Alginate	3D transition metal cation-crosslinked alginate nanogels (Mn^{2+}, Fe^{3+}, and Co^{2+})	Urease	Encapsulation	Fe^{3+}-alginate crosslinked nanogels demonstrated high enzyme activity, efficient enzyme loading, and zeta potential compared to Co^{2+} and Mn^{2+}	266
Alginate	Cu-alginate beads	Trametes Versicolor laccase	Entrapment	Enhanced activity with >96% degradation capacity towards bisphenol at pH 5 and 30 °C in 60 min	268

abundant functional groups.[261–268] For instance, Kamaci *et al.*[264] observed enhanced catalytic activity of immobilized phytase into polyvinyl alcohol-sodium alginate (PVA-SA) electrospun nanofibers. Nanofibers were fabricated by mixing PVA and SA at a 80 : 20 ratio, voltage of 23 kV, and distance of 14 cm *via* electrospinning. Sharma *et al.*[269] prepared bio-based and low-cost hybrid alginate-protein cryogel beads as novel adsorbent materials for the purification of immunoglobulin (Ig)G from human serum (Fig. 24). Due to soft interaction between the protein and IgG, the stability and integrity of the antibody were retained after the desorption step. Similarly,

Fig. 24 Use of hybrid alginate-protein cryogel beads to purify IgG (schematic). From ref. 269.

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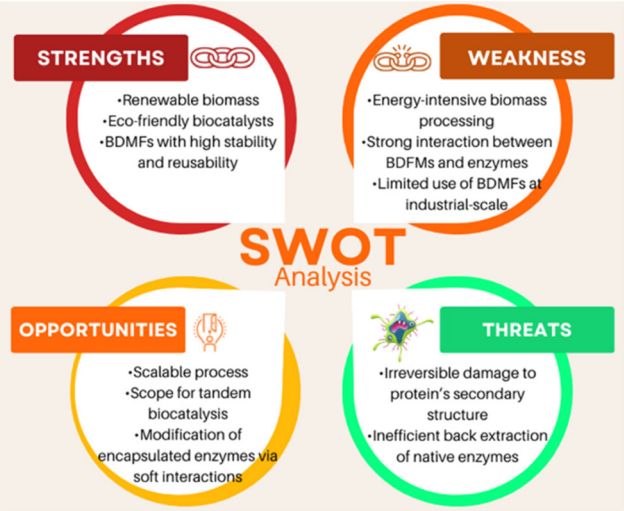

Fig. 25 SWOT analysis of BDFMs for biocatalysis.

upcycling of marine chitin was improved with immobilized chitinolytic enzymes.[270]

8. Conclusions and future prospects

The use of enzyme-based biocatalysts at the industrial scale has garnered interest in recent decades. However, the use of enzymes is limited due to their low thermal stability, complicated reusability, self-cleavage, and aggregation under extreme processing conditions. Immobilization of enzymes on a biocompatible and renewable carrier is a promising method to overcome such drawbacks. BDFMs are promising carriers for enzyme immobilization due to their availability, biodegradability, biocompatibility, and low risk of environmental contamination. In this review, the main types of BDFMs were presented as promising carriers for enzymes to improve their stability and reusability. In addition, the immobilization of enzymes on various biomass-derived carriers (*e.g.*, cellulose, lignin, SF, chitin, chitosan, and ALG) was surveyed. Although various approaches have been developed for BDFM fabrication, studies on the practical applications of these materials are in the early stages. Immobilization/adsorption/entrapment of various enzymes on BDFMs can improve the stability, activity, reusability, and recyclability of biocatalysts compared with those of their native state. In many cases, impressive results have been obtained whereas, in several other cases, the biocatalytic activity must be improved for commercial application. For selection of the best support materials and preparation of an efficient biocatalyst, all reaction conditions must be optimized in terms of monodispersity and surface chemistry. Moreover, the literature is based mainly on enzymes of a single type, which may not be suitable for industrial needs. Therefore, studies with multiple enzyme "cocktails" should be undertaken to lower the cost and increase the utility of biocatalysts.

Fig. 25 provides an overview of the analysis of the strength, weakness, opportunities and threats (SWOT) of BDFMs as carriers for enzymes. These challenges should be considered when selecting the biomass matrix and immobilization methods for direct industrial application. Cost-effective sources (*e.g.*, non-commercial biomass, waste biomass, non-wood forest products, and biomass from unconventional resources) as support materials as well as controlled immobilization methods with soft interactions between enzymes and BDFMs must be selected. This review also provides an opportunity to understand the availability of different types of biomass as catalyst supports to develop efficient biocatalytic systems. The recyclability and reusability of enzymes is another crucial parameter that ensures the practicality of a biocatalytic process. We believe that this review will aid use of BDFMs for further studies with different enzymes under extreme conditions (*e.g.*, high temperature, pH, denaturing substances). We attempted to collect all the information on the immobilization of enzymes on macro- or microscopic biomass-derived materials as carriers to improve the enzyme activity, stability, and recovery of biocatalysts. Therefore, this review aims to create economic value from various biomass and biocatalytic processes to strengthen the economy, with high scientific, technological, and societal impacts. This review also aids the use of biomaterials as supports for the development of immobilized biocatalysts for applications based on energy, environmental issues, and chemical syntheses. From the perspective of green and sustainable chemistry, this review offers a promising approach for effective evaluation of biomass.

Abbreviations

BDFM	Biomass-derived functional material
AA	Alginic acid
MOF	Metal–organic framework
WOS	Web of science
BGU	β-Glucuronidase
CHM	Cellulose hydrogel microsphere
CNC	Cellulose nanocrystal
CNW	Cellulose nanowhisker
NFC	Nanofibrillated cellulose
MCC	Microcrystalline cellulose
CBD	Cellulose-binding domain
GOx	Glucose oxidase
CMC	Carboxymethyl cellulose
IL	Ionic liquid
PPL	Porcine pancreatic lipase
HNT	Halloyite nanotube
MCM	Cellulose porous microsphere
PGA	Penicillin G acylase
TLFCH	Tendril-like functional carbon helix
DES	Deep eutectic solvent
Cyt c	Cytochrome c
[Emim][Ac]	1-Ethyl-3-methylimidazolium acetate
CRL	*Candida rugosa* lipase

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OPH	Organophosphorus hydrolase
BTDE	1,4-Butanediol diglycidyl ether
EMBR	Enzymatic membrane bioreactor
αCF	α-Cellulose fiber
α-CT	α-Chymotrypsin
PDDA	Poly(diallyldimethylammonium chloride)
L-DOPA	3,4-Dihydroxyphenyl-L-alanine
LNP	Lignin nanoparticle
SET-LRP	Single electron transfer-living radical polymerization
SF	Silk fibroin
HRP	Horseradish peroxidase
ChOx	Cholesterol oxidase
CA	Carbonic anhydrase
ASNase	L-Asparaginase
CadA	Lysine decarboxylase
ChBD	Chitin-binding domain
CHNW	Chitin nanowhisker
AuNP	Gold nanoparticle
AgNp	Silver nanoparticle
CNF	Chitin nanofibril
TMB	3,3′,5,5′-Tetramethylbenzidine
GO	Graphene oxide
RGO	Reduced graphene oxide
BSS	Bamboo shoot shell
LBL	Layer-by-layer
SGP	Seal gastric protease
CAT	Catalase
$NaIO_4$	Sodium periodate
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl radical
SNF	Silk nanofiber
ONPG	*o*-Nitrophenyl-β-D-galactopyranoside
DWCNT	Double-walled carbon nanotube
LDH	Lactate dehydrogenase
PDMS	Polydimethylsiloxane
PEO	Polyethylene oxide
ALDC	α-Acetolactate decarboxylase
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
NHS	*N*-Hydroxysuccinimide
$t_{1/2}$	Half life
PEG	Polyethylene glycol
Ca-ALG	Calcium-alginate
AAC	Alginate-derived carbon
SiNT	Silica nanotube
TMOS	Tetramethoxysilane
BCA	Bovine carbonic anhydrase
SA	Sodium alginate
XA	Xanthan-alginate
PVA-SA	Polyvinyl alcohol-sodium alginate
MMT	Montmorillonite
DMC	Dialdehyde-modified cellulose nanocrystal
MDC	Magnetic dialdehyde cellulose nanoparticle
MCNC	Magnetic chitin nanofiber composite
CS-Nps	Chitosan nanoparticle
ALG-SiNT	Alginate-silica nanotube
CMNP	Chitosan magnetic nanoparticle
MnP	Manganese peroxidase

Conflicts of interest

There are no conflicts to declare.

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References

1 Y. Liu, J. Chen, B. Cui, P. Yin and C. Zhang, *C*, 2018, **4**, 53.
2 R. A. Sheldon and S. van Pelt, *Chem. Soc. Rev.*, 2013, **42**, 6223–6235.
3 P. Manzanares, *Acta Innov.*, 2020, 47–56.
4 P. Morone, *Acta Innov.*, 2020, 5–16.
5 D. M. Kochepka, L. P. Dill, D. H. Fockink and R. M. Łukasik, *Acta Innov.*, 2020, **35**, 29–56.
6 L. P. Ramos, M. J. Suota, D. H. Fockink, G. Pavaneli, T. A. da Silva and R. M. Łukasik, *Recent Advances in Bioconversion of Lignocellulose to Biofuels and Value-Added Chemicals within the Biorefinery Concept*, Elsevier, 2020, pp. 61–100.
7 H. Suo, L. Xu, Y. Xue, X. Qiu, H. Huang and Y. Hu, *Carbohydr. Polym.*, 2020, **234**, 115914.
8 S. Doonan, E. W. Abel, D. Phillips, J. D. Woollins and A. G. Davies, *Peptides and Proteins*, Royal Society of Chemistry, 2002, vol. 15, DOI: 10.1039/9781847551634m3-X001.
9 C. I. Branden and J. Tooze, *Introduction to protein structure*, Garland Science, 2012.
10 S. Ohnishi and K. Takano, *Cell. Mol. Life Sci.*, 2004, **61**, 511–524.
11 A. Lesk, *Introduction to protein science: architecture, function, and genomics*, Oxford university press, 2010.
12 A. Sindhu, S. Kumar and P. Venkatesu, *ACS Sustainable Chem. Eng.*, 2022, **10**, 4323–4344.
13 R. A. Sheldon, *Adv. Synth. Catal.*, 2007, **349**, 1289–1307.
14 S. van Pelt, F. van Rantwijk and R. A. Sheldon, *Adv. Synth. Catal.*, 2009, **351**, 397–404.
15 M. L. Verma, S. Kumar, A. Das, J. S. Randhawa and M. Chamundeeswari, *Environ. Chem. Lett.*, 2020, **18**, 315–323.
16 H. T. Imam, P. C. Marr and A. C. Marr, *Green Chem.*, 2021, **23**, 4980–5005.
17 S. Arola, T. Tammelin, H. Setälä, A. Tullila and M. B. Linder, *Biomacromolecules*, 2012, **13**, 594–603.
18 K. Xu, B. Appiah, B.-W. Zhang, Z.-H. Yang and C. Quan, *J. Catal.*, 2023, **418**, 31–39.

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This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

19 J. Meyer, L. E. Meyer and S. Kara, *Eng. Life Sci.*, 2022, **22**, 165–177.
20 V. C. Badgujar and B. M. Bhanage, *Curr. Opin. Green Sustain. Chem.*, 2022, 100621.
21 U. T. Bornscheuer, in *Biotechnology for the Future*, ed. J. Nielsen, Springer Berlin Heidelberg, Berlin, Heidelberg, 2005, pp. 181–203, DOI: 10.1007/b136413.
22 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.
23 S. Lu, X. Wang, Q. Lu, X. Hu, N. Uppal, F. G. Omenetto and D. L. Kaplan, *Biomacromolecules*, 2009, **10**, 1032–1042.
24 S. Lv, *Molecules*, 2020, **25**, 4929.
25 Y. A. Rodríguez-Restrepo and C. E. Orrego, *Environ. Chem. Lett.*, 2020, **18**, 787–806.
26 H. J. Kim, S. Park, S. H. Kim, J. H. Kim, H. Yu, H. J. Kim, Y.-H. Yang, E. Kan, Y. H. Kim and S. H. Lee, *J. Mol. Catal. B: Enzym.*, 2015, **122**, 170–178.
27 R. S. Varma, *ACS Sustainable Chem. Eng.*, 2019, **7**, 6458–6470.
28 V. Incani, C. Danumah and Y. Boluk, *Cellulose*, 2013, **20**, 191–200.
29 H. Liu, A. D. Štiglic, T. Mohan, R. Kargl, K. S. Kleinschek and B. Nidetzky, *Int. J. Biol. Macromol.*, 2022, **222**, 217–227.
30 Y. Liu and J. Y. Chen, *J. Bioact. Compat. Polym.*, 2016, **31**, 553–567.
31 W. Sheng-Chi and L. Ying-Ke, *J. Mol. Catal. B: Enzym.*, 2008, **54**, 103–108.
32 A. Gennari, A. J. Führ, G. Volpato and C. F. V. de Souza, *Carbohydr. Polym.*, 2020, **246**, 116646.
33 M. P. Klein, C. W. Scheeren, A. S. G. Lorenzoni, J. Dupont, J. Frazzon and P. F. Hertz, *Process Biochem.*, 2011, **46**, 1375–1379.
34 S. Sulaiman, M. N. Mokhtar, M. N. Naim, A. S. Baharuddin and A. Sulaiman, *Appl. Biochem. Biotechnol.*, 2015, **175**, 1817–1842.
35 I. B. V. Erramuspe, E. Fazeli, T. Näreoja, J. Trygg, P. Hänninen, T. Heinze and P. Fardim, *Biomacromolecules*, 2016, **17**, 3188–3197.
36 M. Bagheri, H. Rodríguez, R. P. Swatloski, S. K. Spear, D. T. Daly and R. D. Rogers, *Biomacromolecules*, 2008, **9**, 381–387.
37 N. F. Vasconcelos, F. K. Andrade, L. d. A. P. Vieira, R. S. Vieira, J. M. Vaz, P. Chevallier, D. Mantovani, M. d. F. Borges and M. d. F. Rosa, *Cellulose*, 2020, **27**, 3055–3083.
38 H. Chen and Y.-L. Hsieh, *Biotechnol. Bioeng.*, 2005, **90**, 405–413.
39 E. Mascheroni, G. Capretti, M. Marengo, S. Iametti, L. Mora, L. Piergiovanni and F. Bonomi, *Packag. Technol. Sci.*, 2010, **23**, 47–57.
40 S. Martins de Oliveira, S. Velasco-Lozano, A. H. Orrego, J. Rocha-Martín, S. Moreno-Pérez, J. M. Fraile, F. López Gallego and J. M. Guisán, *Biomacromolecules*, 2021, **22**, 927–937.
41 K. A. Mahmoud, E. Lam, S. Hrapovic and J. H. T. Luong, *ACS Appl. Mater. Interfaces*, 2013, **5**, 4978–4985.
42 C. Goldhahn, I. Burgert and M. Chanana, *Adv. Mater. Interfaces*, 2019, **6**, 1900437.
43 Q. Luan, H. Zhang, Y. Lei, Y. Cai, Y. Bao, Y. Li, H. Tang and X. Li, *Cellulose*, 2021, **28**, 5735–5744.
44 S.-L. Cao, X.-H. Li, W.-Y. Lou and M.-H. Zong, *J. Mater. Chem. B*, 2014, **2**, 5522–5530.
45 Y. Ren, L. Zhang, T. Sun, Y. Yin and Q. Wang, *ACS Sustainable Chem. Eng.*, 2022, **10**, 6244–6254.
46 E. Motamedi, S. F. S. Motahar, M. Maleki, K. Kavousi, S. Ariaeenejad, A. A. Moosavi-Movahedi and G. H. Salekdeh, *Cellulose*, 2021, **28**, 3485–3503.
47 C. Uth, S. Zielonka, S. Hörner, N. Rasche, A. Plog, H. Orelma, O. Avrutina, K. Zhang and H. Kolmar, *Angew. Chem., Int. Ed.*, 2014, **53**, 12618–12623.
48 D. Sillu and S. Agnihotri, *ACS Sustainable Chem. Eng.*, 2020, **8**, 900–913.
49 X. Luo and L. Zhang, *Biomacromolecules*, 2010, **11**, 2896–2903.
50 K. Aruchamy, M. Bisht, P. Venkatesu, D. Kalpana, N. R. Maalige, N. Singh, D. Ghosh, D. Mondal and S. K. Nataraj, *Green Chem.*, 2018, **20**, 3711–3716.
51 S. Jo, S. Park, Y. Oh, J. Hong, H. J. Kim, K. J. Kim, K. K. Oh and S. H. Lee, *Biotechnol. Bioprocess Eng.*, 2019, **24**, 145–154.
52 S. Park, S. H. Kim, J. H. Kim, H. Yu, H. J. Kim, Y.-H. Yang, H. Kim, Y. H. Kim, S. H. Ha and S. H. Lee, *J. Mol. Catal. B: Enzym.*, 2015, **119**, 33–39.
53 M. H. Kim, S. An, K. Won, H. J. Kim and S. H. Lee, *J. Mol. Catal. B: Enzym.*, 2012, **75**, 68–72.
54 M. Sharifi, S.-M. Robatjazi, M. Sadri and J. M. Mosaabadi, *React. Funct. Polym.*, 2018, **124**, 162–170.
55 S. Yabuki, Y. Hirata, Y. Sato and S. Iijima, *Anal. Sci.*, 2012, **28**, 373–377.
56 L. Hao, R. Wang, Y. Zhao, K. Fang and Y. Cai, *Cellulose*, 2018, **25**, 6759–6769.
57 N. Tanja, K. Mirjana, P. Jovana, P. Biljana, P. Zivomir and S. Petar, *Carbohydr. Polym.*, 2010, **82**, 976–981.
58 R. Gong, J. Zhang, J. Zhu, J. Wang, Q. Lai and B. Jiang, *Korean J. Chem. Eng.*, 2013, **30**, 1620–1625.
59 A. Gennari, R. Simon, N. D. de Moura Sperotto, C. V. Bizarro, L. A. Basso, P. Machado, E. V. Benvenutti, G. Renard, J. M. Chies, G. Volpato and C. F. De Souza, *Int. J. Biol. Macromol.*, 2022, **199**, 307–317.
60 C. Liu, D. Saeki and H. Matsuyama, *RSC Adv.*, 2017, **7**, 48199–48207.
61 S. G. Gajanan, Y. Jiwook, S. S. Surendra, M. M. Bhupendra, K. Dae-Young, S. Jung-Suk and A. K. Avinash, *Bioresour. Technol.*, 2018, **261**, 420–427.
62 X. Xing, Y. Han, Q. Jiang, Y. Sun, X. Wang, G. Qu, G. Sun and Y. Li, *Cellulose*, 2021, **28**, 4793–4805.
63 G. Hui, L. Bingshuang, Y. Jianwei, C. Yunfei and Q. Junqing, *Int. J. Biol. Macromol.*, 2021, **185**, 287–296.
64 E. Restiawaty, F. A. Yatasya, N. T. U. Culsum and Y. W. Budhi, InIOP Conference Series: Materials Science and Engineering, IOP Publishing, 2021, vol. 1143, p. 012009.
65 S. S. S. Putra, W. J. Basirun, A. A. M. Elgharbawy, A. Hayyan, M. Hayyan and M. A. Mohammed, *Mol. Catal.*, 2022, **528**, 112422.

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66 D. Califano, B. L. Patenall, M. A. S. Kadowaki, D. Mattia, J. L. Scott and K. J. Edler, *Biomacromolecules*, 2021, **22**, 754–762.
67 J. V. Edwards, N. T. Prevost, B. Condon and A. French, *Cellulose*, 2011, **18**, 1239–1249.
68 V. Singh and S. Ahmad, *Cellulose*, 2012, **19**, 1759–1769.
69 A. Girelli, L. Salvagni and A. Tarola, *J. Braz. Chem. Soc.*, 2012, **23**, 585–592.
70 H. H. Je, S. Noh, S.-G. Hong, Y. Ju, J. Kim and D. S. Hwang, *Chem. Eng. J.*, 2017, **323**, 425–433.
71 Z. Hao, L. Qian, L. Yan, W. Jiahui, B. Yuping, T. Hu and H. Fenghong, *Int. J. Biol. Macromol.*, 2022, **199**, 61–68.
72 V. N. Kumar and R. Neera, *Int. J. Biol. Macromol.*, 2022, **200**, 618–625.
73 Q. Weichuan, Z. Ziyan, Q. Yi, X. Lijie and G. He, *Colloids Surf., A*, 2022, **632**, 127818.
74 A. Gennari, R. Simon, N. D. de Moura Sperotto, C. V. Bizarro, L. A. Basso, P. Machado, E. V. Benvenutti, A. D. Viegas, S. Nicolodi, G. Renard and J. M. Chies, *Bioresour. Technol.*, 2022, **345**, 126497.
75 S. Ariaeenejad, E. Motamedi and G. H. Salekdeh, *Bioresour. Technol.*, 2022, **349**, 126833.
76 Z. Zhihong, J. Mengchen, C. Guiru, L. Jiandu, W. Luying and G. Jun, *Green Chem. Eng.*, 2023, **4**(1), 39–48.
77 K. A. Mahmoud, K. B. Male, S. Hrapovic and J. H. T. Luong, *ACS Appl. Mater. Interfaces*, 2009, **1**, 1383–1386.
78 N. E. Kotel'nikova, S. A. Mikhailova and E. N. Vlasova, *Russ. J. Appl. Chem.*, 2007, **80**, 322–329.
79 Z. Liu, H. Wang, B. Li, C. Liu, Y. Jiang, G. Yu and X. Mu, *J. Mater. Chem.*, 2012, **22**, 15085–15091.
80 H. Xiao-Jun, C. Peng-Cheng, H. Fu, O. Yang, C. Ming-Rui and X. Zhi-Kang, *J. Mol. Catal. B: Enzym.*, 2011, **70**, 95–100.
81 B. Muhammad and M. N. I. Hafiz, *Int. J. Biol. Macromol.*, 2019, **130**, 462–482.
82 P. Bayazidi, H. Almasi and A. K. Asl, *Int. J. Biol. Macromol.*, 2018, **107**, 2544–2551.
83 S. P. de Souza, I. I. Junior, G. M. A. Silva, L. S. M. Miranda, M. F. Santiago, F. L.-Y. Lam, A. Dawood, U. T. Bornscheuer and R. O. M. A. de Souza, *RSC Adv.*, 2016, **6**, 6665–6671.
84 S. A. Fatma, M. Monier and H. E. Nadia, *Int. J. Biol. Macromol.*, 2018, **114**, 1018–1025.
85 C. J. R. Frazão, N. H. C. Silva, C. S. R. Freire, A. J. D. Silvestre, A. M. R. B. Xavier and A. P. M. Tavares, *Eng. Life Sci.*, 2014, **14**, 500–508.
86 G. Weihua, R. Zhanxiang, Y. Fayin and Z. Guohua, *Food Chem.*, 2017, **228**, 455–462.
87 J. Bebić, K. Banjanac, J. Rusmirović, M. Ćorović, A. Milivojević, M. Simović, A. Marinković and D. Bezbradica, *RSC Adv.*, 2020, **10**, 21495–21508.
88 C. E. de A. Padilha, C. da C. Nogueira, D. F. de S. Souza, J. A. de Oliveira and E. S. dos Santos, *Ind. Crops Prod.*, 2020, **146**, 112167.
89 K. Łukasz, Z. Jakub and J. Teofil, *Colloids Surf., B*, 2018, **162**, 90–97.
90 E. Capecchi, D. Piccinino, B. M. Bizzarri, D. Avitabile, C. Pelosi, C. Colantonio, G. Calabrò and R. Saladino, *Biomacromolecules*, 2019, **20**, 1975–1988.
91 E. Tomaino, E. Capecchi, D. Piccinino and R. Saladino, *ChemCatChem*, 2022, **14**, e202200380.
92 A. Moreno and M. H. Sipponen, *Nat. Commun.*, 2020, **11**, 5599.
93 A. I. Benítez-Mateos, S. Bertella, J. Behaghel de Bueren, J. S. Luterbacher and F. Paradisi, *ChemSusChem*, 2021, **14**, 3198–3207.
94 M. Stanisz, K. Bachosz, K. Siwińska-Ciesielczyk, Ł. Klapiszewski, J. Zdarta and T. Jesionowski, *Catalysts*, 2022, **12**, 1031.
95 Y. Takahashi, M. Gehoh and K. Yuzuriha, *J. Polym. Sci., Part B: Polym. Phys.*, 1991, **29**, 889–891.
96 S. Kaushik, P. D. Thungon and P. Goswami, *ACS Biomater. Sci. Eng.*, 2020, **6**, 4337–4355.
97 L. Grasset, D. Cordier and A. Ville, *Biotechnol. Bioeng.*, 1977, **19**, 611–618.
98 M. A. Tomeh, R. Hadianamrei and X. Zhao, *Pharmaceutics*, 2019, **11**, 494.
99 K. H. Lee, C. S. Ki, D. H. Baek, G. D. Kang, D.-W. Ihm and Y. H. Park, *Fibers Polym.*, 2005, **6**, 181–185.
100 S. Chatterjee, L. Barbora, S. S. Cameotra, P. Mahanta and P. Goswami, *Appl. Biochem. Biotechnol.*, 2009, **157**, 593–600.
101 B. Chen, C. Yin, Y. Cheng, W. Li, Z. Cao and T. Tan, *Biomass Bioenergy*, 2012, **39**, 59–66.
102 C. Acharya, V. Kumar, R. Sen and S. C. Kundu, *Biotechnol. J.*, 2008, **3**, 226–233.
103 U. Saxena and P. Goswami, *Appl. Biochem. Biotechnol.*, 2010, **162**, 1122–1131.
104 Y. Han, S. Yu, L. Liu, S. Zhao, T. Yang, Y. Yang, Y. Fang and S. Lv, *Mol. Catal.*, 2018, **457**, 24–32.
105 S. Miyairi, M. Sugiura and S. Fukui, *Agric. Biol. Chem.*, 1978, **42**, 1661–1667.
106 P. B. Dennis, A. Y. Walker, M. B. Dickerson, D. L. Kaplan and R. R. Naik, *Biomacromolecules*, 2012, **13**, 2037–2045.
107 H. Yoshimizu and T. Asakura, *J. Appl. Polym. Sci.*, 1990, **40**, 127–134.
108 Y.-Q. Zhang, W.-L. Zhou, W.-D. Shen, Y.-H. Chen, X.-M. Zha, K. Shirai and K. Kiguchi, *J. Biotechnol.*, 2005, **120**, 315–326.
109 L. Lichao, H. Chengzhi, X. Menglin, A. Zeyu and L. Shanshan, *Appl. Catal., A*, 2020, **597**, 117541.
110 E. M. Pritchard, P. B. Dennis, F. Omenetto, R. R. Naik and D. L. Kaplan, *Biopolymers*, 2012, **97**, 479–498.
111 Z. Chen, F. Hu, Z. Lin, J. Hu, R. Shen, Y. Lin and X. Y. Liu, *Small Sci.*, 2021, **1**, 2000049.
112 Y. Fu, X. Xie, Y. Wang, J. Liu, Z. Zheng, D. L. Kaplan and X. Wang, *ACS Biomater. Sci. Eng.*, 2021, **7**, 2734–2744.
113 M. Denise, D. Aleksander, I. Heiko, K. Nadja, Z. Max, S. Ralf, K. Marius, K. Alexander and J. S. Michael, *Biosens. Bioelectron.*, 2021, **183**, 113204.
114 C. Bungthong, C. Wrigley, T. Sonteera and S. Siriamornpun, *Molecules*, 2021, **26**, 3455.

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115 M. d. C. de Castro, P. S. Garcia, M. M. Andrade, M. V. E. Grossmann, B. M. Simões, R. B. Samulewski and A. M. Baron, *Biotechnol. Appl. Biochem.*, 2022, **69**, 660–667.
116 Q. Jianghong, L. Yongcheng, L. Haiying, Y. Tongyin and D. Jiaqi, *Anal. Biochem.*, 1996, **236**, 208–214.
117 D. Makoto and A. Tetsuo, *J. Membr. Sci.*, 1991, **59**, 39–52.
118 K. Akio, A. Tetsuo, T. Ryuzo and M. Tadashi, *J. Biotechnol.*, 1987, **5**, 199–207.
119 D. Cordier, R. Couturier, L. Grasset and A. Ville, *Enzyme Microb. Technol.*, 1982, **4**, 249–255.
120 S. Inoue, Y. Matsunaga, H. Iwane, M. Sotomura and T. Nose, *Biochem. Biophys. Res. Commun.*, 1986, **141**, 165–170.
121 Y. Liu, X. Chen, J. Qian, H. Liu, Z. Shao, J. Deng and T. Yu, *Appl. Biochem. Biotechnol.*, 1997, **62**, 105–117.
122 B. Liang, L. Fang, Y. Hu, G. Yang, Q. Zhu and X. Ye, *Nanoscale*, 2014, **6**, 4264–4274.
123 A. Einbu, S. N. Naess, A. Elgsaeter and K. M. Vårum, *Biomacromolecules*, 2004, **5**, 2048–2054.
124 R. Marguerite, *Prog. Polym. Sci.*, 2006, **31**, 603–632.
125 E. Cabib, in *Plant Carbohydrates II: Extracellular Carbohydrates*, ed. W. Tanner and F. A. Loewus, Springer Berlin Heidelberg, Berlin, Heidelberg, 1981, pp. 395–415. DOI: 10.1007/978-3-642-68234-6_16.
126 V. Farkaš, *Acta Biotechnol.*, 1990, **10**, 225–238.
127 D. Knorr, *Food Technol.*, 1984, **38**, 92–97.
128 B. Amit and S. Mika, *Adv. Colloid Interface Sci.*, 2009, **152**, 26–38.
129 A. A. M. Riccardo, *Carbohydr. Polym.*, 2009, **76**, 167–182.
130 T. N. Nwagu, B. Okolo and H. Aoyagi, *Bioresour. Technol. Rep.*, 2021, **13**, 100645.
131 J. Synowiecki, Z. E. Sikorski and M. Naczk, *Biotechnol. Bioeng.*, 1981, **23**, 2211–2215.
132 M. T. Xavier, V. F. Soares, D. G. Freire, C. P. Moreira, M. F. Mendes and E. Bon, *Biomass*, 1987, **13**, 25–32.
133 O. Eiji and K. Tsuyoshi, *Anal. Chim. Acta*, 1992, **262**, 19–25.
134 H. Xiao-Qing and S. Fereidoon, *Food Chem.*, 1995, **52**, 71–76.
135 F. M. Gomes, E. B. Pereira and H. F. de Castro, *Biomacromolecules*, 2004, **5**, 17–23.
136 G. Leissy, L. R. Hector, L. V. Maria, H. Junior and V. Reynaldo, *Enzyme Microb. Technol.*, 2006, **38**, 22–27.
137 L. Gomez, H. L. Ramirez, G. Cabrera, B. K. Simpson and R. Villalonga, *J. Food Biochem.*, 2008, **32**, 264–277.
138 S. Manthiriyappan and L. Cheng-Kang, *Carbohydr. Polym.*, 2011, **84**, 775–780.
139 N. Zhou, A. Zhang, G. Wei, S. Yang, S. Xu, K. Chen and P. Ouyang, *Front. Bioeng. Biotechnol.*, 2020, **8**, 103.
140 H. L. Ramirez, L. G. Brizuela, J. Ú. Iranzo, M. Arevalo-Villena and A. I. B. Pérez, *J. Food Process Eng.*, 2016, **39**, 97–104.
141 J. Suisui, Q. Yang, Y. Jie, L. Man, X. Liu and S. Qingjie, *Food Chem.*, 2017, **221**, 1507–1513.
142 W. A. Mehdi, A. A. Mehde, M. Özacar and Z. Özacar, *Int. J. Biol. Macromol.*, 2018, **117**, 947–958.
143 H. Yao, F. Yan, C. Lingyun, L. Ang and Z. Lina, *Chem. Eng. J.*, 2017, **315**, 573–582.
144 M. Chen, H. Wu, Z. Li, K. Wu, Y. Jiao and C. Zhou, *J. Porous Mater.*, 2020, **27**, 549–554.
145 F. Shahid, A. Ansari, A. Aman and S. A. U. Qader, *Catal. Lett.*, 2020, **150**, 613–622.
146 W.-C. Huang, W. Wang, C. Xue and X. Mao, *ACS Sustainable Chem. Eng.*, 2018, **6**, 8118–8124.
147 Y. Yandri, T. Suhartati, H. Satria, S. Karlinasari, S. D. Yuwono and S. Hadi, *J. Adv. Pharm. Educ. Res.*, 2021, **11**, 63–69.
148 T. Machałowski, K. Jankowska, K. Bachosz, W. Smułek, H. Ehrlich, E. Kaczorek, J. Zdarta and T. Jesionowski, *Molecules*, 2022, **27**, 1354.
149 Y. Yandri, E. R. Tiarsa, T. Suhartati, B. Irawan and S. Hadi, *Emerg. Sci. J.*, 2022, **7**, 77–89.
150 E. R. Tiarsa, Y. Yandri, T. Suhartati, H. Satria, B. Irawan and S. Hadi, *Biochem. Res. Int.*, 2022, **2022**, 5692438.
151 H. Ibrahim and E. El-Zairy, *Concepts, compounds and the alternatives of antibacterials*, 2015, vol. 1, pp. 81–101.
152 S. K. Arya, M. Manohar, G. Singh and W. A. Siddiqui, *Chitosan: Derivatives, Composites and Applications*, 2017, pp. 151–165.
153 N. C. Ricardi, L. T. Arenas, E. V. Benvenutti, R. Hinrichs, E. E. E. Flores, P. F. Hertz and T. M. H. Costa, *Food Chem.*, 2021, **359**, 129890.
154 D. R. Baidamshina, V. A. Koroleva, S. S. Olshannikova, E. Y. Trizna, M. I. Bogachev, V. G. Artyukhov, M. G. Holyavka and A. R. Kayumov, *Mar. Drugs*, 2021, **19**, 197.
155 T. A. Costa-Silva, A. K. F. Carvalho, C. R. F. Souza, H. F. De Castro, L. Bachmann, S. Said and W. P. Oliveira, *Appl. Catal., A*, 2021, **622**, 118217.
156 A. Sadia, A. Muhammad, K. N. Ahmad and B. Muhammad, *J. Water Process. Eng.*, 2021, **40**, 101971.
157 A. Apriceno, I. Silvestro, A. Girelli, I. Francolini, L. Pietrelli and A. Piozzi, *Polymers*, 2021, **13**, 1453.
158 P. C. Lima, I. Gazoni, A. M. G. de Carvalho, D. Bresolin, D. Cavalheiro, D. de Oliveira and E. Rigo, *Food Chem.*, 2021, **349**, 129050.
159 H. Marina, F. Dzhigangir, K. Victoria, O. Svetlana, Z. Nataliya, Z. Yuriy, K. Maxim, Z. Ekaterina and A. Valeriy, *Int. J. Biol. Macromol.*, 2021, **180**, 161–176.
160 K. Belho and P. Ambasht, *J. Sci. Res.*, 2021, **65**, 111–119.
161 L. Zhong, J. Li, D. Tian, J. Cai, H. Wang and Q. Ma, *Water Sci. Technol.*, 2021, **83**, 906–921.
162 V. Archana and K. Awanish, *J. Drug Delivery Sci. Technol.*, 2021, **61**, 102231.
163 M. I. G. Siso, E. Lang, B. Carrenõ-Gómez, M. Becerra, F. O. Espinar and J. B. Méndez, *Process Biochem.*, 1997, **32**, 211–216.
164 S. Dumitriu and E. Chornet, *Biotechnol. Prog.*, 1997, **13**, 539–545.
165 M. Chellapandian and M. R. V. Krishnan, *Process Biochem.*, 1998, **33**, 595–600.
166 E. Pereira, G. Zanin and H. Castro, *Braz. J. Chem. Eng.*, 2003, **20**, 343–355.
167 Ş. A. Çetinus and H. N. Öztop, *Enzyme Microb. Technol.*, 2003, **32**, 889–894.

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168 Y. M. Yang, J. W. Wang and R. X. Tan, *Enzyme Microb. Technol.*, 2004, **34**, 126–131.
169 C. Min-Yun and J. Ruey-Shin, *Enzyme Microb. Technol.*, 2005, **36**, 75–82.
170 J. De-Sheng, L. Sheng-Ya, H. Jun, X. Hai-Yan and Z. Ju-Ying, *Biochem. Eng. J.*, 2005, **25**, 15–23.
171 W. Adriano, J. Silva, R. Giordano and L. Gonçalves, *Braz. J. Chem. Eng.*, 2005, **22**, 529–538.
172 X. Mao, G. Guo, J. Huang, Z. Du, Z. Huang, L. Ma, P. Li and L. Gu, *J. Chem. Technol. Biotechnol.*, 2006, **81**, 189–195.
173 T. Zhen-Xing, Q. Jun-Qing and E. S. Lu, *Process Biochem.*, 2006, **41**, 1193–1197.
174 Z. X. Tang, J. Q. Qian and L. E. Shi, *Mater. Lett.*, 2007, **61**, 37–40.
175 Ş. A. Çetinus, H. N. Öztop and D. Saraydin, *Enzyme Microb. Technol.*, 2007, **41**, 447–454.
176 G. D. Altun and S. A. Cetinus, *Food Chem.*, 2007, **100**, 964–971.
177 A. Dinçer and A. Telefoncu, *J. Mol. Catal. B: Enzym.*, 2007, **45**, 10–14.
178 X.-J. Huang, D. Ge and Z.-K. Xu, *Eur. Polym. J.*, 2007, **43**, 3710–3718.
179 B. Krajewska, M. Leszko and W. Zaborska, *J. Chem. Technol. Biotechnol.*, 1990, **48**, 337–350.
180 C. Min-Yun and J. Ruey-Shin, *Biochem. Eng. J.*, 2007, **35**, 93–98.
181 W. S. Adriano, D. B. Mendonça, D. S. Rodrigues, E. J. Mammarella and R. L. C. Giordano, *Biomacromolecules*, 2008, **9**, 2170–2179.
182 E. E. E. Flores, F. D. Cardoso, L. B. Siqueira, N. C. Ricardi, T. H. Costa, R. C. Rodrigues, M. P. Klein and P. F. Hertz, *Process Biochem.*, 2019, **84**, 73–80.
183 S. R. Dasciana, A. M. Adriano, S. A. Wellington, R. B. G. Luciana and L. C. G. Raquel, *J. Mol. Catal. B: Enzym.*, 2008, **51**, 100–109.
184 R. Wang, B. Xia, B.-J. Li, S.-L. Peng, L.-S. Ding and S. Zhang, *Int. J. Pharm.*, 2008, **364**, 102–107.
185 R. S. Singh, R. P. Singh and J. F. Kennedy, *Int. J. Biol. Macromol.*, 2017, **95**, 87–93.
186 Ş. A. Çetinus, E. Şahin and D. Saraydin, *Food Chem.*, 2009, **114**, 962–969.
187 H. Fang, J. Huang, L. Ding, M. Li and Z. Chen, *J. Wuhan Univ. Technol., Mater. Sci. Ed.*, 2009, **24**, 42–47.
188 M. Monier, D. M. Ayad, Y. Wei and A. A. Sarhan, *Int. J. Biol. Macromol.*, 2010, **46**, 324–330.
189 S. Wanjari, C. Prabhu, R. Yadav, T. Satyanarayana, N. Labhsetwar and S. Rayalu, *Process Biochem.*, 2011, **46**, 1010–1018.
190 N. A. Kalkan, S. Aksoy, E. A. Aksoy and N. Hasirci, *J. Appl. Polym. Sci.*, 2012, **123**, 707–716.
191 M. P. Klein, M. R. Nunes, R. C. Rodrigues, E. V. Benvenutti, T. M. H. Costa, P. F. Hertz and J. L. Ninow, *Biomacromolecules*, 2012, **13**, 2456–2464.
192 D. Ayşe, B. Seda and A. Tülin, *Int. J. Biol. Macromol.*, 2012, **50**, 815–820.
193 K. Chia-Hung, L. Yung-Chuan, J. C. Chieh-Ming, C. Jiann-Hwa, C. Cheng and S. Chwen-Jen, *Carbohydr. Polym.*, 2012, **87**, 2538–2545.
194 M. Kamburov and I. Lalov, *Biotechnol. Biotechnol. Equip.*, 2012, **26**, 156–163.
195 R. Zhai, B. Zhang, Y. Wan, C. Li, J. Wang and J. Liu, *Chem. Eng. J.*, 2013, **214**, 304–309.
196 P. Kristyna and S. Ivo, *Carbohydr. Polym.*, 2013, **96**, 545–548.
197 J. Missau, A. J. Scheid, E. L. Foletto, S. L. Jahn, M. A. Mazutti and R. C. Kuhn, *Sustainable Chem. Processes*, 2014, **2**, 13.
198 L. Zang, J. Qiu, X. Wu, W. Zhang, E. Sakai and Y. Wei, *Ind. Eng. Chem. Res.*, 2014, **53**, 3448–3454.
199 H. J. Kim, J. N. Jin, E. Kan, K. J. Kim and S. H. Lee, *Biotechnol. Bioprocess Eng.*, 2017, **22**, 89–94.
200 V. S. Uttam, S. N. Shamraja and K. R. Virendra, *Carbohydr. Polym.*, 2017, **157**, 677–685.
201 E. Demirkan, T. Avci and Y. Aykut, *J. Ind. Text.*, 2018, **47**, 2092–2111.
202 T. Mardani, M. S. Khiabani, R. R. Mokarram and H. Hamishehkar, *Int. J. Biol. Macromol.*, 2018, **120**, 354–360.
203 K.-M. Yeon, J. You, M. D. Adhikari, S.-G. Hong, I. Lee, H. S. Kim, L. N. Kim, J. Nam, S.-J. Kwon, M. I. Kim, W. Sajomsang, J. S. Dordick and J. Kim, *Biomacromolecules*, 2019, **20**, 2477–2485.
204 C. Ilaria, L. Claudio, B. Ilaria and E. Marco, *J. Mater. Res. Technol.*, 2019, **8**, 3644–3652.
205 S. Hongbo, G. Zhen, X. Lili, X. Chao, Y. Dinghua, X. Xinran, H. He and H. Yi, *Mater. Sci. Eng., C*, 2019, **96**, 356–364.
206 W. Deqiang and J. Weifeng, *Int. J. Biol. Macromol.*, 2019, **126**, 1125–1132.
207 A. M. Girelli, L. Quattrocchi and F. R. Scuto, *J. Biotechnol.*, 2020, **318**, 45–50.
208 M. Prasad and P. Palanivelu, *Biotechnol. Appl. Biochem.*, 2015, **62**, 523–529.
209 J. Ruey-Shin, W. Feng-Chin and T. Ru-Ling, *Adv. Environ. Res.*, 2002, **6**, 171–177.
210 F. Maryam and I. Ani, *Renewable Energy*, 2022, **185**, 1362–1375.
211 Ç. Esin and Ö. Seçil, *React. Funct. Polym.*, 2022, **172**, 105181.
212 M. G. Holyavka, S. M. Pankova, Y. M. Vyshkvorkina, A. N. Lukin, M. S. Kondratyev and V. G. Artyukhov, *Biophysics*, 2022, **67**, 365–373.
213 K. K. Gali, N. Soundararajan, V. Katiyar and S. Sivaprakasam, *J. Mater. Res. Technol.*, 2021, **13**, 686–699.
214 M. B. Pereira, B. L. Nogueira, I. D. C. Montano, D. D. S. Rodrigues and C. A. G. Suarez, *Cellul. Chem. Technol.*, 2021, **55**, 829–837.
215 K. Matsushima, H. Minoshima, H. Kawanami, Y. Ikushima, M. Nishizawa, A. Kawamukai and K. Hara, *Ind. Eng. Chem. Res.*, 2005, **44**, 9626–9630.
216 H. Zimmermann, F. Ehrhart, D. Zimmermann, K. Müller, A. Katsen-Globa, M. Behringer, P. J. Feilen, P. Gessner, G. Zimmermann, S. G. Shirley, M. M. Weber, J. Metze and U. Zimmermann, *Appl. Phys. A*, 2007, **89**, 909–922.
217 I. P. S. Fernando, W. Lee, E. J. Han and G. Ahn, *Chem. Eng. J.*, 2020, **391**, 123823.

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218 C. H. Goh, P. W. S. Heng and L. W. Chan, *Carbohydr. Polym.*, 2012, **88**, 1–12.
219 R. Yavaşer and A. A. Karagözler, *Process Biochem.*, 2021, **101**, 137–146.
220 A. O. Ali, M. S. Abdalla, Y. E. Shahein, A. Shokeer, H. M. Sharada and K. A. Ali, *3 Biotech.*, 2021, **11**, 341.
221 R. Guo, X. Zheng, Y. Wang, Y. Yang, Y. Ma, D. Zou and Y. Liu, *Appl. Biochem. Biotechnol.*, 2021, **193**, 2043–2060.
222 Y. Q. Almulaiky and S. A. Al-Harbi, *Catal. Lett.*, 2022, **152**, 28–42.
223 A. B. A. Mohammed, A. E. Hegazy and A. Salah, *Appl. Nanosci.*, 2023, **13**(1), 641–649.
224 N. Popović, D. Pržulj, M. Mladenović, O. Prodanović, S. Ece, K. I. Đurđić, R. Ostafe, R. Fischer and R. Prodanović, *Int. J. Biol. Macromol.*, 2021, **181**, 1072–1080.
225 M. Jonović, M. Žuža, V. Đorđević, N. Šekuljica, M. Milivojević, B. Jugović, B. Bugarski and Z. Knežević-Jugović, *Catalysts*, 2021, **11**, 305.
226 W. Yang, F. Chenyu, G. Rongxin, M. Yifang, Y. Yu and L. Yanping, *Process Biochem.*, 2021, **107**, 38–47.
227 N. Pantić, R. Prodanović, K. I. Đurđić, N. Polović, M. Spasojević and O. Prodanović, *Environ. Technol. Innovation*, 2021, **21**, 101211.
228 I. W. Marwa, E. H. Mohamed and A. A. Korany, *Biocatal. Biotransform.*, 2021, **39**, 138–151.
229 J. Moriyama and M. Yoshimoto, *ACS Omega*, 2021, **6**, 6368–6378.
230 G. Palmieri, P. Giardina, B. Desiderio, L. Marzullo, M. Giamberini and G. Sannia, *Enzyme Microb. Technol.*, 1994, **16**, 151–158.
231 Y. M. Elçin, *Biomaterials*, 1995, **16**, 1157–1161.
232 N. Yadav, M. H. Mruthunjayappa, M. Bisht, S. K. Nataraj, P. Venkatesu and D. Mondal, *Chem. Commun.*, 2020, **56**, 9659–9662.
233 A. Blandino, M. Macías and D. Cantero, *Process Biochem.*, 2001, **36**, 601–606.
234 P. Rilling, T. Walter, R. Pommersheim and W. Vogt, *J. Membr. Sci.*, 1997, **129**, 283–287.
235 A. Tanriseven and Ş. Doğan, *Process Biochem.*, 2001, **36**, 1081–1083.
236 T. Vu and V. Le, *ASEAN Food J.*, 2008, **15**, 73–78.
237 N. W. Fadnavis, G. Sheelu, B. M. Kumar, M. U. Bhalerao and A. A. Deshpande, *Biotechnol. Prog.*, 2003, **19**, 557–564.
238 E. Taqieddin and M. Amiji, *Biomaterials*, 2004, **25**, 1937–1945.
239 J.-Y. Wang, H.-R. Yu, R. Xie, X.-J. Ju, Y.-L. Yu, L.-Y. Chu and Z. Zhang, *AIChE J.*, 2013, **59**, 380–389.
240 L. Lu, M. Zhao and Y. Wang, *World J. Microbiol. Biotechnol.*, 2007, **23**, 159–166.
241 S. Xu, Y. Lu, Z. Jiang and H. Wu, *J. Mol. Catal. B: Enzym.*, 2006, **43**, 68–73.
242 S. M. Shet, M. Bisht, S. Pramanik, S. Roy, S. K. Thayallath, S. K. Nataraj, D. Mondal and S. Bhandari, *Adv. Opt. Mater.*, 2020, **8**, 1902022.
243 L. Ma, J. Wen, W. Lu, Q. Caiyin and Y. Liang, *Enzyme Microb. Technol.*, 2008, **42**, 235–241.
244 I. Bhushan, R. Parshad, G. N. Qazi and V. K. Gupta, *J. Bioact. Compat. Polym.*, 2008, **23**, 552–562.
245 A. Anwar, S. A. U. Qader, A. Raiz, S. Iqbal and A. Azhar, *World Appl. Sci. J.*, 2009, **7**, 1281–1286.
246 T. Q. Viet, N. P. Minh and D. T. A. Dao, *Am. J. Res. Commun.*, 2013, **1**, 254–267.
247 M. Bilal and M. Asgher, *BMC Biotechnol.*, 2015, **15**, 1–14.
248 M. A. P. Nunes, H. Vila-Real, P. C. B. Fernandes and M. H. L. Ribeiro, *Appl. Biochem. Biotechnol.*, 2010, **160**, 2129–2147.
249 B. M. Bonine, P. P. Polizelli and G. O. Bonilla-Rodriguez, *Enzyme Res.*, 2014, **2014**, 738739.
250 N. Ortega, M. Perez-Mateos, M. C. Pilar and M. D. Busto, *J. Agric. Food Chem.*, 2009, **57**, 109–115.
251 M. S. M. Eldin, E. I. Seuror, M. A. Nasr and H. A. Tieama, *Appl. Biochem. Biotechnol.*, 2011, **164**, 45–57.
252 X. Wang, K.-X. Zhu and H.-M. Zhou, *Int. J. Mol. Sci.*, 2011, **12**, 3042–3054.
253 F. Zhao, H. Li, X. Wang, L. Wu, T. Hou, J. Guan, Y. Jiang, H. Xu and X. Mu, *J. Mater. Chem. B*, 2015, **3**, 9315–9322.
254 M. A. Abd El-Ghaffar and M. S. Hashem, *Carbohydr. Polym.*, 2013, **92**, 2095–2102.
255 K. S. Muthuvelu, R. Rajarathinam, N. K. Manickam and S. Uthandi, *Bioresour. Technol.*, 2018, **269**, 227–236.
256 Y. İ. Doğaç, I. Deveci, B. Mercimek and M. Teke, *Int. J. Biol. Macromol.*, 2017, **96**, 302–311.
257 F. Zhao, Q. Wang, J. Dong, M. Xian, J. Yu, H. Yin, Z. Chang, X. Mu, T. Hou and J. Wang, *Process Biochem.*, 2017, **57**, 87–94.
258 S. Kumar, I. Haq, J. Prakash and A. Raj, *Int. J. Biol. Macromol.*, 2017, **98**, 24–33.
259 D. K. Bedade, Y. B. Sutar and R. S. Singhal, *Food Chem.*, 2019, **275**, 95–104.
260 M. Mohammadi, M. K. Heshmati, K. Sarabandi, M. Fathi, L.-T. Lim and H. Hamishehkar, *Int. J. Biol. Macromol.*, 2019, **137**, 253–260.
261 Z. Zhi-de, L. Gui-yin and L. Yuan-jian, *Int. J. Biol. Macromol.*, 2010, **47**, 21–26.
262 Q. Ai, D. Yang, Y. Zhu and Z. Jiang, *Ind. Eng. Chem. Res.*, 2013, **52**, 14898–14905.
263 C.-T. Tsai and A. S. Meyer, *Molecules*, 2014, **19**, 19390–19406.
264 U. D. Kamaci and A. Peksel, *Catal. Lett.*, 2021, **151**, 821–831.
265 C. Benjamas, J. Prawit and H. K. Aran, *J. Mol. Catal. B: Enzym.*, 2009, **59**, 206–211.
266 A. Saxena, S. Sharda, S. Kumar, B. Kumar, S. Shirodkar, P. Dahiya and R. Sahney, *Polymers*, 2022, **14**, 1277.
267 A. Omar and Q. A. Yaaser, *Biocatal. Biotransform.*, 2022, 1–10.
268 L. Abdul, M. Ahsan, S. Kai and S. Youbin, *J. Environ. Chem. Eng.*, 2022, **10**, 107089.
269 M. Sharma, A. P. M. Tavares, J. C. F. Nunes, N. Singh, D. Mondal, M. C. Neves, K. Prasad and M. G. Freire, *Green Chem.*, 2020, **22**, 2225–2233.
270 A. Charoenpol, D. Crespy, A. Schulte and W. Suginta, *Green Chem.*, 2023, **25**, 467–489.

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