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Enzyme immobilization advances: a key to unlocking renewable bioenergy potential

Mohamed E. Hassan,†[a,b] Xuhai Zhu, †[a] Evanildo F. de Souza, Jr.,[a] Magdy M. Elnashar[b,c] and Fang Lu *[a]

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This review provides an in-depth analysis of enzyme immobilization techniques and their application in catalyzing the transformation of biomass into high-value chemicals. The utilization of immobilized enzymes in biomass conversion demonstrates distinctive catalytic properties, enabling a more sustainable and efficient process for converting lignocellulosic materials into platform chemicals and biofuels. Immobilization enhances enzyme stability, facilitates repeated use, improves reaction control, reduces enzyme consumption, and minimizes operational costs. These attributes position immobilized enzymes as promising candidates for scalable, environmentally friendly biomass refining technologies. Furthermore, they contribute to higher yields and reduce environmental impact by decreasing reliance on harsh chemicals and simplifying downstream processing. Overall, enzyme immobilization, both technically and in terms of market opportunities, holds significant promise for advancing sustainable development in biorefineries.

Green foundation

1. The review highlights enzyme immobilization methods enabling sustainable biomass conversion into high-value chemicals, emphasizing enhanced stability, reusability, and cost-efficiency. This reduces reliance on harsh chemicals and improves catalytic precision for biofuels and platform chemical synthesis.
2. Immobilized enzymes align with circular economy goals by transforming renewable lignocellulosic waste into marketable products. Their scalability and lowered environmental footprint offer solutions for industries seeking greener alternatives to petrochemical-based processes.
3. Optimizing immobilization techniques (*e.g.*, nanocarrier design and modular reactors) could revolutionize biorefineries. By addressing enzyme durability, process integration, and lifecycle analysis, this review guides strategies to accelerate industrial adoption, reduce carbon impacts, and advance sustainable biomanufacturing. In response to the challenge of economic cost, future research will focus on developing more advanced materials and microreactors using AI.

1 Introduction

Lignocellulosic biomass (LB) constitutes the most abundant, renewable, and recyclable terrestrial resource, characterized by a structural composition predominantly comprising cellulose, hemicellulose, and lignin (Fig. 1). Owing to its rich carbon content and polymeric complexity, LB offers a critical pathway amid the escalating imperative for global decarbonization.[1–3] As the environmental, economic, and health-related consequences of fossil fuel dependency become increasingly untenable, the strategic valorization of LB has emerged as a cornerstone of the bioeconomy. This multidisciplinary paradigm synergizes industrial innovation, scientific research, and technological advancement to foster a sustainable future. Within this framework, biorefineries are positioned as pivotal infrastructures, enabling the systematic conversion of underutilized biomass into a diverse array of high-value products while minimizing environmental impacts.

Although the strategic potential of biomass was recognized as early as the energy crises of the 1970s, recent intensifications of climate change, geopolitical instabilities, and resource depletion have markedly amplified the urgency of biomass-based solutions.[1,4] Distinguished by its high carbon content and diverse chemical functionalities, LB is a versatile feedstock for the sustainable production of biofuels, biochemicals, and bioproducts with a substantially lower carbon footprint than that of its fossil-based counterparts.[5–7] Composed predominantly of plant-derived residues—including agricultural wastes, forestry byproducts, and agro-industrial effluents—LB could meet up to 30% of global gasoline demand by 2030

[a]State Key Laboratory of Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road Dalian, Dalian 110623, China. E-mail: lufang@dicp.ac.cn; Tel: +86-0411-8437-9846
[b]Pharmaceutical and Drug Industries Research Institute, National Research Centre, Cairo 12622, Egypt
[c]School of Medicine, Curtin University, Perth 6102, Australia
†These authors contributed equally to this work.

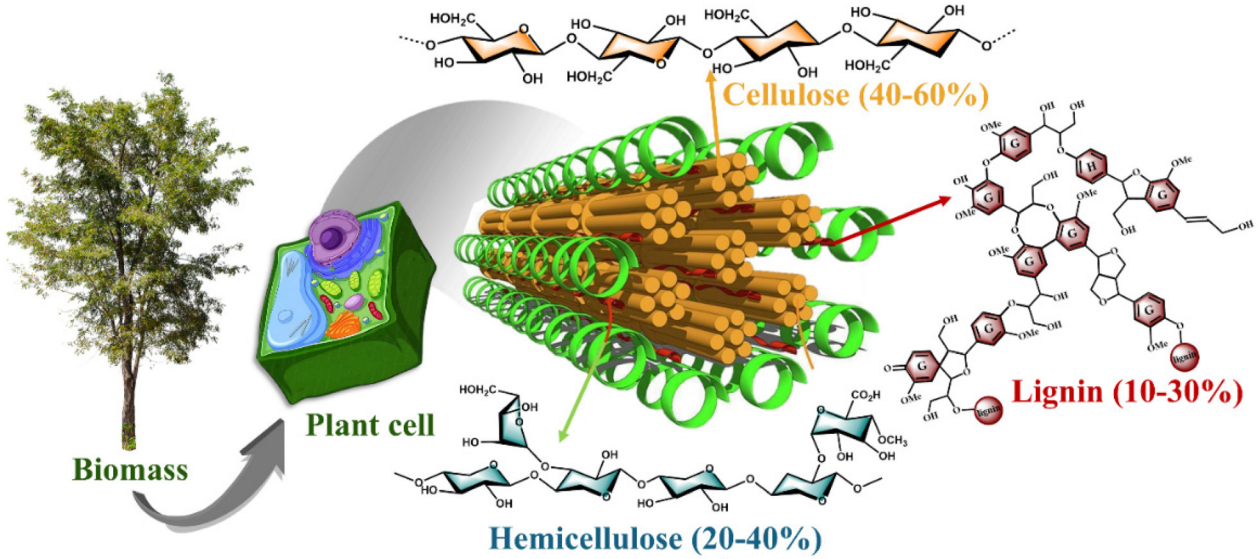

Fig. 1 Structural composition of lignocellulosic biomass, highlighting its main fractions.

through integrated biorefinery models.[8] Its effective valorization transcends energy substitution, representing a fundamental pillar for greenhouse gas mitigation, resource circularity, and the realization of a low-carbon, resilient global economy.

Nonetheless, lignocellulosic matrices' inherent structural complexity and recalcitrance present formidable challenges to efficient bioconversion.[9] Although effective in disrupting biomass architecture, conventional chemical and physical pretreatment methods are frequently associated with high energy demands, generation of inhibitory byproducts, and elevated processing costs.[10] In contrast, biocatalytic strategies—particularly those employing enzymatic systems—offer superior specificity, operate under milder conditions, and exhibit reduced environmental burdens, aligning more closely with green chemistry and industrial sustainability principles.[3,6]

However, despite their promise, the industrial deployment of enzymatic bioconversion processes remains constrained by factors such as enzyme instability, elevated production costs, and limited reusability. In this context, enzyme immobilization technologies have gained prominence as transformative approaches, significantly enhancing catalytic stability, enabling efficient catalyst recovery, and facilitating the seamless integration of enzymes into continuous processing systems. However, LB's structural recalcitrance necessitates efficient conversion technologies, where enzyme immobilization has become a game-changing solution to three key challenges: (1) overcoming natural enzyme instability in industrial environments, (2) enabling continuous bioprocessing through reusable catalysts, and (3) reducing biocatalyst costs by >60% through enhanced durability. While traditional pretreatment methods (steam explosion, acid hydrolysis) remain energy-intensive and inhibitor-generating, immobilized enzymes offer precision biomass deconstruction under mild conditions–cellulases on magnetic MOFs achieve 85% sugar yields at 50% lower energy input compared to thermal methods.

This review presents a critical advancement in the field of sustainable biocatalysis by systematically bridging cutting-edge enzyme immobilization technologies with practical biorefinery applications. Unlike previous works that treat these domains separately, we provide the first integrated analysis of how modern immobilization strategies (MOFs, microreactor systems) can overcome key challenges in biomass valorization. Our novel contributions include: (1) quantitative comparison of immobilization techniques for lignin valorization, revealing optimal support–enzyme combinations; (2) industrial implementation frameworks featuring decision matrices and economic thresholds for scalable adoption; and (3) pioneering lifecycle assessments demonstrating 40–60% reductions in water usage and 35% lower energy demands compared to conventional methods. By consolidating these advances with original roadmaps for cost-competitive biofuel production (<$2.50 per kg), this work serves as both a fundamental reference for biocatalyst design and a practical guide for implementing sustainable biotransformation platforms. The review specifically addresses three critical gaps in the current literature: the technology integration gap between immobilization science and biorefinery engineering, the industrial translation gap in scaling immobilized enzymes, and the sustainability metrics gap in assessing environmental impacts of biocatalytic processes.

2 Overview of raw biomass

Structurally, LB is composed of a diverse array of organic compounds, including lipids, cellulose, sugars, hemicelluloses, starches, hydrocarbons, and water, along with a range of bio-

active molecules such as carotenoids, flavonoids, lignans, and antioxidants.[11] Among these components, LB, a widely studied feedstock for biorefineries, is primarily made up of three structural biopolymers: cellulose (~60% w/w), hemicellulose (~40% w/w), and lignin (~30% w/w), as illustrated in Fig. 1.[11,12] These proportions, however, are not fixed and can vary significantly depending on factors such as botanical origin, climatic conditions, geographical location, and seasonal influences, as shown in Table S1.[6,12–14]

At the molecular level, cellulose is a highly ordered, linear homopolymer of glucose units linked by β-(1 → 4) glycosidic bonds.[12] These chains assemble into crystalline microfibrils, which provide tensile strength and structural rigidity to plant cell walls.[14] In contrast, hemicellulose is a heterogeneous, branched heteropolysaccharide matrix composed of various monosaccharides, including xylose, arabinose, mannose, and glucose. These sugar units are interconnected predominantly through β-(1 → 4) and β-(1 → 6) glycosidic bonds.[12] Due to its amorphous structure, hemicellulose exhibits greater chemical reactivity and accessibility to enzymatic hydrolysis than cellulose, playing a critical role in modulating the porosity and flexibility of the cell wall matrix. Lignin is a highly complex, heterogeneous aromatic polymer crucial in conferring mechanical strength, hydrophobicity, and resistance to microbial attack in plant cell walls.[15,16] Its molecular architecture is primarily derived from the oxidative polymerization of three monolignols: *p*-hydroxycinnamyl (coumaryl) alcohol, 4-hydroxy-3-methoxycinnamyl (coniferyl) alcohol, and 3,5-dimethoxy-4-hydroxycinnamyl (sinapyl) alcohol. These monolignols give rise to *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively, which are interconnected through a complex array of ether (C–O) and carbon–carbon (C–C) linkages.[17,18] In contrast to cellulose (C5) and hemicellulose (C5 and C6), the enzymatic depolymerization and conversion of which into fermentable sugars is well established, lignin's irregular, cross-linked macromolecular network, characterized by extensive branching and structural diversity, creates a rigid, hydrophobic matrix that severely limits the enzymatic and microbial accessibility of polysaccharide components embedded within the plant cell wall. As a result, lignin represents a significant barrier to efficient biomass deconstruction, necessitating targeted strategies for its selective modification and valorization in biorefinery applications. Despite its abundance and aromatic richness, over 90% of industrial lignin (particularly kraft lignin) is still burned for low-grade energy due to the recalcitrance imposed by its high C–C bond content.[19,20] Recent efforts have increasingly focused on lignin-first strategies and selective depolymerization approaches, reflecting its emerging status as the next frontier in biorefinery development.[21] Advancing lignin valorization is therefore critical for achieving integrated, high-efficiency bioprocesses and unlocking the full potential of LB.[19,22] However, biomass is not a homogeneous resource. Its complexity extends far beyond lignocellulose and encompasses diverse feedstocks from agricultural, industrial, municipal, marine, and microbial sources, as shown in Table S1 and Fig. 2.[6,11,13,14,23]

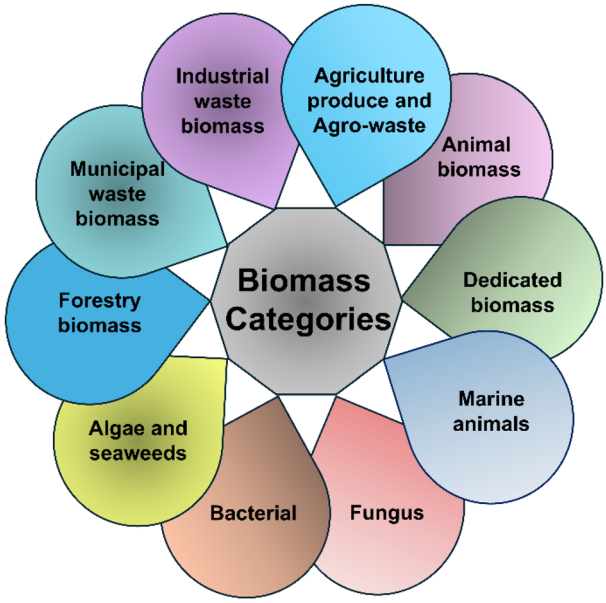

Fig. 2 Raw biomass sources utilized in biorefineries.

The expanding portfolio of biomass feedstocks demands a tailored, feedstock-specific approach in the design of integrated biorefineries. Conventional agricultural residues such as straw, husks, and oilseed cakes remain widely utilized for the production of bio-oil, biodiesel, biochar, and biogas,[23,24] while high-yield, chemically uniform energy crops—such as *Miscanthus*, switchgrass, sugarcane, and fast-growing woody species like willow and hybrid poplar—are particularly suited for second-generation ethanol and bioelectricity.[25] Beyond these, underutilized resources are gaining relevance, including animal-derived biomass (*e.g.*, tallow, manure, and fish oil), which offers lipid-rich profiles for transesterification and anaerobic digestion,[9,26] and concentrated industrial residues such as black liquor, sawdust, and organic effluents that are geographically co-located with the existing processing infrastructure. Municipal solid waste (food scraps, yard trimmings, sewage sludge, and waste cooking oil) plays an increasingly strategic role in urban circular economy frameworks, contributing to bioelectricity, biogas, and biohydrogen production.[9] Additionally, marine and microbial biomass sources represent emerging platforms for bioconversion: algal genera such as *Spirulina*, *Gracilaria*, and *Ulva* are particularly valued for their rapid growth, lipid content, and compatibility with non-arable land, while fungal and bacterial biomass, often byproducts of fermentation processes, can be further valorized into bioenergy carriers and platform chemicals.[2,6,9,27–29] Given this diversity, critical parameters such as physicochemical composition, moisture content, spatial distribution, and process compatibility must guide pretreatment and conversion strategies.[1,9,13] Ultimately, raw biomass should not be viewed merely as an energy alternative but rather as a multifunctional input at the core of sustainable industrial innovation, waste valorization, and the advancement of low-carbon technologies.

2.1 Raw biomass conversion

Fig. 3 reveals pronounced geographic asymmetries in the scientific output of biomass valorization. While China (3787 documents) and the United States (2599) lead global research efforts, countries such as India (991) and Brazil (469) show contrasting trajectories. India has made remarkable strides in scientific productivity and industrial application, aligning with its growing biofuel output and supportive national policies. In contrast, despite being one of the world's largest producers of LB, Brazil has not matched its scientific potential with equivalent industrial integration. This discrepancy becomes more evident when considering that in recent years, China and India have increased biofuel production annually by 15.3% and 11.8%, respectively. At the same time, Brazil continues to underexploit its substantial biomass reserves, much of which is either burned or discarded without added value.[28]

Despite the substantial quantities of agricultural biomass generated annually on a global scale, the majority is still burned as a means of disposal, with only a minor fraction repurposed for mulching, fodder, or direct fuel use.[6,13,16] When appropriately valorized, however, these lignocellulosic materials constitute a sustainable and versatile feedstock for producing liquid and gaseous fuels and electrical energy *via* thermochemical or biochemical conversion pathways, as illustrated in Fig. 4.[6,13,16] Nevertheless, despite its abundance and renewability, the effective transformation of raw biomass (see Fig. 2) into value-added products remains a central challenge in biorefinery development. The crystalline structure of cellulose, the heterogeneous architecture of hemicellulose, and the aromatic, highly cross-linked complexity of lignin form a tightly bound and recalcitrant matrix that severely restricts enzymatic and microbial accessibility.[19]

Consequently, efficiently exploiting these resources necessitates a coordinated, multi-stage strategy. This process begins with carefully selecting appropriate biomass types, followed by applying pretreatment methodologies aimed at disrupting the native plant cell wall architecture. These pretreatments are broadly classified into physical (*e.g.*, milling, grinding, extrusion, microwave irradiation, hydrodynamic cavitation, and freeze pretreatment), chemical (*e.g.*, acid hydrolysis, alkaline treatment, ozonolysis, organosolv processes, and ionic liquid extraction), physicochemical (*e.g.*, liquid hot water, steam explosion, wet oxidation, CO_2 explosion, ammonia recycle percolation, and ammonia fiber explosion), and biological approaches (*e.g.*, microbial attack and enzymatic pretreatment).[9] Each modality seeks to enhance the accessibility of structural polysaccharides and mitigate biomass recalcitrance. Subsequently, the process involves generating or applying specialized lignocellulose-degrading enzymes, including cellulases, hemicellulases, ligninases, and critical accessory proteins tailored to the biochemical complexity of the pretreated substrate. Final bioprocessing stages encompass the fermentation of liberated hexoses and pentoses, the selective bio-fractionation of lignin, and comprehensive downstream operations for product recovery, purification, and valorization.[2,6,28–31] The optimization of each of these interconnected stages is pivotal for unlocking the full potential of LB within integrated, scalable, and economically viable biorefinery platforms.

Building upon recent insights into the synergistic potential of thermomechanical and biochemical processing routes, a promising pathway for biomass conversion lies in the strategic integration of thermochemical pretreatment with enzyme-assisted and microbial biotransformations (see Fig. 4).[32,33] Thermochemical methods efficiently disrupt the compact lignocellulosic matrix. Still, they often require high energy input and chemical additives. Conversely, biochemical routes offer greater specificity and sustainability but suffer from slow reaction rates and limited accessibility to native biomass due to structural recalcitrance.

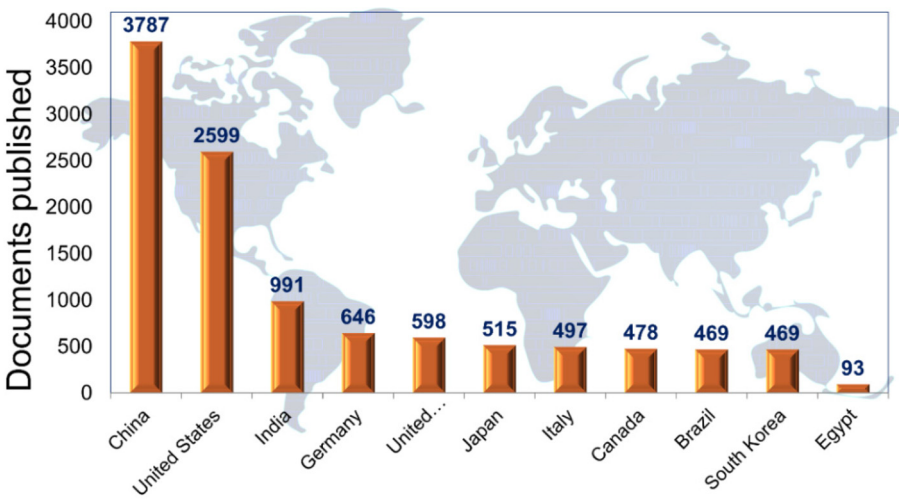

Fig. 3 Papers published per country on biomass conversion were indexed in the SCOPUS database from 2000 to 2024 (keywords: biomass; conversion).

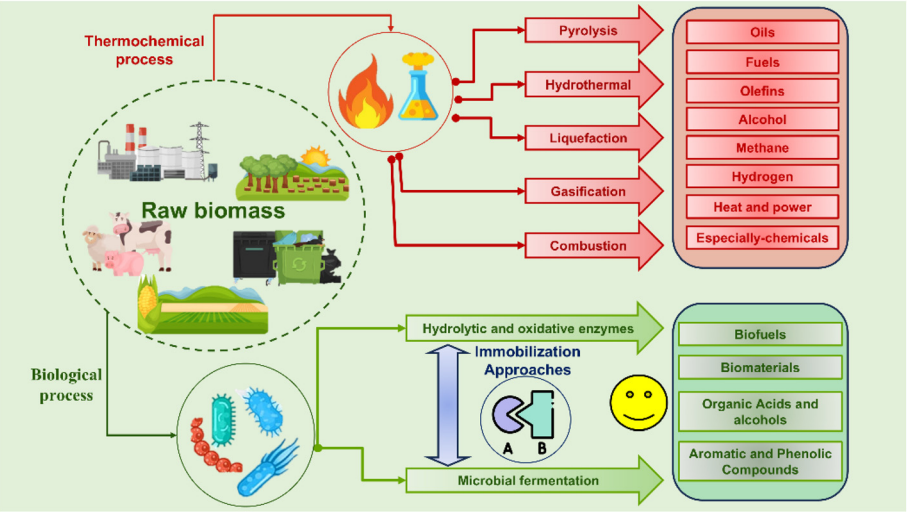

Fig. 4 Schematic representation of an integrated biomass conversion platform combining thermochemical and biochemical routes. Thermochemical processing enables the production of fuels, gases, and specialty chemicals, while biochemical conversion leverages immobilized enzymes and microbial systems for enhanced hydrolysis and fermentation. Feedback loops represent the reuse of thermochemical byproducts (*e.g.*, biochar and gases) as energy sources for biochemical steps, reinforcing energy efficiency within a circular bioeconomy framework.

The hybrid bioprocessing model leverages mild hydrothermal pretreatments to enhance biomass digestibility while minimizing inhibitor formation and preserving critical fractions for downstream valorization.[32] This framework allows immobilized enzymatic systems to be strategically integrated post-thermal disruption, exploiting their stability and reusability under residual solvent and moderate pH conditions. These biocatalysts, tailored for operational resilience, bridge thermal and biological phases, ensuring catalytic continuity across unit operations. Currently, adaptive microbial consortia capable of tolerating or detoxifying residual inhibitors extend substrate flexibility and enhance process robustness. Reinforcing the circular bioeconomy, recycling hydrolysates, nutrient streams, and valorizing lignin-rich side products into bioplastics, biosurfactants, or aromatics maximize resource efficiency. Despite engineering complexities, contamination risks, and the necessity for customized bioreactor configurations, integrating thermochemical disruption with selective biocatalysis represents a scalable pathway toward sustainable biomass valorization. Future optimization must focus on synergizing enzyme immobilization strategies, microbial robustness, and modular process compatibility to fully realize the potential of unified bioprocess platforms.

2.2 Importance of catalytic conversion in biomass

Catalytic conversion is pivotal in transforming LB into a wide range of bio-based products, including simple sugars, alcohols, platform chemicals, bio-oils, and advanced biomaterials.[21,34,35] This strategy is central to developing sustainable biorefinery models, as it allows the selective depolymerization of complex biomass polymers through energy-efficient and environmentally responsible pathways. Compared to traditional thermochemical processes as mentioned above, catalytic routes—whether chemical, enzymatic, or hybrid—offer enhanced selectivity, milder operational conditions, and reduced formation of inhibitory by-products, thus improving the overall technoeconomic feasibility of biomass valorization.[32,33,36]

Enzymatic catalysis has received considerable attention due to its green profile, operating under low temperature and pressure with minimal chemical input.[33,37,38] Enzymatic hydrolysis, in particular, is considered one of the most effective pretreatment techniques for breaking down C5 and C6 into fermentable sugars.[34] However, its industrial scalability remains limited by several critical constraints, including the low intrinsic activity of cellulolytic enzymes, high enzyme dosage requirements, nonspecific adsorption onto lignin-rich substrates, and progressive enzyme deactivation during processing.[39] To address these challenges, enzyme immobilization has emerged as a robust and economically viable approach, offering improved enzyme stability, reduced operational cost, and easier separation and reuse.[8,40–42]

Nevertheless, adsorption-based immobilization techniques often underperform in heterogeneous systems due to competitive binding of non-target proteins or residual contaminants, which may compromise the enzyme's orientation and structural conformation at the interface.[42] Recent advances in immobilization methods, such as sol–gel encapsulation, provide a more favourable microenvironment for enzymes and redox mediators, maintaining their functional integrity and enhancing catalytic lifetime.[41,43] These hybrid materials stabilize the catalytic entities and facilitate downstream product recovery and process integration.[25,40,41] When effectively combined with catalytic systems, these immobilization strategies contribute to the selective degradation of recalcitrant biomass fractions and the production of high-value biofuels and chemi-

cals, such as ethanol, xylitol, biochar, and biogas—key building blocks for a circular and low-carbon bioeconomy.[36,41,44] Ultimately, catalytic conversion—particularly when integrated with engineered enzymatic systems—transcends its role as a mere reactive step and becomes a cornerstone of green chemistry and industrial biotechnology. The interplay between catalyst design, substrate compatibility, and process optimization is essential for unlocking the full potential of biomass as a multifunctional feedstock.

2.3 Challenges in raw biomass conversion

Despite the growing maturity of bio-based technologies, the large-scale conversion of LB into value-added products remains a complex challenge. While raw biomass is abundant and renewable, it demonstrates significant heterogeneity in structural, compositional, and physicochemical characteristics, subsequently affecting its bioconversion potential. Agricultural and forestry operations generate vast quantities of residual biomass, which presents considerable challenges related to efficient hydrolysis, process integration, and logistical handling despite their promise to be lignocellulosic feedstocks. In particular, the effective deconstruction and treatment of such heterogeneous materials remain significant scientific and technical hurdles.[45] Biotechnological efforts have increasingly focused on optimizing enzymatic systems capable of deconstructing these materials under industrial conditions.[37]

Unlike most industrial enzymes, which are typically produced through genetic engineering, naturally occurring enzymes are derived from fungi, bacteria, and certain higher organisms.[45] These unicellular organisms depend on sunlight, minerals, and organic matter for growth, resulting in relatively low initial production costs. However, a series of substantial limitations hinders the use of naturally occurring enzymes in industrial biomass conversion. These enzymes often suffer from inadequate catalytic potency, operational inefficiency, and instability, particularly under the harsh conditions prevalent in industrial processes, thus rendering their application less reliable and efficient than that of their genetically engineered counterparts.[46,47] A key challenge is requiring diverse enzyme cocktails to achieve complete hydrolysis, complicating the preparatory technologies. Furthermore, industrial-scale operations typically involve elevated temperatures, which induce enzyme denaturation and aggregation, severely restricting the feasibility of these enzymes in large-scale applications.[48]

Scaling up enzymatic hydrolysis processes presents significant challenges. High-solid loading, crucial for industrial viability, reduces hydrolysis rates and yields due to the high-solid effect driven by water limitations within the biomass matrix, hindering enzyme diffusion and activity.[2,47] Additionally, the rheological properties of high-solid slurries impede mass and heat transfer, further diminishing process efficiency.[49] Logistical hurdles also complicate large-scale biomass conversion; low bulk density, widespread distribution, and seasonal variability of biomass feedstocks create transportation and storage difficulties, while fluctuations in biomass composition challenge supply chain consistency and feedstock predictability.[50]

3 Enzymatic catalysis in biomass conversion

Enzymes, which are proteins that catalyze nearly all biochemical reactions, are central to the conversion of LB. Without enzymatic catalysis, most biochemical reactions would occur so slowly that life, as we know it, would not be feasible.[51] Enzymes accelerate reactions by orders of magnitude—often more than a million-fold—enabling sluggish reactions to occur within a fraction of a second.[51] In the context of LB conversion, the efficient breakdown of raw biomass into fermentable sugars and high-value bioproducts depends on a coordinated action of multiple enzymes, each targeting and breaking down distinct bonds in complex lignocellulosic polymers.

Enzymes that degrade lignocellulose employ a variety of catalytic mechanisms to enhance their efficiency. Glycoside hydrolases (GHs), such as cellulases and hemicellulases, act primarily through inverting or retaining mechanisms; the latter proceeds *via* a two-step pathway involving the transient formation of a covalent glycosyl–enzyme intermediate.[52] Knott *et al.* demonstrated, through structural and computational analyses of GH Family 7 cellobiohydrolases, that the catalytic mechanism involves conformational changes in the nucleophile and a product-assisted deglycosylation step, in which cellobiose positions a water molecule to hydrolyze the glycosyl–enzyme intermediate.[52] Lignin-active enzymes, such as peroxidases and laccases, depend on metal cofactors like Cu^{2+} and Mn^{2+} to facilitate electron transfer, promoting radicalization and cleavage of lignin's aromatic bonds.[53,54] These catalytic mechanisms, combined with the modularity of lignocellulose-degrading enzymes, which often contain catalytic cores alongside non-catalytic domains such as carbohydrate-binding modules (CBMs), enable precise targeting and effective breakdown of the structurally complex lignocellulosic biomass.[39,55]

Despite these biochemical advances, enzymatic hydrolysis still faces significant barriers related to low catalytic turnover and high enzyme loading requirements. To address these limitations, protein engineering strategies have focused on the development of multifunctional fusion enzymes. Du *et al.* reported that a chimeric construct combining swollenin and xylanase from *Trichoderma reesei* significantly enhanced sugar release from LB, with domain orientation and linker flexibility playing crucial roles in catalytic performance. The optimized configuration (S-2×) led to a 42% increase in reducing sugars compared to xylanase alone and further improved yields when paired with endoglucanase.[55] In parallel, oxidative enzymes such as lytic polysaccharide monooxygenases (LPMOs) have emerged as critical components in next-generation enzyme cocktails. These copper-dependent catalysts cleave polysaccharides oxidatively by inserting oxygen at the C1 and/or C4

positions, thereby weakening the cellulose structure and enhancing accessibility.[56]

3.1 Mechanism of enzymatic catalysis

The efficacy of enzymes, including cellulases, β-glucosidases, and lytic polysaccharide monooxygenases (LPMOs), in the degradation of cellulose, hemicellulose, and lignin is contingent upon several catalytic mechanisms. These mechanisms encompass acid–base catalysis, wherein proton donors such as glutamic acid (Glu) and aspartic acid (Asp) stabilize transition states; covalent catalysis, which entails nucleophilic attacks that yield glycosyl–enzyme intermediates; and metal ion coordination, as exemplified by LPMOs that utilize Cu^{2+} to effectuate the oxidative cleavage of crystalline cellulose.[56–58] The efficacy of these processes is further augmented by carbohydrate-binding modules (CBMs) and the processive movement of enzymes along polysaccharide chains.[59,60] To enhance industrial efficiency, various strategies can be employed, including directed evolution to optimize catalytic residues, engineering stability across pH and temperature variations, optimizing electron donors for LPMOs, and developing synergistic enzyme cocktails.[61,62] Moreover, advancements in computational design, quantum mechanical (QM)/molecular mechanics (MM) simulations, and metagenomic mining offer promising avenues for the development of hyper-efficient enzymes specifically tailored for biofuel production, waste processing, and other large-scale applications.[63–65]

The enzymatic conversion of LB involves integrated steps combining structural disruption with molecular-level specificity. As depicted in Fig. 5, the complex architecture of the plant cell wall poses significant physicochemical barriers to enzyme accessibility.[29] As discussed earlier, various pretreatment strategies are employed to overcome this recalcitrance.[29]

These treatments partially disrupt the lignin–carbohydrate matrix, thereby increasing the porosity and solubility of hemicellulose and cellulose chains, which become more accessible to enzymatic attack.[66] Once exposed, specific enzymes (E) selectively recognize and bind to distinct structural domains by forming enzyme–substrate complexes (ES). This recognition begins with a specific adsorption step, where the substrate interacts with the enzyme's active site, a structurally defined region whose conformation and electrochemical properties confer high specificity. The interaction is governed by noncovalent forces—including hydrogen bonds, electrostatic interactions, van der Waals forces, and hydrophobic effects—which stabilize the substrate and orient it precisely for catalytic transformation.[39,51]

Following adsorption, enzymes facilitate the catalytic conversion of substrates by lowering the activation energy required to reach the transition state.[51] This is accomplished by stabilizing the high-energy intermediate configuration, accelerating the reaction rate under relatively mild processing conditions. After product formation, the E releases the product and returns to its initial state, remaining available for subsequent catalytic cycles. While enzymes enhance the kinetic rate of both forward and reverse reactions, they do not alter the thermodynamic equilibrium, which is determined solely by the intrinsic free energies of reactants and products.[51,67]

The molecular efficiency of enzymatic catalysis is intrinsically tied to the dynamics of substrate recognition. Classical models, such as the lock-and-key hypothesis, describe substrate binding as a rigid and geometrically complementary interaction. However, more nuanced theories, including the induced-fit model, propose that substrate binding induces conformational rearrangements in the enzyme, thereby optimizing the positioning of catalytic residues.[68] Conversely, the conformational selection model, known as the population-shift model, suggests that the enzyme pre-exists in multiple conformational states, with the substrate preferentially binding to the most catalytically competent form.[35,39,67,68] These mechanisms are particularly relevant for lignocellulolytic enzymes, such as cellobiohydrolases (CBH) and lignolytic enzymes, which often require flexible active sites to accommodate heterogeneous, high-molecular-weight substrates.[39,52,55]

Although these models are not mutually exclusive and may operate simultaneously or sequentially depending on the system, the precise mechanism of ligand binding remains an active area of research. Computational and structural studies

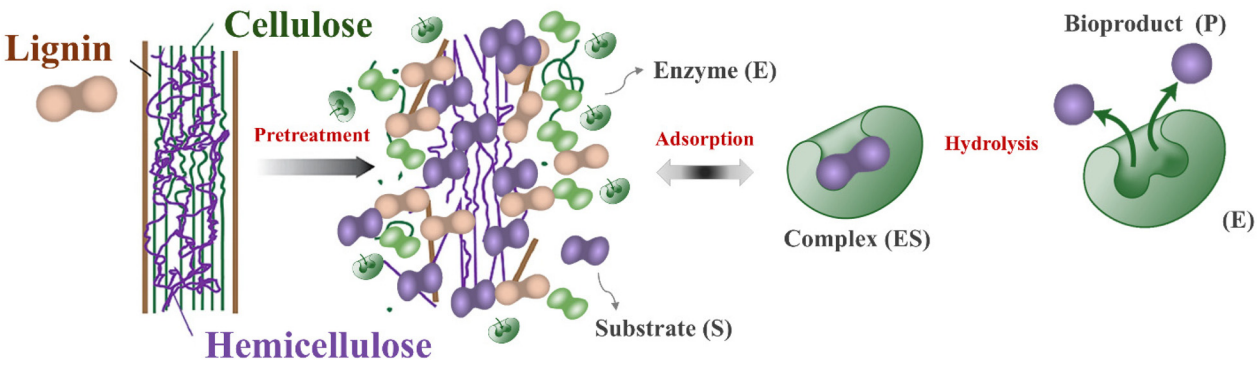

Fig. 5 Schematic representation of the enzymatic conversion of lignocellulosic biomass. Following pretreatment to disrupt the complex lignin–carbohydrate matrix, specific enzymes (E) adsorb onto accessible substrates (S), forming enzyme–substrate complexes (ES). Subsequent hydrolysis releases bioproducts (P), enabling the valorization of cellulose and hemicellulose into fermentable sugars or other valuable compounds.

have contributed significantly to this field, revealing that conformational plasticity is a feature of substrate binding, cofactor interaction, allosteric regulation, and inhibitor recognition.[34,69,70] Therefore, enhancing theoretical and computational ability to model these dynamic enzyme–ligand interactions is essential for improving the design and engineering of efficient biocatalysts for biomass valorization.[67,69,70]

3.2 Enzymes used in biomass conversion

Various types of enzymes are employed to target these different LB components, as outlined in Table 1. Among them, cellulases are the most commercially significant, given their central role in deconstructing crystalline and amorphous cellulose. The enzymatic degradation of cellulose is orchestrated by a synergistic system comprising *endo*-β-1,4-glucanases (EGs), cellobiohydrolases (CBHs, also referred to as exoglucanases), and β-glucosidases (BGs). EGs initiate the process by randomly cleaving internal β-1,4-glycosidic bonds within the amorphous regions of the cellulose polymer, generating new chain ends. Subsequently, CBHs act processively on these chain ends, releasing cellobiose units by hydrolyzing the glycosidic linkages near the crystalline regions. Finally, BGs catalyze the hydrolysis of cellobiose and other soluble oligosaccharides into glucose monomers. This coordinated enzymatic cascade effectively depolymerizes the recalcitrant cellulose structure into fermentable sugars suitable for downstream bioconversion processes.[31,71,72] Notably, BGs modulate cellulose hydrolysis under specific processing conditions, such as those involving ionic liquids, by relieving product inhibition and enabling continuous depolymerization. These enzymes are typically classified within glycoside hydrolase (GH) families such as GH1, GH5, GH9, and GH48.[73] Their synergistic interplay is essential to maximize conversion rates, especially in substrates with highly ordered microfibrillar structures.

In contrast to cellulose, the enzymatic deconstruction of hemicellulose presents a higher level of complexity due to its heterogeneous structure, which comprises a wide array of sugar monomers (*e.g.*, xylose, mannose, arabinose, and galactose) and diverse glycosidic and ester linkages.[31,72] This structural variability necessitates a broader and more specialized enzymatic arsenal, involving core hydrolases such as *endo*-xylanases, *endo*-mannanases, and an array of accessory enzymes. Among these, acetylxylan esterases (AXEs) are essential for removing acetyl substituents from the xylan backbone, a modification that increases substrate accessibility by exposing xylose residues for subsequent hydrolysis by xylanases. More broadly, esterases disrupt ester bonds that otherwise impede enzymatic access to the hemicellulosic matrix. Microbial xylan, a key hemicellulosic component particularly abundant in LB treated with *Trichoderma reesei*, *Penicillium funiculosum*, *Humicola insolens*, and select yeast strains, is a significant sub-

Table 1 Key enzymes involved in lignocellulosic biomass deconstruction, including cellulolytic, hemicellulolytic, ligninolytic, and oxidative auxiliary activities targeting plant cell wall components

Enzyme group	Enzyme class	Function	Ref.
Cellulose-degrading enzymes	EG	Hydrolyzes internal β-1,4-glycosidic bonds in cellulose	44 and 74
	CBH/EOG	Removes cellobiose units from the ends of cellulose chains	75–77
	BG	Converts cellobiose into glucose, alleviating feedback inhibition	74, 77 and 78
Hemicellulose-degrading enzymes	Endo-hemicellulases	Hydrolyzes internal bonds of hemicellulose polymers	75 and 79
	EXY	Cleaves internal β-1,4-xylosidic linkages in the xylan backbone	75–77
	EMN	Hydrolyzes β-1,4-mannosidic linkages in mannans and glucomannans	72 and 79
	AXE	Removes acetyl groups from acetylated xylan	44 and 79
	FAE	Hydrolyzes ester bonds between arabinoxylans and ferulic acid	79
Lignin-modifying enzymes	LiP	Oxidizes non-phenolic and phenolic lignin structures *via* radical formation	80–84
	MnP	Oxidizes Mn^{2+} to Mn^{3+}, which diffuses and oxidizes lignin	
	VP	Oxidizes Mn^{2+} and high-redox substrates; combines LiP and MnP activity	
	Laccases	Catalyzes one-electron oxidation of phenolic compounds using oxygen	
Auxiliary enzymes	P2O	Oxidizes pyranose sugars, producing H_2O_2	79 and 85–88
	LPMOs	Oxidative cleavage of polysaccharides using O_2 or H_2O_2 enhances GHs	
	CDH	Oxidizes cellobiose and transfers electrons to LPMOs	
	VAO	Oxidizes vanillyl alcohol to vanillin	
	CROs	Generates H_2O_2 by oxidizing alcohols/amines, assisting lignin breakdown	
	GOX	Produces H_2O_2 *via* glucose oxidation; supports peroxidases	
	GLOX	Generates H_2O_2 by oxidizing aldehydes; supports MnP and LiP	
	AAO	Oxidizes aryl alcohols to aldehydes, producing H_2O_2 for lignin degradation	

Abbreviations: EG (endoglucanases); (BG) β-glucosidases; CBH/EOG (exoglucanases); EXY (*endo*-xylanase); EMN (*endo*-mannanase); AXE (acetyl xylan esterase); FAE (ferulic acid esterase); LiP (lignin peroxidase); MnP (manganese peroxidase); VP (versatile peroxidase); P2O (pyranose-2-oxidase); LPMOs (lytic polysaccharide monooxygenases); CDH (cellobiose dehydrogenase); VAO (vanillyl alcohol oxidase); CROs (copper radical oxidases); GOX (glucose oxidase); GLOX (glyoxal oxidase); AAO (aryl alcohol oxidase).

strate for these hemicellulolytic enzymes.[30] These enzymes are primarily associated with GH families GH10 and GH11 and carbohydrate esterase (CE) families. Their concerted activity is indispensable for efficiently liberating pentoses, especially xylose and arabinose, crucial intermediates in industrial fermentations targeting bioethanol and xylitol production.[28] These enzymatic systems, detailed in Table 1, demonstrate the complexity and precision required for effective bioconversion of LB substrates.

Lignin depolymerization remains one of the primary bottlenecks in the efficient conversion of LB, mainly due to its heterogeneous, highly branched, and recalcitrant polymeric structure, characterized by a complex network of β-O-4 and C–C linkages among various substituted phenylpropanoid units.[89] The degradation of this intricate matrix necessitates the action of specialized oxidative biocatalysts, broadly categorized as lignin-modifying enzymes (LMEs). These include class II heme peroxidases—such as lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP)—and copper-containing oxidases, notably laccases.[17]

These enzymes operate through redox-based mechanisms involving high-valent metal intermediates and radical-mediated reactions, enabling the cleavage of phenolic and non-phenolic structures with the aid of mediators.[90–92] Laccases are noteworthy for their operational simplicity, as they use molecular oxygen as the terminal electron acceptor and can be applied under milder reaction conditions. When coupled with redox mediators (*e.g.*, ABTS, HBT, and violuric acid), laccases can extend their oxidative capacity beyond native phenolic substrates, facilitating the breakdown of complex lignin-derived molecules, industrial dyes, endocrine-disrupting compounds, and other environmental pollutants.[93,94] Their biological production is commonly associated with white-rot fungi such as *Trametes versicolor*, *Pleurotus ostreatus*, and *Pycnoporus sanguineus*, organisms known for their extensive ligninolytic systems and ecological role in wood decay.[82,95,96]

In addition to enzymatic strategies, physicochemical and integrated chemo-enzymatic routes have become complementary approaches for lignin valorization. These include: (i) solvent-assisted lignin solubilization, particularly using deep eutectic solvents (DESs) and ionic liquids (ILs),[97] which disrupt lignin–carbohydrate complexes and enhance enzymatic accessibility; (ii) oxidative pretreatments using mild Fenton chemistry or transition-metal catalysts[98] to introduce reactive sites for enzymatic attack; (iii) microbial consortia[98] capable of sequential or co-metabolic lignin conversion; and (iv) electrochemical or photocatalytic oxidation[99] which generates reactive oxygen species to initiate lignin bond cleavage and support downstream enzymatic transformations. Collectively, these enzymatic and hybrid approaches contribute to increasing the porosity and functional accessibility of the LB structure, thereby improving the efficiency of subsequent carbohydrate hydrolysis and enabling the integrated valorization of lignin into aromatic chemicals, platform molecules (*e.g.*, vanillin and syringaldehyde), and high-performance materials.

To support these core enzymatic systems, an array of oxidative auxiliary enzymes facilitates redox cycling and the generation of reactive oxygen species (ROS), which further destabilize the lignocellulosic matrix.[44] Among these, LPMOs catalyze oxidative cleavage of glycosidic bonds in crystalline cellulose, frequently in concert with electron donors such as cellobiose dehydrogenase (CDH).[100] Additional contributors include aryl alcohol oxidases (AAOs), glyoxal oxidases (GLOXs), pyranose-2-oxidase (P2O), vanillyl alcohol oxidase (VAO), copper radical oxidases (CROs), and glucose oxidase (GOX).[79,85,86] These enzymes are commonly found in auxiliary activity (AA) families of the CAZy classification and perform roles such as generation of H_2O_2 for peroxidase activation or modification of lignin-derived compounds to facilitate downstream catalysis.[85,101] The integration of these auxiliary systems significantly enhances the efficiency of primary enzymatic processes and is critical for robust performance under industrially relevant conditions.

3.3 Industrial feasibility of enzyme immobilization in biomass conversion

The industrial adoption of immobilized enzymes for biomass conversion hinges on balancing technical performance with economic and operational considerations. While covalent binding and MOF-based systems demonstrate superior stability (>20 cycles) and selectivity in lab studies, their scalability is often limited by high carrier costs (>$500 per kg for MOFs) and complex reactor requirements.[102] In contrast, magnetic nanoparticles and cross-linked enzyme aggregates (CLEAs) have reached higher technology readiness levels (TRL7–8),[103] with demonstrated success in commercial cellulosic ethanol plants (*e.g.*, 1 ton per day continuous systems in Brazil).[104] Key challenges include (1) reducing enzyme production costs to < $15 per kg through improved immobilization yields,[105] (2) developing standardized recovery protocols to minimize carrier loss, and (3) integrating immobilized systems with existing pretreatment infrastructure. Recent advances in continuous-flow microreactors and 3D-printed scaffolds show promise for reducing capital expenditures by 30–40%,[106] while hybrid approaches (*e.g.*, magnetic CLEAs) address the activity–stability trade-off.[107] Regulatory compliance (*e.g.*, FDA leaching limits <0.1 ppm) and life cycle assessments confirm that immobilized systems can reduce water/energy use by 40–60% *versus* free enzymes, though further optimization is needed for lignocellulosic applications where substrate heterogeneity remains a bottleneck.[108–110]

3.4 Effect of pH and temperature on enzymatic catalysis

The enzymatic conversion of LB depends on the operational stability and activity of enzymes under specific physicochemical conditions. As illustrated in Fig. 6, the optimal performance of cellulolytic enzymes typically occurs within moderate acidic pH (4.5–6.0) and elevated temperature ranges (45–60 °C), which facilitates the hydrolysis of crystalline and amorphous cellulose regions.[77,111] Hemicellulases, often derived from thermophilic microorganisms, exhibit greater

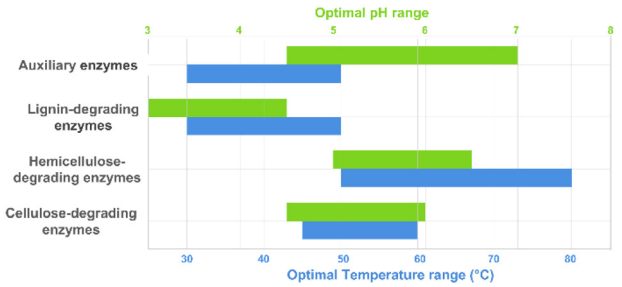

Fig. 6 Optimal pH and temperature ranges for major enzyme groups involved in LB conversion.

thermal tolerance (up to 80 °C), expanding their applicability in high-temperature bioreactor configurations.[112] In contrast, LMEs display activity under more acidic conditions (pH 3.0–4.5) and moderate temperatures (30–50 °C), reflecting their evolutionary adaptation to fungal oxidative environments. Auxiliary enzymes, including LPMOs, oxidases, and dehydrogenases, present broader pH flexibility (4.5–7.0) but similarly moderate thermal optima (30–50 °C), enabling synergistic interactions with core hydrolases and enhancing overall biomass deconstruction.

Understanding these distinct operational windows is essential for rationalizing the design of enzymatic cocktails and optimizing biorefinery conditions. Inadequate alignment between enzymatic activity profiles and processing parameters can compromise catalytic efficiency, often resulting in enzyme inactivation or non-productive interactions, reinforcing the importance of enzyme engineering and environmental control.

4 Immobilization technology

Immobilization is a natural phenomenon observed in various environmental contexts. Microorganisms in nature are often unevenly distributed, forming biofilms—complex microbial communities that adhere to surfaces and consist of multiple layers of cells embedded within hydrated matrices.[113] Biofilms have been studied extensively since the 1940s, but their ubiquity across nearly all natural environments was not fully recognized until the 1970s. Examples of biofilm formation can be found on submerged rocks, medical implants, teeth, water pipes, and other surfaces.[114] This natural phenomenon has inspired human exploration of its potential applications. An immobilized molecule is one whose spatial movement is restricted, either entirely or confined to a specific area, due to its attachment to a solid structure. The term "immobilization" typically refers to the process of limiting or halting molecular mobility, thereby effectively slowing or stopping movement.[50]

4.1 Overview of biomolecule immobilization

Biomolecules have become central to immobilization strategies due to their broad applications across biomedical, biotechnological, and biomass valorization fields.[115] Among them, biomolecules—such as lipids, proteins, enzymes, and nucleic acids—constitute the structural and functional backbone of cellular systems, participating in diverse biochemical and physiological pathways. As illustrated in Fig. 7, this molecular diversity has been systematically explored for immobilization, reflecting their central role in biosensing, drug delivery, and the development of bioinspired materials.

In drug delivery applications, immobilization techniques allow precise control over release kinetics, improving therapeutic efficacy and reducing burst release, as demonstrated by the encapsulation of antibiotics in mesoporous carriers embedded in electrospun polymeric matrices.[115] In biosensing, immobilizing ligands such as avidin and biotin onto nanostructured substrates has enabled high-affinity detection platforms. In particular, gold nanoparticle (AuNP)-modified graphene sheets have shown enhanced biocompatibility and electrical conductivity, facilitating the stable and sensitive immobilization of recognition probes on field-effect transistor (FET)-based sensors.[115–118] Similar advances have been achieved with nucleic acids, where immobilization strategies using DNA nanostructures and functional nanomaterials have allowed controlled probe spacing and improved signal amplification in electrochemical and optical sensors.[119] Peptides, especially antimicrobial peptides, have also been immobilized on various surfaces, such as gold nanoparticles and medical devices, to combine antibacterial activity with pro-angiogenic functions while minimizing cytotoxicity.[120]

However, immobilization may modulate their bioactivity and cellular signaling.[120] For proteins, including enzymes, antibodies, and serum proteins, immobilization has been extensively explored using supports like graphene oxide, reduced graphene oxide, and polymer-coated magnetic nanoparticles. These offer high surface area and functionalization

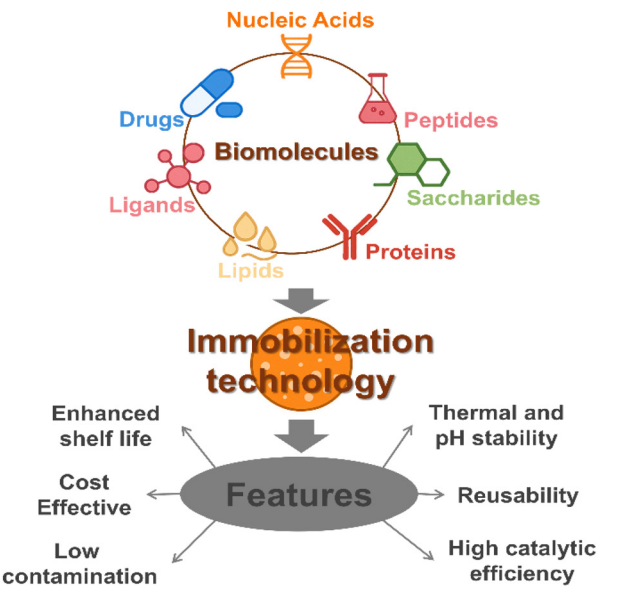

Fig. 7 Major classes of biomolecules commonly immobilized and their typical applications.

potential for physical and covalent attachment.[121,122] Conversely, lipids are immobilized to mimic cellular membranes and support biocompatible interfaces in biosensors and drug formulations.[123] Additionally, saccharides have gained prominence in immobilization systems due to their structural versatility. Galactooligosaccharides, for example, can be synthesized using immobilized microbial cells that express β-galactosidase, showing increased thermal stability, high conversion efficiency, and operational reusability for up to 20 cycles.[124] Beyond biological molecules, immobilization strategies have also proven effective in the development of solid acid catalysts, such as zirconia-modified halloysite nanoclays, enabling efficient monosaccharide conversions into value-added chemicals like 5-hydroxymethylfurfural with high yield and recyclability.[125]

Altogether, the growing sophistication of immobilization techniques—whether for therapeutic, diagnostic, or catalytic purposes—underscores their transformative role in advancing biotechnological platforms. The careful selection of immobilization methods and support materials ensures biomolecule functionality and stability and drives innovation across a broad spectrum of applications aligned with efficiency, reusability, and sustainability principles.

4.2 Immobilization methods of enzymes

Immobilization strategies are broadly classified into physical and chemical methods, as illustrated in detail in Fig. 8.[8,10,112,126] Physical techniques such as adsorption and entrapment rely on non-covalent forces, including hydrogen bonding, electrostatic interactions, and van der Waals forces, which render the process simpler and often reversible.[8,126] Adsorption is among the most straightforward methods, enabling enzyme attachment to carriers like activated carbon, ion-exchange resins, and alumina.[8,40,41] Despite its cost-effectiveness, enzyme desorption can occur under fluctuating pH or ionic strength, limiting long-term operational stability.[126] Conversely, entrapment confines enzymes within porous or polymeric materials, offering greater protection and improved resistance to environmental changes. Notable advances in this category include the use of mesoporous MOFs such as PCN-888 and PCN-333, which allow spatial compartmentalization of multiple enzymes, thereby enabling cascade reactions and *in situ* cofactor regeneration.[127,128]

Microencapsulation technologies further expand the scope of physical immobilization by creating semi-permeable microenvironments that enhance enzyme stability and allow controlled molecular diffusion. Hydrogels, sol–gels, and polymersomes are frequently used to develop core–shell structured microcapsules capable of hosting enzymes for complex reactions like biomass deconstruction.[129] However, these techniques must contend with mass transfer limitations depending on the architecture and porosity of the capsule matrix.[8,41,130]

Chemical immobilization methods (see Fig. 8), including covalent bonding and cross-linking, provide stronger enzyme–support interactions, resulting in robust biocatalysts suitable for industrial conditions. Covalent attachment typically involves coupling enzymes to supports *via* bifunctional agents such as glutaraldehyde, carbodiimides, or silanes. While this improves enzyme retention and activity under extreme pH or temperature conditions, the process requires precise control to avoid losing enzymatic functionality.[131,132] Cross-linked enzyme aggregates (CLEAs) and crystals (CLECs) represent a support-free strategy with high enzyme packing density and operational stability. The emergence of Multi-CLEAs and

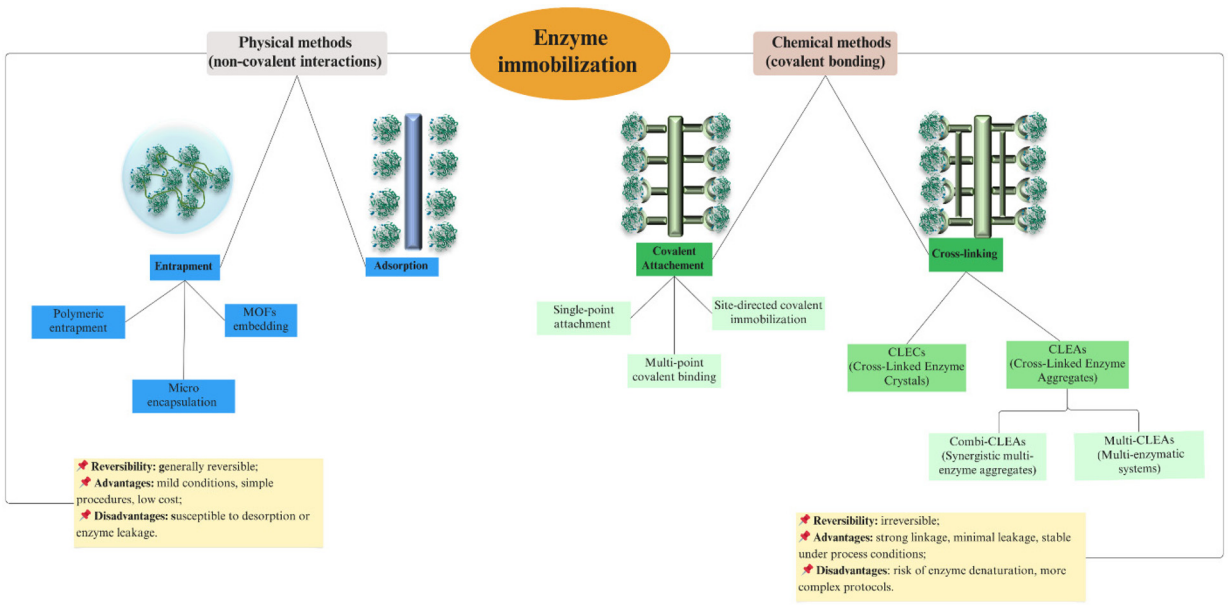

Fig. 8 Classification of enzyme immobilization methods based on the nature of the interaction between the enzyme and support.

Combi-CLEAs has proven especially valuable in LB degradation due to the synergistic action of multi-enzyme systems.[131,132] Recent developments in polymer chemistry have led to the design of biohybrid materials through layer-by-layer deposition, emulsion polymerization, and polymerization-induced self-assembly. These strategies leverage amphiphilic block copolymers and micellar systems to create tailored nanoscale environments, improving enzyme orientation, mass transfer, and compatibility with aqueous–organic systems.[133] Polyamide-based materials have gained interest as greener immobilization platforms, avoiding toxic reagents while maintaining high enzyme entrapment and long-term stability.[131]

4.3 Immobilized enzyme systems for LB conversion

Enzyme immobilization has emerged as a cornerstone strategy in LB valorization, offering solutions to the limitations associated with free enzymes, such as thermal instability, sensitivity to pH and solvents, and challenges in recovery and reuse. Immobilized biocatalysts enable enzyme recovery *via* centrifugation, filtration, or magnetic separation, enhancing activity retention, storage stability, and tolerance to harsh operational environments.[5,134,135] The immobilization approach—from physical adsorption and entrapment to covalent attachment—must be tailored to enzyme characteristics and process conditions, such as pH, temperature, and solvent compatibility.[136,137]

Among physical techniques, adsorption and entrapment remain attractive due to their simplicity and mild operational conditions. However, they often suffer from enzyme leakage and limited mechanical stability. Though more complex, covalent immobilization strategies offer stronger binding, reduced leaching, and better operational durability. Recent studies exemplify the importance of support architecture and chemistry in maximizing enzymatic performance. For instance, Costantini *et al.* demonstrated that cellulase immobilized on wrinkled silica nanoparticles (WSNs) with tailored inter-wrinkle spacing retained ~80% of its catalytic performance over five hydrolysis cycles, underscoring the role of nanostructured matrices in preserving enzyme conformation and accessibility.[138] Likewise, Amari *et al.* reported the immobilization of laccase on magnetic MOF supports (Fe_3O_4-NH_2@MIL-101(Cr)), achieving 88% residual activity after 28 days and notable dye degradation performance, driven by synergistic sorption and catalytic mechanisms.[139]

Entrapment-based systems also hold promise, especially with biopolymers like alginate and chitosan. Ortega *et al.* showed that β-glucosidase entrapped in polyacrylamide gels maintained kinetic behavior like its free form, while alginate-induced substrate inhibition, indicating mass transfer limitations inherent to some polymeric matrices.[140]

The immobilization of lipases for biodiesel synthesis has advanced through microencapsulation techniques. Guzmán-Martínez *et al.* successfully applied a jet break-up method to entrap lipases in Ca^{2+}-alginate beads, yielding up to 95% fatty acid ethyl esters (FAEEs) from *Jatropha curcas* oil, meeting international biodiesel standards (ASTM D6751, EN14214).[141]

To summarize the comparison between the immobilization techniques, physical adsorption and entrapment offer cost-effective, mild-condition solutions but often compromise stability due to enzyme leakage. Covalent immobilization, though more expensive, ensures superior durability (*e.g.*, 80% activity retention over 5 cycles for cellulase on WSNs; 88% for laccase on MOFs after 28 days) and reusability, while entrapment in biopolymers (*e.g.*, alginate) balances simplicity and performance but may introduce mass transfer limitations. Advanced supports like MOFs and functionalized silicas enhance catalytic efficiency (*e.g.*, 74% bagasse conversion; 359.89 mg g^{-1} enzyme loading) and stability (>70% activity after 10 cycles), whereas microreactor systems excel in rapid conversions (88% cellulose in 1 hour) and scalability. Cross-linked aggregates (CLEAs) and co-immobilized systems further boost synergistic action and stress resilience. Overall, covalent methods and engineered supports dominate for high-value applications, while physical techniques suit cost-sensitive, batch processes. Table 2 provides a detailed comparison of different enzyme immobilization techniques, summarizing their advantages, disadvantages, optimal use cases, performance metrics, and cost considerations.[142–151]

Support selection and design play a critical role beyond immobilization chemistry. Biocomposite and inorganic materials, such as glyoxyl agarose, metal–organic frameworks (MOFs), and functionalized silicas, contribute to enhanced mass transfer, enzyme loading, and operational robustness. These materials have been particularly valuable in continuous processing systems and microreactors, where immobilized enzymes contribute to high substrate turnover under mild and scalable conditions.[152–154] Encapsulation strategies, including enzyme entrapment in porous hydrogels and nanostructured matrices, further offer protective microenvironments that reduce diffusional resistance and maintain enzyme structure. These approaches have proven effective in immobilizing laccases, xylanases, and chitosanases, especially when exposed to lignin derivatives, salts, or organic co-solvents.[155,156]

Table 3 compiles recent advances in enzyme immobilization technologies applied to biomass conversion, showcasing a diverse range of enzyme–support configurations and process outcomes. Cellulases covalently immobilized on IOMNP@SiO_2–NH_2 nanocarriers achieved a 74.19% conversion yield from sugarcane bagasse with stable reuse performance over multiple cycles.[157] Immobilization on Fe_3O_4@ZIF-8-NH_2 enabled high enzyme loading (359.89 mg g^{-1}) and >70% activity retention after ten reuse cycles.[158] Costantini *et al.* demonstrated that WSNs facilitated excellent glucose yields with minimal enzyme deactivation.[130] In lignin bioconversion, co-immobilized laccase and peroxidase on magnetic silica microspheres improved vanillin yields 1.2-fold, and laccase immobilized on agarose beads retained 80.43% of its activity over 45 days, significantly enhancing lignocellulose digestibility.[90,159] In microreactor systems, laccase co-deposited with $CuSO_4$, H_2O_2, and DOPA achieved 88.1% cellulose conversion within one hour, aided by vanillin as a co-substrate.[160] Similarly, microreactor-based cellulase immobilized

Table 2 Advanced comparison of enzyme immobilization methods for biomass valorization

	Immobilization method				
Characteristic	Physical adsorption	Covalent binding	Entrapping method	Encapsulation	Cross-linking method
Preparation	Easy	Difficult	Difficult	Easy	Difficult
Enzyme activity	Low	High	High	High	Moderate
Optimal enzymes	Delicate enzymes (lipases, some glycosidases)	Robust enzymes (cellulases, laccases)	Multi-enzyme systems	Multi-enzyme systems	Oxidoreductases, hydrolases
Substrate specificity	Unchangeable	Changeable	Unchangeable	Unchangeable	Changeable
Binding force	Weak	Strong	Strong	Moderate	Strong
Regeneration	Possible	Impossible	Impossible	Possible	Impossible
General applicability	Low	Moderate	High	Moderate	Low
Cost of immobilization	Low	High	Low	Low	Moderate
Key advantages	• Simple procedure • Preserves enzyme activity	• High stability (15–20 cycles) • Minimal enzyme loss	• Protects enzymes from harsh conditions	• Protects enzymes from harsh conditions	• Carrier-free • High activity recovery
Limitations	• Low stability (3–5 cycles) • Enzyme leakage	• Harsh conditions may reduce activity	• Mass transfer limitations • Low loading capacity	• Mass transfer limitations • Low loading capacity	• Irregular particle size • Aggregation issues
Optimal use cases	• Lab-scale research • Sensitive enzymes	• Industrial processes • Robust enzymes (cellulases)	• Whole-cell systems • Multi-enzyme cascades	• Whole-cell systems • Multi-enzyme cascades	• Multi-enzyme systems • Biodiesel production
Performance metrics	• 30–60% activity retention • Fast immobilization	• 70–95% initial activity • Low leakage (<5%)	• 50–80% yield • Moderate reusability (5–8 cycles)	• 50–80% yield • Moderate reusability (5–8 cycles)	• 60–85% stability • 10–15 reuse cycles

via dopamine–PEI coating demonstrated a 97.2% increase in glucose productivity and retained 84.4% of its activity after 20 days.[161]

Hemicellulose valorization has also benefited from advances in immobilization. Xylanases immobilized on Cu-MOFs or glyoxyl-activated agarose supports showed high xylooligosaccharide (XOS) yields, superior thermal stability, and reduced byproduct formation.[144,162] Purolite-supported MpXyn10 maintained over 80% conversion efficiency after six reuse cycles.[163] Multifunctional systems, such as β-glucosidase and endoglucanase co-immobilized on Fe_3O_4 nanoparticles, revealed synergistic action and enhanced catalytic throughput.[164] Furthermore, cross-linked enzyme aggregates (Combi-CLEAs) combining GOx and HRP formed hollow nanoscale aggregates with high redox activity and improved resilience to oxidative stress.[41] Additional systems include engineered xylanases (XylCg) covalently bound to silica nanoparticles, achieving 99.5% xylan conversion and a fourfold increase in thermal stability;[165] YADH immobilized on capped amine-functionalized resins with 94% activity after 20 reuse cycles;[166] and β-galactosidase immobilized on halloysite–cellulose nanocrystals or silica nanospheres, demonstrating extended shelf-life and catalytic enhancement under shifted pH and temperature conditions.[167,168]

Immobilized oxidative enzyme systems have shown remarkable potential in biocatalysis and the selective degradation of complex aromatic pollutants and lignin-derived residues.[121] From a biomass processing perspective, removing or modifying lignin is a crucial step to enhance the accessibility of cellulose and hemicellulose for downstream enzymatic saccharification. Encapsulated oxidative enzymes have further demonstrated resilience in removing endocrine-disrupting compounds (EDCs) and lignin-like aromatic structures under environmentally relevant conditions. For instance, laccase from *Myceliophthora thermophila* encapsulated in sol–gel matrices retained 80% of its catalytic activity after ten cycles and was capable of removing up to 85% of model EDCs, reinforcing the relevance of immobilized enzyme systems in both environmental biotechnology and biomass valorization pipelines.[43,139] Lipase microcapsules produced *via* jet break-up exhibited high esterification efficiency from unrefined feedstock and feasibility for continuous-scale applications in biodiesel production.[141]

Altogether, these findings reinforce the central role of enzyme immobilization in designing robust, scalable, and process-resilient biocatalysts. As lignocellulosic biorefineries evolve toward greater integration and environmental compliance, immobilized enzyme systems will remain pivotal in driving efficient biofuel and biochemical production from diverse biomass sources.

4.4 Technological and economic advantages of enzyme immobilization

The performance of immobilized biocatalysts can be assessed by comparing hydrolysis rates or reaction kinetics relative to their

Table 3 Recent advances in enzyme immobilization techniques applied to biomass

Enzyme(s)	Immobilization technique	Support material	Substrate	Key findings	Ref.
Cellulase (Cellic CTec2)	Covalent bonding	IOMNP@SiO_2–NH_2	Sugarcane bagasse	74.19% yield; 52.6% retained activity after 3 cycles	157
Cellulase	Adsorption	Fe_3O_4@ZIF-8-NH_2	Cellulose	359.89 mg g^{-1} loading; 71.03% activity after 10 cycles	158
		WSNs	CMC	High reusability: ~80% glucose yield retained after 5 cycles	138
Laccase + VP	Co-immobilization	Magnetic silica microspheres	Lignin	1.2× vanillin yield *vs.* free enzymes	149
Laccase	Adsorption + crosslinking	Agarose beads	Crop residues	80.43% activity after 45 days; improved digestibility	90
	Co-deposition (DOPA/H_2O_2/$CuSO_4$)	Microreactor	Lignocellulose + vanillin	88.1% cellulose conversion in 1 h; 47.3% increase in accessibility	150
Cellulase	Co-deposition	DOPA + PEI (microreactor)	CMC	97.2% increase in glucose productivity; 84.4% activity after 20 days	161
Xylanase	Covalent bonding	Cu-BTC MOF	Xylan	High XOS yield; low xylose contamination	154
		Glyoxal-activated agarose	Birchwood xylan	8600-fold increase in thermal stability; 23% XOS yield in 24 h	162
Xylanase (MpXyn10)		Purolite	Hydrothermal liquor of eucalyptus wood	>80% XOS yield after 6 cycles	163
β-Glucosidase + Endoglucanase		Fe_3O_4 nanoparticles	CMC and pNP-Glc	Synergistic action; high catalytic efficiency	164
GOx + HRP	Cross-linked enzyme aggregates (Combi-CLEAs)	Self-aggregated	Glucose + ABTS	Hollow spheres (~250 nm); 10.5 μM min^{-1} rate; 81.3% activity in catalase presence	38
Xylanase (XylCg)	Covalent bonding (engineered lysines)	SiO_2 nanoparticles	Xylan	4× thermal stability; 99.5% yield; 135% efficiency for mutant	165
YADH	Covalent bonding	Amine-functionalized resin (capped)	Ethanol + Furfural	94% activity after 20 cycles; enhanced longevity and reuse	166
β-Galactosidase	Adsorption + entrapment	Halloysite + cellulose nanocrystals	Lactose	75.8% activity after 60 days; pH and T shift to 7.5 and 55 °C	167
	Entrapment in silica nanospheres	Silicon support		3.5× activity boost; 80% activity after 10 days	168
Laccase	Covalent + adsorption	Fe_3O_4-NH_2@MIL-101 (Cr)	Reactive black 5 and alizarin red S dyes	88% retained after 28 days; 49% activity at 85 °C; 92% and 73% dye removal after 5 cycles	139
Laccase (*M. thermophila*)	Sol–gel encapsulation	MTMS/TMOS	ABTS, dyes, estrogens	80% retained activity after 10 cycles: up to 85% EDC removal	43
Lipase	Microencapsulation (jet break-up)	Ca^{2+}-alginate beads	*Jatropha curcas* L. oil	~95% FAEE yield	141

Abbreviations: ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); CMC: carboxymethyl cellulose; DOPA: dopamine; EDC: endocrine disrupting chemicals; FAEE: fatty acid ethyl ester; Fe_3O_4: magnetite (iron(II,III) oxide); Fe_3O_4-NH_2@MIL-101(Cr): amino-functionalized iron oxide integrated with MIL-101(Cr) MOF; GOx: glucose oxidase; HRP: horseradish peroxidase; IOMNP: iron oxide magnetic nanoparticles; MOF: metal–organic framework; MpXyn10: *endo*-1,4-β-xylanase from *Malbranchea pulchella*; MTMS: methyltrimethoxysilane; PEI: polyethyleneimine; SiO_2: silicon dioxide; SiO_2–NH_2: amino-functionalized silica; TMOS: tetramethoxysilane; VP: versatile peroxidase; WSNs: wrinkled silica nanoparticles; XOS: xylooligosaccharides; XylCg: xylanase from *Chaetomium globosum*; YADH: yeast alcohol dehydrogenase.

free counterparts, with enzyme loading playing a decisive role in catalytic output.[169] Adsorption isotherms assist in determining the equilibrium between bound and unbound enzymes, helping to define the maximum hydrolytic potential.[170] In parallel, using crude enzymatic extracts in biomass hydrolysis offers economic advantages by lowering production costs. In systems involving complex substrates and synergistic enzyme actions, such as those combining β-glucosidases, xylanases, and esterases, crude preparations often outperform purified single enzymes. Additionally, the physicochemical properties of each biomass type can significantly influence overall enzymatic performance.

Standard reactor configurations employed in enzyme-catalyzed processes include stirred-tank reactors (STRs), packed-bed, fluidized-bed, retentostat, and membrane reactors, each offering distinct operational profiles and material compatibility.[83] Among these, STRs are widely used but often result in considerable operational costs due to enzyme loss and inactivation. The repeated need for fresh enzyme additions significantly impacts process economics (Fig. 9a).[171] In contrast, enzyme immobilization provides a powerful alternative by effectively separating and reusing biocatalysts across multiple cycles, thereby reducing enzyme consumption and production costs, as illustrated in Fig. 9b.[121] When immobilized onto suitable carrier matrices, enzymes exhibit improved catalytic stability and selectivity, enhancing reusability and overall robustness of the process.[134]

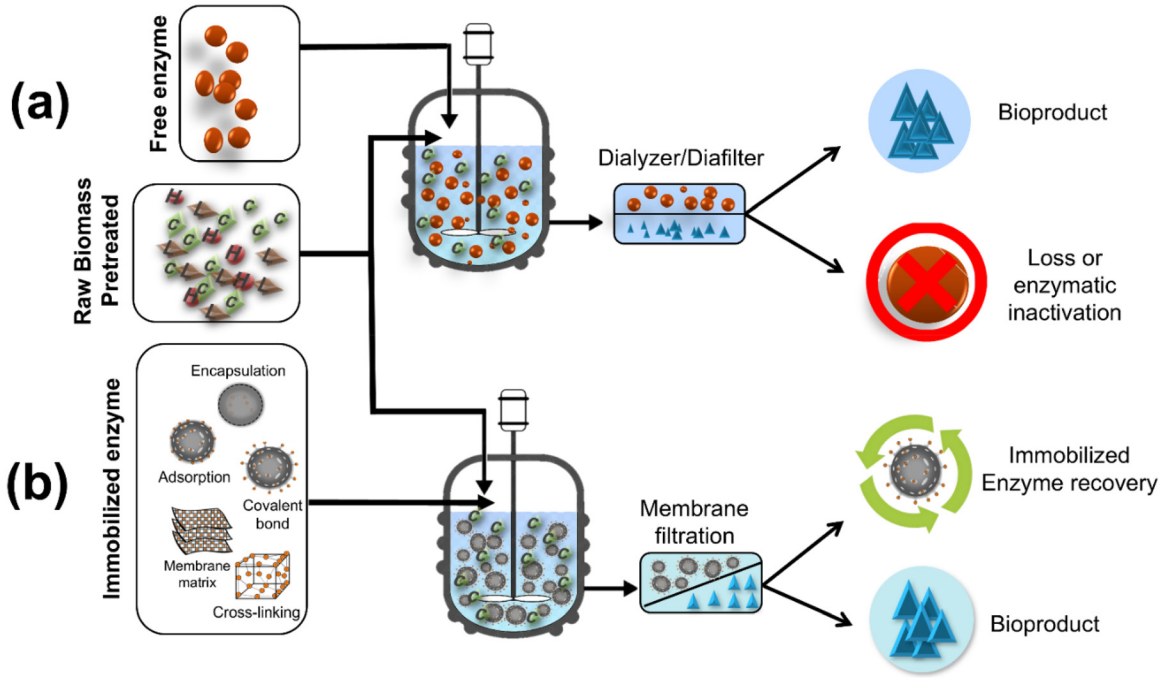

Fig. 9 Schematic comparison between the use of free enzymes and immobilized enzymes in biocatalytic processes. (a) Use of free enzymes leads to product formation, but enzyme loss or inactivation occurs during downstream processing, requiring frequent replacement and increasing operational costs. (b) Immobilized enzymes retained *via* strategies such as encapsulation, adsorption, covalent binding, or cross-linking enable improved recovery and reuse through membrane filtration, reducing enzyme loss and enhancing overall process sustainability and cost-effectiveness.

Immobilized enzyme systems have attracted growing interest for their superior operational stability and ease of recovery. Various immobilization strategies are tailored to different reactor designs and substrate types. In STRs, mechanical shear may cause enzyme particle attrition, whereas packed-bed and fluidized-bed reactors offer more stable environments. Packed beds are ideal for continuous operation but are susceptible to clogging when processing insoluble LB.[172] Fluidized beds provide better flow dynamics with solid-containing media, though enzyme retention becomes critical. Recent innovations, such as rotating bed and perfusion basket bioreactors, address these challenges by reducing mechanical stress and enzyme washout.[7,83]

Magnetic reactors further support enzyme recovery by enabling spatial control of enzyme supports under external magnetic fields, offering process flexibility and minimizing losses.[173] Microfluidic immobilized enzyme reactors (μ-IMERs) have emerged as a next-generation solution for high-efficiency, small-scale biocatalysis. With high surface-to-volume ratios and precise control over residence time, pH, and temperature, these systems reduce enzyme denaturation while enhancing mass transfer. Their modular nature facilitates scale-out and scale-up, enabling industrial applications in continuous hydrolysis and synthesis.[174,175] Additionally, immobilization can be extended to whole-cell biocatalysts, further enhancing catalytic efficiency, selectivity, and reusability in preparative fermentations. These systems offer operational flexibility and have been scaled for high-density processes with successful integration into continuous flows.[172]

The key advantages of enzyme immobilization are as follows:

i. **Rapid reaction termination** – immobilized enzymes can be readily removed from reaction mixtures, enabling precise control of process timing.

ii. **Prevention of product contamination** – as enzymes are bound to a support, they do not mix with or contaminate the final product, ensuring purity in sensitive industries like food and pharmaceuticals.

iii. **Simplified enzyme separation** – this facilitates enzyme removal post-reaction, streamlines purification, and reduces the risk of residual catalyst contamination.

iv. **Improved enzyme stability** – immobilized enzymes exhibit increased resistance to harsh operational conditions, prolonging functional lifespan and expanding their industrial applicability.

v. **Cost-effectiveness and sustainability** – multi-cycle reuse lowers enzyme consumption and contributes to greener, more economical manufacturing practices.

vi. **Enhanced reaction efficiency** – optimized enzyme orientation and reduced denaturation lead to better catalytic performance and higher product yields.

These developments underscore the critical role of enzyme immobilization in advancing industrial biocatalysis. By combining technological innovation with economic and environmental advantages, immobilization strategies continue to enable more sustainable, efficient, and scalable processes for bioproduct synthesis.

4.5 Drawbacks of enzyme immobilization

While enzyme immobilization has significantly advanced biocatalysis by improving operational stability, reusability, and process control,[176] it introduces technical and economic barriers that cannot be overlooked. Despite the catalytic advantages of free enzymes, their low stability, high production costs, and poor recovery profiles have driven the development of immobilization strategies.[177] However, immobilization often leads to partial loss of enzyme activity, diffusion limitations, enzyme leaching, and structural instability, particularly when covalent agents like glutaraldehyde are used.[41,178] Moreover, scaling up immobilization remains costly and complex, especially considering the need for robust carrier production and reactor adaptation.[179]

Diffusion constraints, particularly in porous or overloaded systems, further reduce catalytic efficiency. While nanocarrier surface modification and low protein loading strategies have been proposed, they introduce new complexities and do not entirely eliminate mass transfer resistance.[180] Co-immobilization of multiple enzymes, although promising for cascade reactions, often faces antagonistic interactions and spatial misalignment, complicating real-world applications. Applying immobilized cellulases to raw biomass hydrolysis highlights these limitations: substrate insolubility, viscosity, and restricted diffusivity sharply reduce enzymatic performance.[130,181] Although immobilization enhances resistance to pH, temperature, and solvent fluctuations, the initial cost, diffusion barriers, and potential toxicity of support materials remain serious obstacles.[172] Advances in support design, enzyme engineering, and co-immobilization are being pursued, but universal solutions remain elusive.[41,178] Consequently, in many industrial settings, the use of crude, unpurified enzymes is still preferred to balance catalytic efficiency and economic feasibility.[179] Improving enzymatic catalysis thus requires enhanced activity and effective stabilization strategies,[182] including buffer optimization and incorporation of protective agents such as SDS, BSA, DTT, gramicidin, and heat shock proteins.[113] A critical, case-specific approach is essential to ensure that immobilization contributes meaningfully to process efficiency rather than introducing new bottlenecks.

The efficacy of enzymes such as cellulases, β-glucosidases, and lytic polysaccharide monooxygenases (LPMOs) in industrial applications is contingent upon their catalytic mechanisms, which encompass acid–base catalysis, the formation of covalent intermediates, and metal-ion redox reactions, along with their substrate binding and processivity.[57–59] However, the immobilization of these enzymes—achieved through attachment to solid supports to enhance reuse or stability—entails specific trade-offs. These trade-offs include mass transfer limitations that impede substrate diffusion to active sites, as well as conformational constraints that restrict enzyme flexibility and potentially diminish catalytic activity.[40,129] While immobilization improves reusability and stability across varying pH and temperature conditions, and facilitates product separation, it may also induce inefficiencies. Therefore, the optimization of support materials, enzyme orientation, and pore structure is essential to reconcile catalytic performance with practical applications in biorefineries and waste valorization processes.[130,183]

5 Immobilized enzyme-based bioconversion of LB into biofuels

Enzyme immobilization has become a key strategy in LB conversion, enhancing stability, reusability, and process efficiency for more sustainable biofuel production.[40,41,184–186] Recent developments have revealed a marked shift toward the integration of nanomaterials, particularly MNPs, as advanced supports for immobilization. For instance, lipase from *Bacillus subtilis*, covalently immobilized onto Fe_3O_4 nanoparticles, retained high catalytic activity (58 U mg^{-1}) under alkaline and thermal conditions (60 °C), and achieved a biodiesel yield of approximately 45% within one hour, underscoring its robustness and potential for continuous biotransformations.[185] Similarly, Khanpanuek *et al.* showed that bacterial cellulose, with its high surface area and biocompatibility, has been used to co-immobilize α-amylase, glucoamylase, and *Saccharomyces cerevisiae*, enabling efficient simultaneous saccharification and fermentation (SSF) of cassava pulp.[187] This system not only sustained repeated-batch operations but also achieved an ethanol yield of 0.28 g/g of cassava pulp and a saccharification efficiency of 0.74 g glucose/gCP, outperforming conventional SSF methods.[188]

The potential of immobilization extends beyond bacterial systems to fungal platforms. Alabdalall *et al.* demonstrated that cellulases from *Aspergillus niger* and *A. flavus*, when immobilized and applied to coffee pulp, maintained consistent carboxymethyl cellulase (CMCase) activity over six weeks, while co-immobilized *S. cerevisiae* enhanced ethanol production up to 71.39 mg mL^{-1}.[176] These findings underscore the resilience and scalability of immobilized fungal systems under semi-solid fermentation conditions.

Nanotechnology has further accelerated the functional versatility of immobilized enzymes. Ariaeenejad *et al.* developed a hybrid nano-biocatalyst by anchoring a hydrolytic enzyme cocktail onto dopamine-functionalized cellulose nanocrystals coated with Fe_3O_4 (DA/Fe_3O_4NPs@CNCs).[177] This construct maintained over 50% of its initial activity after ten cycles and achieved up to a 76% increase in saccharification yields on substrates such as rice straw and sugar beet pulp.[177] Building on this, Kong *et al.* reported the co-immobilization of commercial cellulase onto Fe_3O_4@SiO_2–APTES nanoparticles in conjunction with a recombinant *S. cerevisiae* strain engineered to co-express laccase and versatile peroxidase. Operating under high-solid SSF conditions (30% DW, w/w), the system reached an ethanol titer of 79.5 ± 4.3 g L^{-1}, corresponding to 88.2% of the theoretical yield, while minimizing enzyme loading.[177] These hybrid strategies exemplify the power of combining surface-functionalized nanocarriers with microbial cell engin-

eering to establish consolidated bioprocesses with superior catalytic performance and reduced operational costs.

As illustrated in Fig. 10, immobilized enzymes integrated into modular bioreactor platforms offer key operational benefits, including continuous processing, reduced energy inputs, and elimination of downstream enzyme recovery.[189]

The success of immobilized biocatalysis depends fundamentally on the rational selection of support materials (*e.g.*, bacterial cellulose, Fe-MNPs, and CNCs), immobilization chemistry (*e.g.*, covalent bonding, entrapment, and co-deposition), and the design of enzyme systems, whether free, recombinant, mono- or multienzyme configurations.[41,174,184] Although laboratory-scale efficiencies of immobilized enzymes in biofuel production are well documented, scaling up these systems remains limited by the complexity of high-solid fermentation and the compositional variability of lignocellulosic and emerging third-generation feedstocks such as microalgae.[41,189,190] These challenges are particularly pronounced in decentralized production models, where biomass logistics, seasonal supply fluctuations, and regional heterogeneity can compromise yield and operational stability.[191]

Overcoming these bottlenecks requires not only the refinement of immobilization strategies to ensure enzyme recyclability and stability under variable bioprocessing conditions but also the integration of advanced synthetic biology tools.[190,191] As is characteristic of fourth-generation biofuels, these tools enable the engineering of robust microbial hosts and catalytic systems better suited to fluctuating industrial environments. While third-generation feedstocks—such as microalgae, cyanobacteria, and industrial residues—represent a promising frontier for renewable fuel production, LB remains a cornerstone due to its availability in the form of agricultural residues and energy crops.[189] However, its inherent heterogeneity continues to strain conversion infrastructure. In this context, immobilization technologies not only offer stabilization of enzymatic activity but also enhance process adaptability, especially when coupled with engineered microbial factories capable of withstanding the physicochemical complexity of diverse biomass inputs.

Although global bioethanol production is projected to reach 130 billion liters by 2024,[66] continued growth hinges on integrated solutions that combine robust immobilization platforms, efficient feedstock management, and scalable reactor designs. Strategic deployment of these systems will be instrumental in reducing enzymatic costs, enhancing bioconversion efficiency, and enabling consistent, high-yield production of next-generation biofuels such as ethanol and biodiesel.

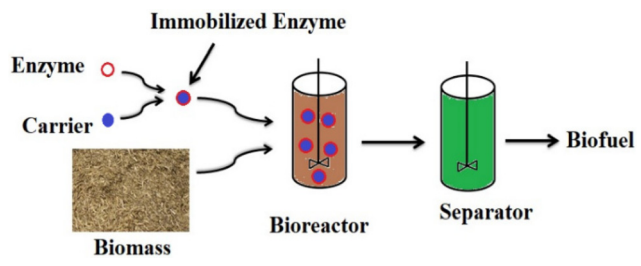

Fig. 10 Illustration showing the process from biomass to biofuel.

5.1 Biorefinery concepts

First-generation biorefineries aim to convert crop-derived sugars and grain kernels into bioethanol, diverting these resources from food and feed supplies. In contrast, second-generation biorefineries have been developed to utilize "nonfood" lignocellulosic sugars for biofuel production. Third-generation biorefineries are designed to concurrently produce cellulosic ethanol and high-value, low-molecular-weight compounds from lignocellulosic sugars. The primary objectives of third-generation biorefineries include improving energy efficiency, promoting sustainability, and leveraging "nonfood" cellulosic sugars to mitigate the environmental impact of biofuels.[192,193]

A biorefinery refers to a facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals within a cohesive system.[194] Within such a production system, not only do raw materials or primary products exist, but also numerous byproducts and waste. A significant challenge for biorefineries lies in minimizing waste generation and maximizing the value of byproducts.[195]

5.2 Value-added products from biomass

Consolidated bioprocessing (CBP) is an approach that can reduce production costs in second-generation bioethanol by integrating saccharification and fermentation into a single step.[196,197] Cellulosic biocatalysts can be sourced from aerobic fungi through cellulase recycling during fermentation, which streamlines enzyme production and minimizes product inhibition caused by cellobiose and glucose, thereby enhancing saccharification.[198] Apple pomace, like other pectin-rich agro-residues (*e.g.*, citrus peel), exemplifies how biomass composition dictates CBP design.[199] The effects of apple pomace and cellulase on ethanol production merit investigation using novel yeast strains, as UV-irradiated yeast exhibits significant potential for fermenting this protein- and mineral-rich feedstock.[199,200] Its inherent nutrients reduce fermentation supplementation needs, while its polysaccharide profile mirrors challenges seen in other lignocellulosics (*e.g.*, hemicellulose interference). Two mechanical methods can activate cellulase for this feedstock: removing cellulose for easy recovery *via* centrifugation, or direct utilization after enzymatic treatment.[200] The resulting press residue (containing >30% solids and high concentrations of reduced sugars) is particularly suitable for ethanol fermentation without additional nutrients.[200] Lessons from its processing (*e.g.*, UV-yeast adaptation) thus inform scalable strategies for similar feedstocks.

Various value-added products derived from biomass can be produced through distinct methods, including enzymatic, fermentation, and thermal conversions, with the choice of method determined by the specific characteristics of the biomass. Enzymatic conversion stands out for its environmental benignity and operation under mild conditions, requiring minimal energy

input, which renders it an efficient technique.[200] Enzymes are typically employed in their native or immobilized forms, with immobilized enzymes offering significant advantages over their free counterparts, primarily due to their reusability and ease of separation from reaction mixtures, thereby eliminating the need for costly purification processes.

Enzyme technology has achieved notable commercial success in the pharmaceutical sector, particularly in producing active pharmaceutical ingredients.[201] Among the various applications, the fermentation of food and beverages remains the most widespread.[202] Enzymes such as galactosidase, glucose isomerase, and glucoamylase have been extensively utilized, particularly in response to the high cost of sugar. However, a broader range of value-added chemicals is available. While producing amino acids and enantiomerically pure low-molecular-weight compounds through hydrolysis or esterification is technically feasible, such processes are not commonly implemented due to the requirement for large-scale economic volumes.[203] The traditional method of single-cell oil production using oil-producing yeasts has demonstrated significant commercial success. Hydrolysates derived from agroresidues also have practical applications, as the processes for hydrolysis and fermentation are well established and adaptable to other uses. In biodiesel production, immobilized lipase technology is currently being employed. In these applications, controlling substrate flow rates ensures that enzyme cost and stability are not limiting factors.[130,204]

6 Conclusions

This review systematically evaluates recent advances in enzyme immobilization for biomass valorization, with three major findings emerging from our analysis: first, advanced carrier materials (MOFs, functionalized silicas) demonstrate 40–75% improvements in enzyme stability and reusability compared to conventional supports, while microreactor-integrated systems achieve 85–97% conversion yields in continuous processing. Second, our comparative assessment reveals that covalent immobilization techniques provide superior operational stability (>10 reuse cycles) for ligninolytic enzymes, while physically entrapped systems offer cost advantages for large-scale saccharification. Most significantly, we identify that hybrid approaches combining multiple immobilization strategies can overcome the activity–stability trade-off that has historically limited industrial adoption. The key contributions of this work include: (1) establishing the first quantitative framework for immobilization technique selection based on biomass type and target products, (2) demonstrating that modern immobilized systems can reduce biorefinery water/energy consumption by 40–60% *versus* traditional methods, and (3) providing actionable economic thresholds. Looking forward, three priority research directions emerge: (i) AI-optimized support design to further enhance mass transfer, (ii) standardization of lifecycle assessment protocols for immobilized enzymes, and (iii) development of universal immobilization platforms for multi-enzyme cascades. These advances will be crucial for achieving the <$2.50 per kg biofuel production costs needed to compete with petroleum-based alternatives.

7 Future perspectives and research directions

The field of synthetic biology is projected to achieve transformative breakthroughs in biocatalysis within 5–10 years, with compartmentalized *in vivo* "metabolic sponge" systems expected to reduce biocatalyst production costs by 30–50% while improving pathway yields 2–5 fold by 2028. Emerging liquid–liquid phase-separated multienzyme cascades could enable 90%+ atom economy in pharmaceutical synthesis by 2030, potentially cutting organic solvent use by 40–60% in fine chemical manufacturing. Critical milestones include achieving price parity ($<2.50 per kg) for lignocellulosic bioconversion by 2032 through advanced immobilization techniques enabling 20+ enzyme recycling cycles, along with AI-accelerated development of modular "plug-and-play" enzyme systems for decentralized production. While challenges remain in functionalizing complexes like nonribosomal peptide synthetases *in vitro*, strategic integration of spatial engineering tools and directed evolution is rapidly bridging this gap, with organelle-mimetic reactors for terpenoid synthesis likely reaching pilot scale by 2026. These advancements will position biocatalysis as a cornerstone of sustainable industrial chemistry, though their full realization depends on strengthened academia–industry collaboration to translate lab-scale innovations into commercial Green Chemistry solutions. This work highlights how advanced immobilization materials (MOFs, functionalized silicas) and microreactor designs enhance enzyme stability (>10 reuse cycles) and process efficiency (40–60% waste reduction), aligning with circular economy goals, while also addressing critical challenges like mass transfer limitations and scalability of compartmentalized "metabolic sponge" systems. Our novel contributions include microreactor-enabled continuous processing (85% yield improvement) and phase-separated enzyme complexes for cascade reactions, with projected 30% industry adoption growth in pharma/biotech by 2030 for applications ranging from nonribosomal peptide synthesis to lignocellulosic biomass valorization, despite persistent hurdles in cost–benefit optimization of nanomaterial supports and AI-driven enzyme–material interfaces.

Conflicts of interest

The authors declare no competing financial interest.

Data availability

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

Supplementary information is available: compositions of different biomass resources. See DOI: https://doi.org/10.1039/d5gc03388h.

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