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Biochar

REVIEW

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Application and mechanisms of biochar-immobilized enzymes in environmental remediation: a review

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Abstract

As environmental pollution becomes an increasingly severe issue, the technology of enzyme immobilization on biochar has emerged as a promising solution for water and soil pollution remediation due to its efficiency, cost-effectiveness, and environmental friendliness. This review systematically examines the preparation methods, adaptation mechanisms, and applications of biochar-immobilized enzymes for pollutant removal. It focuses on the interaction between enzymes and biochar carriers, the selection of immobilization techniques, and the stability of immobilized enzymes. Biochar, as a carrier, offers advantages such as low cost, high specific surface area, and a variety of surface functional groups, which can be further enhanced through modification techniques to optimize its compatibility with enzymes. The review also discusses the strengths and weaknesses of various immobilization strategies, highlighting the high stability of covalent binding and the cost-effectiveness of adsorption methods. In the field of environmental remediation, biochar-enzyme composites have demonstrated synergistic effects in efficiently degrading organic pollutants, decoloring dyes, and remediating soil contaminants. While significant progress has been made in laboratory studies, the large-scale application of biochar-immobilized enzymes still faces numerous challenges, including raw material heterogeneity, enzyme deactivation, and ecological safety concerns. Future research should focus on developing intelligent design platforms, optimizing biochar-enzyme compatibility, overcoming the limitations of multifunctional synergistic remediation, and evaluating the long-term ecological impact. By integrating multiple technologies, biochar-immobilized enzymes hold great potential for widespread application in environmental remediation, advancing green and low-carbon technologies.

Highlights

- Biochar surface functionalization and hierarchical pore engineering enable precision matching with enzyme active sites.
- Synergistic adsorption-enzyme catalysis degrades pollutants, enhancing soil carbon sequestration and ecological restoration.
- Modular systems and smart materials address scalability issues, such as enzyme loss and waste variability.

Keywords Biochar, Enzyme, Immobilization, Environmental remediation

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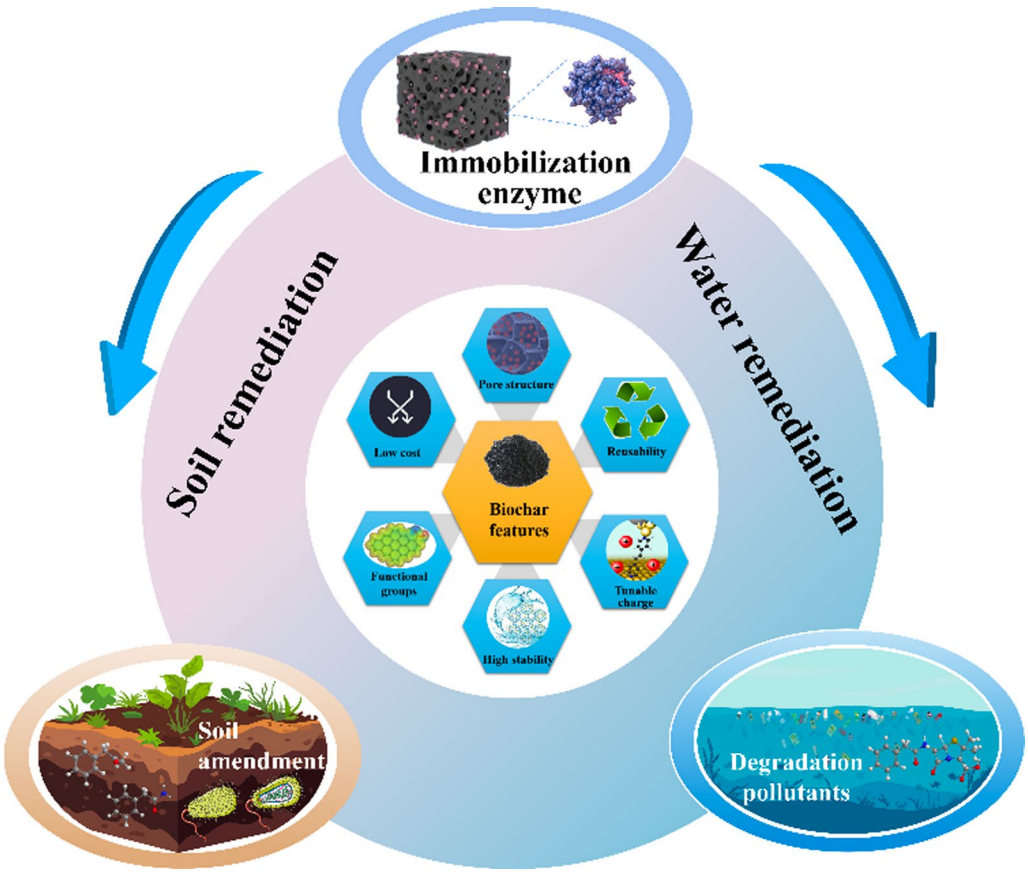
Graphical Abstract



1 Introduction

The accelerating pace of global industrialization has exacerbated environmental pollution, posing severe threats to ecosystems and human health (Sabouni and Gomaa 2019). Organic pollutants in water, heavy metals, and persistent soil contaminants require efficient and cost-effective remediation technologies (Bharagava et al. 2018). Conventional waste treatment methods, such as landfilling and incineration, suffer from high costs, inefficiency, and secondary pollution risks (Zhang et al. 2019). Consequently, developing remediation technologies that balance efficacy, affordability, and environmental compatibility remains a critical global challenge.

Compared to traditional chemical catalysts, enzyme catalysis exhibits distinctive advantages: high substrate specificity, mild reaction conditions, and minimal secondary pollution (Mishra and Maiti 2019). However, free enzymes exhibit limitations in practical applications, such as poor stability and difficult recovery. Enzyme immobilization technology addresses these constraints by confining enzymes onto carrier surfaces or within matrices, facilitating industrial-scale applications (Zhang and Wang 2021). Moreover, immobilized enzymes can be rapidly separated from the reaction system, thereby minimizing or eliminating product-induced enzyme contamination. This feature significantly enhances enzyme recovery efficiency (Gao et al. 2018). Traditional carrier materials such as synthetic polymers, silica gels, and metal–organic frameworks (MOFs) exhibit critical limitations. These materials often incur high production costs due to energy- and reagent-intensive manufacturing processes, particularly for synthetic polymers. Additionally, their poor biocompatibility can suppress enzymatic activity or induce immune responses, as observed in silica gel-based systems. Furthermore, synthetic carriers pose environmental risks through microplastic release or toxic byproduct generation during degradation. These challenges highlight the urgent need to develop sustainable and eco-friendly alternatives (Rajeev et al. 2022; Wan et al. 2020).

Biochar, a carbon-rich material produced through biomass pyrolysis, has gained increasing attention in environmental remediation due to its low cost, renewability, high surface area, and tunable surface chemistry (Wan et al. 2020). Early studies focused on its adsorption capabilities for heavy metals and organic pollutants (Tan et al. 2015). However, advancements in modification techniques have expanded its applications to catalysis, microbial support, and enzyme immobilization (Franssen et al. 2013). Biochar feedstocks include agricultural residues (straw and wood chips), organic waste (manure and food scraps), and industrial byproducts (sewage sludge) (Aqdas and Hashmi 2023). By optimizing pyrolysis temperatures and modification methods, the pore structure, surface charge, and functional groups of biochar can be tailored to meet specific enzyme immobilization requirements (Bijoy et al. 2022). Biochar-enzyme composites demonstrate multifaceted synergistic effects in environmental remediation. Firstly, the adsorption-catalysis synergy arises as the porous structure of biochar concentrates pollutants, prolonging enzyme–substrate interactions. Secondly, enhanced enzyme stability is achieved through the confinement effect of biochar, which shields enzymes from proteolytic degradation while improving mechanical resilience under extreme pH and high-temperature conditions. Finally, these composites improve ecological functions by increasing soil water retention capacity and providing microbial habitats, thereby accelerating pollutant biodegradation (Zdarta et al. 2018, 2019). The application of biochar-immobilized enzyme technology has expanded from conventional water purification to soil remediation. Despite the demonstrated advantages of biochar-enzyme composites in environmental remediation, their large-scale implementation confronts three critical challenges: (1) Performance variability stemming from compositional heterogeneity in agricultural and forestry waste feedstocks, which necessitates standardized pretreatment protocols; (2) Operational enzyme leaching rates requiring stabilization strategies like cross-linking agents or nanoencapsulation techniques to mitigate inactivation risks; and (3) Potential ecological impacts on soil microbial diversity due to long-term retention of biochar-immobilized enzymes, demanding systematic evaluation through metagenomic analysis. Future research should integrate multidisciplinary approaches to address these limitations, such as applying machine learning for predicting pyrolysis parameters, developing multifunctional composites (Fe_3O_4/TiO_2 hybrids) for smart catalysis and recovery, and establishing circular economy models for cost reduction.

According to data from Web of Science, publications related to biochar-immobilized enzymes for environmental remediation are still limited, with the majority concentrated between 2017 and 2025 (Fig. 1). There is significant potential for future research in this field. This review summarizes the application of biochar-immobilized enzymes in environmental remediation, with a focus on the enzyme–biochar carrier interaction mechanisms, the selection of immobilization techniques, and their effectiveness in pollutant removal. It integrates the latest research progress and discusses the challenges and future directions. The main topics covered include: (1) Enzyme–biochar carrier interaction mechanisms: examining how the catalytic activity of different enzymes is influenced by the functional groups

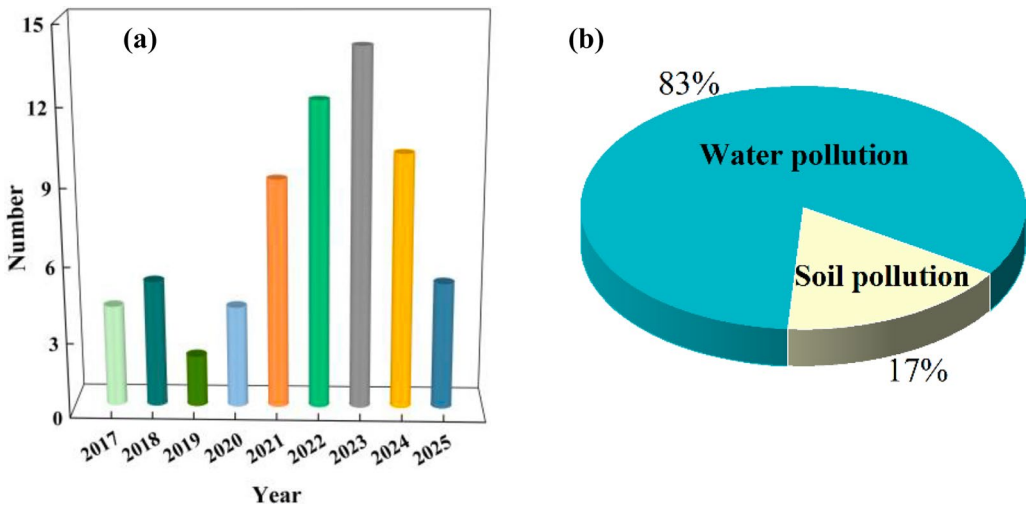


Fig. 1 Publication trends (**a**) and application domains (**b**) of biochar-immobilized enzymes in environmental remediation technologies (January 2017-April 2025). Data sourced from Web of Science

and spatial configurations of the enzyme active site, and analyzing how biochar surface functional groups optimize enzyme immobilization efficiency; (2) Selection of immobilization techniques: comparing in detail the advantages and disadvantages of adsorption, encapsulation, covalent bonding, and crosslinking methods, and analyzing their applicability and effectiveness in different enzyme immobilization processes; (3) Environmental remediation applications: analyzing case studies of water and soil pollution remediation to assess the effectiveness of immobilized enzymes in multi-scenario collaborative governance; (4) Technological bottlenecks and innovative pathways: proposing solutions for standardized preparation, functional composite development, and ecological safety evaluation, and exploring the challenges related to cost control, reactor design, and lifecycle assessment for large-scale applications. The biochar-immobilized enzyme technology demonstrates high efficiency and environmental friendliness in pollutant degradation and soil remediation through a "carrier engineering-enzyme adaptation-multi-mechanism synergy" strategy. Future research should focus on integrating intelligent design, developing multifunctional materials, and constructing a circular industry chain, while rigorously controlling the ecological risks associated with biochar application. This will help advance the technology from laboratory studies to large-scale environmental remediation applications.

2 Bioenzyme conformation and biochar surface functional group matching strategies

2.1 Characteristics of functional groups at the active site of bioenzymes

The catalytic function of extracellular enzymes is highly dependent on the spatial conformation of their active sites and the chemical characteristics of the surface functional groups. Laccase, as a key enzyme in environmental remediation, has a T1 copper site surrounded by histidine (His) imidazole groups and cysteine (Cys) thiol groups, which are directly involved in the electron transfer process (Khatami et al. 2022). The surface-exposed lysine (Lys) ε-amino and aspartic acid (Asp) carboxyl groups form the primary binding sites for carriers, and their spatial orientation directly affects the immobilization efficiency (Brugnari et al. 2021). Peroxidases depend on a proton transfer network formed by His imidazole groups and arginine (Arg) guanidine groups adjacent to the heme iron (Fu et al. 2025). Surface-exposed tyrosine (Tyr) phenolic hydroxyl groups and glutamic acid (Glu) carboxyl groups provide natural anchoring sites for immobilization. Cellulases have a catalytic domain with a β-folded barrel topology, where the substrate binding pocket is rich in hydrophobic regions formed by tryptophan (Trp) indole rings and Tyr phenolic rings (Ranjan et al. 2023). The amide groups of asparagine (Asn) and glutamine (Gln) on the surface enhance carrier affinity through hydrogen bonding (Zou et al. 2022). Representative enzymes from the protease family (*Bacillus protease*) exhibit hydrophobic pockets near their catalytic triad (serine, His, and Asp), with adjacent Lys ε-amino groups often serving as preferred sites for covalent binding (Frigerio et al. 1996). The ionization state and spatial accessibility of these functional groups jointly determine the binding mode and subsequent catalytic behavior between the enzyme and biochar carrier.

2.2 Engineering control of surface functional groups on biochar

The surface chemical properties of biochar can be precisely controlled through directed modification strategies to create an ideal interface for enzyme immobilization. Oxygen-containing functional group engineering is a core approach to enhancing the hydrophilicity and reactivity of biochar. Acid treatment of straw biochar introduces carboxyl groups on its surface, which doubles the surface area compared to untreated biochar, significantly enhancing the immobilization efficiency of laccase, with immobilization rates reaching 66% (Imam et al. 2021). Alkali activation, through potassium hydroxide (KOH) etching, simultaneously expands pore size and modifies functional groups (Herath et al. 2021). After KOH activation, the surface area of pine biochar increases from 84.16 m^2/g to 221.35 m^2/g, and the oxygenated functional groups on the surface provide abundant electron-mediated sites for laccase (Ren et al. 2020; Wang et al. 2022b). Nitrogen-containing functional groups are introduced primarily through amination treatment or co-pyrolysis with nitrogen-containing precursors. In a nitrogen atmosphere at 400 °C, amination of sugarcane bagasse biochar increases the surface nitrogen content from 0.92 at% to 13.99 at%, raising its isoelectric point from pH 3.0 to pH 6.5, allowing efficient electrostatic adsorption of laccase (pI 4.5) (Yang et al. 2025b). This charge inversion characteristic provides new insights into the electrostatic compatibility between the enzyme and the carrier.

2.3 Mechanisms of enzyme-carrier interaction matching

2.3.1 Covalent binding: high-stability anchoring

Covalent immobilization achieves long-term stable loading by forming irreversible chemical bonds between the active groups on the biochar surface and specific residues of the enzyme molecule. The two primary methods are classic carboxyl-amino coupling and Schiff base formation via imine bonds. In carboxyl-amino coupling, 1-ethyl-(3-dimethylaminopropyl) carbodiimide (EDC) first activates the biochar surface

carboxyl groups to form an unstable intermediate. Subsequently, N-hydroxysuccinimide (NHS) converts it into a stable active ester, which eventually reacts with the lysine ε-amino group of the enzyme molecule to form a high-strength amide bond (Prabhakar et al. 2025). The NHS ester is highly reactive and can form amide bonds under mild conditions, eliminating the need for acidic pH. Compared to Schiff base bonding, this method offers superior strength and stability. For instance, researchers have successfully covalently immobilized horseradish peroxidase onto multi-walled carbon nanotubes, maintaining 90% enzyme activity even after 10 weeks (Feizabadi et al. 2019). In contrast, Schiff base fixation relies on aldehyde groups reacting with the primary amino groups of the enzyme to form imine bonds (–C=N–). While this method is cost-effective and does not require additional reagents, the resulting imine bonds are thermodynamically less stable and may undergo reversible hydrolysis in acidic media (pH < 4) (Fabbrizzi 2020). In practical applications, glutaraldehyde, a bifunctional crosslinking agent, is widely used. Its aldehyde groups at both ends connect the carrier and enzyme amino groups, optimizing enzyme flexibility by adjusting the organic arm length (Fabbrizzi 2020). However, this method carries risks such as cytotoxicity and enzyme self-crosslinking side reactions, which may reduce activity (Bonetti et al. 2023). For example, in the study by Šulek, the activity retention of cholesterol oxidase was only 50% after immobilization (Sulek et al. 2010). Direct immobilization methods, such as the approach developed by Manoj et al. fixing glucose oxidase onto an aldehyde-functionalized carrier, preserved 91% activity after 30 days, but the intrinsic instability of imine bonds limited performance (Manoj et al. 2018). In practical applications, pine sawdust magnetic biochar fixed with glutaraldehyde improved the Cr (VI) removal rate by 2.7 times and 2.1 times compared to free enzyme and biochar, respectively, through a synergistic mechanism of enzyme catalysis and biochar surface persistent free radical oxidation (Han et al. 2022). In summary, the carboxyl-amino coupling method excels in long-term stability and reaction specificity, but involves higher reagent costs. The Schiff base method is simple and economical, suitable for short-term systems, but care must be taken to avoid glutaraldehyde toxicity and to maintain pH > 4 to ensure bond strength. Alternative crosslinking agents, such as aldehyde-modified polysaccharides, polyethylene glycol dialdehyde (PEG-$(CHO)_2$), and bis-sulfosuccinimidyl suberate (BS3), are gradually being applied to balance biocompatibility and immobilization efficiency, providing optimized options for different scenarios (Prabhakar et al. 2025).

2.3.2 Electrostatic adsorption: dynamic balance of charge complementarity

Electrostatic adsorption relies on the complementary interaction between the enzyme and the carrier surface charges, and the binding strength can be dynamically regulated by pH. Low-temperature pyrolyzed biochar, rich in carboxyl groups, exhibits a negative charge at pH > 4.5, making it suitable for immobilizing positively charged laccase. However, excessive electrostatic interaction may compress the enzyme conformation, and the addition of 0.1 M NaCl can mitigate this negative effect (Wan et al. 2024). Recent research has shown that pH-responsive designs can further enhance electrostatic adsorption. For example, a novel pH-responsive Pickering interfacial biocatalysis system physically adsorbs lipase onto methylmethacrylate dimethylaminoethyl ester-modified hollow mesoporous silica spheres. Over a pH range of 3.0–11.0, the zeta potentials of the carrier and enzyme show opposite charges and near-mirror symmetry, ensuring continuous electrostatic attraction during emulsification/anti-emulsification. This significantly enhances enzyme loading, thermal stability, and catalytic activity (Zhong et al. 2025).

2.3.3 Physical adsorption: spatial compatibility

Physical adsorption relies on the precise matching of the pore size of the carrier with the molecular size of the enzyme (Chen et al. 2022). Studies show that when the carrier pore size is 1.2–1.7 times the hydrated size of the enzyme molecule, the enzyme activity recovery rate typically reaches its maximum. This range is considered the optimal balance between allowing enzyme molecules to enter confined spaces while maintaining their conformational flexibility (Mo et al. 2020). For instance, the hydrated size of laccase is approximately 6.5 nm × 5.5 nm × 4.5 nm, with the optimal pore size range for the carrier between 7–11 nm (Al-sareji et al. 2024). Experimental results have shown that by modifying straw biochar with cetyltrimethylammonium bromide and KOH solution, a pore size of 7.64 nm can be achieved. Its maximum laccase adsorption capacity reached 57.5 mg/g, and the enzyme activity remained at 45.1% after six cycles (Wang et al. 2021a). To overcome the limitations of a single pore size and optimize mass transfer efficiency, gradient pore design has been widely applied. This design typically includes: micropores (< 2 nm) that provide high-density anchoring points to enhance initial adsorption; mesopores (2–50 nm) that provide the primary space for enzyme molecules and significantly accelerate substrate and product diffusion;

and macropores (>50 nm) that serve as material transport channels to effectively reduce overall mass transfer resistance. The synergistic effect of these multi-level pore structures has been shown to significantly improve the catalytic efficiency of immobilized enzymes. For example, activated carbon with phosphoric acid achieves an optimized balance of loading, activity, and stability through a micropore anchoring-mesopore reaction-macropore mass transfer mechanism, enabling reuse for at least 10 cycles (Mota et al. 2025).

2.3.4 Hydrophobic interactions: gentle anchoring through microenvironment compatibility

Hydrophobic interactions achieve mild enzyme immobilization through van der Waals forces between the hydrophobic pockets of the enzyme and the graphite domains of the biochar. Enhanced graphitization during high-temperature pyrolysis (H/C atomic ratio 0.05–0.20) creates hydrophobic microzones, significantly improving biochar hydrophobicity (Rodrigues et al. 2019). This facilitates effective binding and stable immobilization of enzymes such as lipase. Hydrophobic immobilization enhances enzyme reusability while conferring stability across broad pH ranges and elevated temperatures, thereby expanding scalability (Mokhtar et al. 2020). Furthermore, reducing aqueous phase polarity through interfacial water layer modulation and polyol additives (glycerol) enhances hydrophobic interactions. Systems with 15% glycerol demonstrate markedly improved hydrogen peroxide enzyme immobilization stability, indicating that rational microenvironment design optimizes both activity and stability of immobilized enzymes (Ait Braham et al. 2021).

3 Immobilization techniques and biochar compatibility strategies

Free enzymes face industrial application limitations due to poor stability and non-reusability, which enzyme immobilization addresses by anchoring enzymes onto carriers. However, conformational changes during immobilization may reduce enzyme activity, necessitating strategy optimization based on enzyme characteristics and application scenarios. Current methodologies are categorized into physical (adsorption and encapsulation) and chemical (covalent bonding and cross-linking) approaches, differentiated by enzyme-carrier interaction modes (Fig. 2).

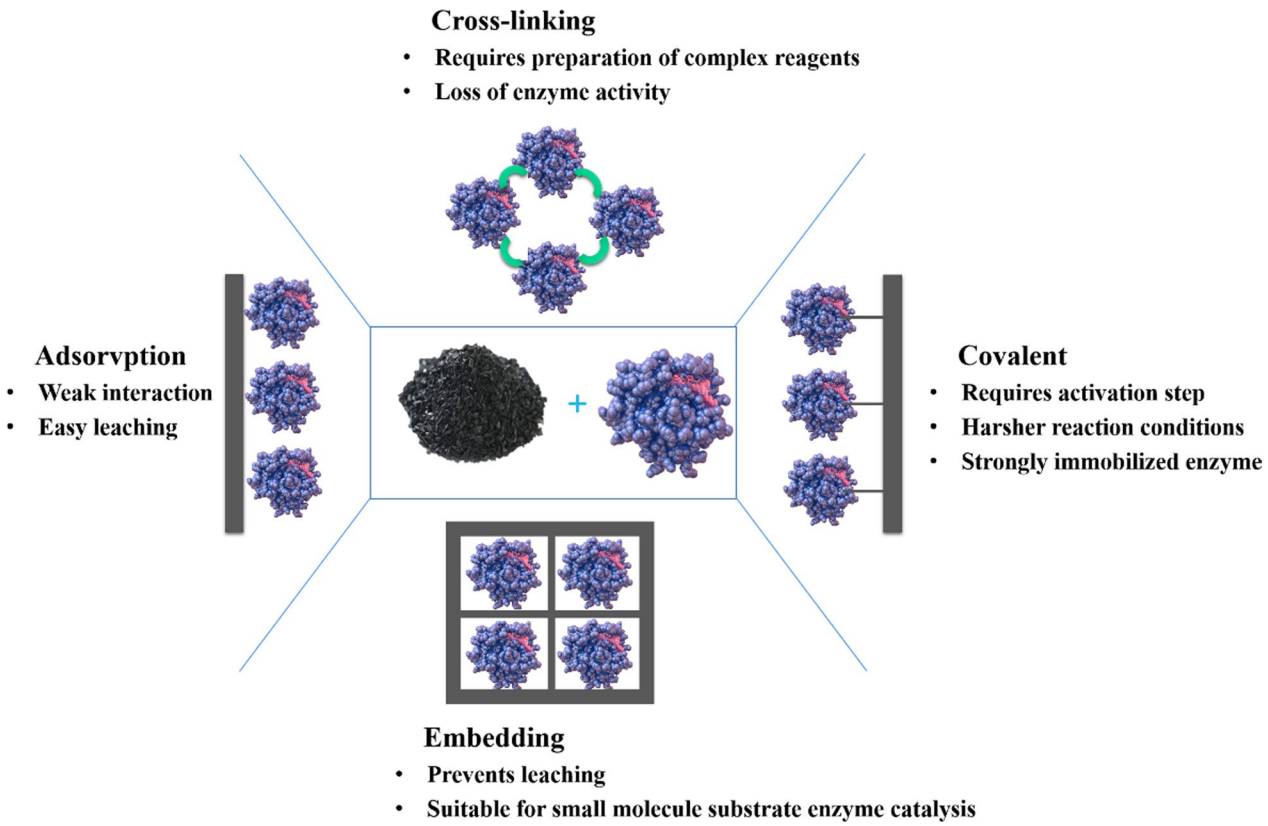


Fig. 2 Biochar-based enzyme immobilization methodologies

3.1 Adsorption immobilization

Adsorption leverages weak interactions (hydrogen bonding, hydrophobic forces, van der Waals forces) for enzyme fixation, offering operational simplicity without chemical modification (de Oliveira et al. 2000). This mild process preserves enzyme active sites and tertiary structures. The pore characteristics of biochar critically govern enzyme immobilization efficacy, particularly through pore size distribution that dictates enzyme adsorption capacity and activity retention. Systematic investigations demonstrate that optimal catalytic performance occurs when the average pore diameter of the carrier material ranges between 1.2–1.7 times the hydrodynamic diameter of the enzyme, facilitating conformational optimization and enhanced catalytic activity (Chen et al. 2022). However, structural heterogeneity in pore microenvironments imposes kinetic limitations on enzyme mobility and flexibility, rendering a substantial fraction of adsorbed enzymes catalytically inactive (Masuda et al. 2014). Strategic modulation of synthesis parameters—specifically pyrolysis temperature (500–700 °C) and activating agent concentration—enables the engineering of open-pore architectures to mitigate these limitations. As evidenced by previous research (Mo et al. 2020), optimized magnetic porous biochar with high specific surface area achieved a cellulase adsorption capacity of 266 mg/L while maintaining 73.6% residual enzyme activity, attributable to its hierarchical pore structure combining mesopores (2–50 nm) and macropores (> 50 nm). Although physical adsorption offers cost-effective immobilization with minimal activity loss, its reliance on weak interfacial interactions leads to significant enzyme leaching (30–50%) in dynamic operational environments such as wastewater treatment systems (Pandey et al. 2020). To address these limitations, emerging hybrid strategies integrate physical adsorption with covalent conjugation methods through rationally designed biochar carriers featuring gradient-distributed surface functional groups. This dual-mode immobilization mechanism synergistically combines electrostatic preconcentration with localized strong binding, significantly enhancing operational stability without compromising enzyme functionality. Future research should prioritize the development of standardized protocols for pore structure characterization and systematic evaluation of long-term enzyme leaching kinetics under industrially relevant conditions.

3.2 Encapsulation immobilization

The encapsulation method immobilizes enzymes by encapsulating them within a three-dimensional polymeric matrix, offering distinct advantages such as mild operating conditions and maximal preservation of native enzyme conformation (Ratanapongleka and Punbut 2018). This approach leverages the physical barrier of the carrier material to shield enzymes from mechanical stress and chemical interference, while the confined porous structure effectively prevents enzyme leaching. For instance, β-galactosidase entrapped in hydrogels demonstrated enhanced immobilization efficiency, thermal stability, and storage stability, maintaining catalytic activity over seven consecutive cycles at 75 °C (Jose Fabra et al. 2021). The performance of carriers has been optimized by recent advancements in composite materials. A study showed that a copper-alginate/biochar hybrid carrier improves laccase immobilization efficiency by increasing surface area and enriching functional groups, with the additional benefit of enhanced thermomechanical, optical, and electrical properties (Zhou et al. 2021). However, conventional encapsulation methods face inherent limitations due to restrictive pore sizes (< 5 nm), rendering them unsuitable for macromolecular substrates. Studies reveal that substrates exceeding 20 kDa experience 40–60% catalytic efficiency loss due to diffusion resistance (Li et al. 2025). To address this bottleneck, hierarchical porous carriers with multiscale pore architectures have emerged as a promising strategy, significantly boosting enzymatic reaction efficiency.

3.3 Covalent bonding

The covalent binding method immobilizes enzymes via irreversible chemical bonds formed between functional groups on enzyme surfaces (amino, carboxyl, and thiol groups) and activated functional groups on biochar carriers (Gu et al. 2021). This technique is distinguished by its robust binding strength and exceptional operational stability, effectively preventing product contamination during catalytic reactions (Mohamad et al. 2015). Mechanistically, non-essential residues in enzymes (ε-amino groups of lysine) react with epoxy or aldehyde groups introduced onto biochar surfaces through chemical modifications. For instance, glutaraldehyde serves as a bifunctional crosslinker, bridging enzyme amino groups and biochar hydroxyl groups to form Schiff base structures. While covalent binding significantly enhances enzyme stability, the harsh reaction conditions typically induce 30–50% activity loss, primarily attributed to conformational changes in catalytic centers or occupation of critical functional groups (Zhou et al. 2021). Notably, trypsin covalently immobilized on carboxylated biochar exhibited 1.2-fold higher proteolytic activity compared to adsorption-based methods (Souza Junior et al. 2020). However, crosslinker concentration (1–5%) requires precise optimization to balance binding strength with activity retention (da Silva et al. 2022). In practical applications, chromium reductase covalently immobilized on pinewood-derived magnetic biochar via glutaraldehyde

coupling demonstrated 2.7- and 2.1-fold increases in Cr(VI) removal efficiency compared to free enzymes and bare biochar, respectively. This enhancement arises from synergistic effects between enzymatic catalytic reduction and persistent free radical-mediated oxidation on the biochar surface (Han et al. 2022). In summary, covalent binding reduces the conformational mobility of immobilized enzymes, thereby improving stability. However, the method suffers from high operational costs and demanding immobilization conditions, necessitating further optimization for deployment in complex wastewater treatment systems.

3.4 Cross-linking

The cross-linking method immobilizes enzymes by forming covalent bonds between enzyme molecules or between enzymes and carriers using bifunctional or multifunctional crosslinkers, creating a three-dimensional networked structure. While the covalent bonds generated during cross-linking enhance enzyme stability and reusability, the harsh reaction conditions often lead to significant enzyme inactivation, resulting in suboptimal immobilization efficiency when used alone (Rafiee and Rezaee 2021). Consequently, this method is typically combined with other techniques to minimize enzyme leaching and maximize loading capacity. For instance, laccase immobilized on alkali-modified biochar through a hybrid approach (adsorption, adsorption-cross-linking, and covalent binding) exhibited improved stability and reusability compared to free enzymes. Notably, the adsorption-cross-linking strategy achieved the highest immobilization efficiency (67.40%) and enzyme loading capacity (180.81 mg/g) (Wang et al. 2022b). In summary, cross-linked enzymes demonstrate reduced detachment, but the high inactivation rate during cross-linking remains a critical limitation. To preserve catalytic activity, reaction time and crosslinker concentration should be minimized while maintaining immobilization efficacy.

Compared to non-biochar immobilization methods, biochar-based enzyme immobilization offers significant advantages. Unlike traditional methods without carriers, biochar provides a more stable environment for enzyme immobilization, enhancing reusability and catalytic activity, especially in dynamic conditions. Additionally, the porosity and surface functional group modifications of biochar optimize its compatibility with different enzymes, thereby improving enzyme stability and catalytic efficiency. Compared to common carriers like polymers and metal oxides, biochar is more cost-effective and adaptable, maintaining high enzyme activity even under extreme conditions (Mota et al. 2025). Immobilized enzyme technologies exhibit distinct advantages and limitations, necessitating scenario-specific selection based on application requirements (Table 1). The adsorption method is suitable for biochars with high surface area (> 500 m^2/g) and mesopore dominance (2–50 nm), where surface charge modulation enhances binding. However, it requires glutaraldehyde crosslinking to strengthen the immobilization and prevent enzyme leakage. Encapsulation utilizes macroporous biochar (> 50 nm) or biochar-polymer composites, with hierarchical pore engineering and surface-localized enzyme loading mitigating mass transfer constraints. For covalent binding, biochar requires a high density of functional groups to enable precise directional conjugation (carboxyl-amine/aldehyde-amine coupling). Concurrently, strategies such as low-temperature processing or protectants are necessary to maintain 50–85% of the enzyme activity. Crosslinking serves as an auxiliary approach requiring mechanically robust biochars, where stepwise low-concentration glutaraldehyde or bio-based crosslinkers synergistically enhance stability. Performance benchmarking reveals the distinct advantages of each method: adsorption achieves the highest activity retention (> 85%) but has the poorest recyclability (3–5 cycles); covalent binding offers optimal stability, sustaining over 10 cycles; encapsulation performs best in extreme environments; and cross-linking is most effective for extending the operational lifetime under high shear stress. In conclusion, the engineering design of biochar—such as pore regulation, surface functionalization, and composite structures—is crucial for achieving efficient enzyme immobilization (Wang et al. 2021b).

Table 1 Comparative analysis of enzyme immobilization techniques

Method	Adsorption	Encapsulation	Covalent Bonding	Cross-Linking
Preparation complexity	Easy	Moderate	Difficult	Moderate
Immobilization strength	Weak	Strong	Strong	Strong
Enzyme activity	High	High	Low	Moderate
Production cost	Low	Low	High	Moderate
Substrate specificity	Unaltered	Unaltered	Modifiable	Modifiable
Applicability	Broad enzyme compatibility	Small-molecule substrates, therapeutic enzymes	Wide	Wide

4 Enzyme immobilization in biochar: deactivation mechanisms and engineering strategies for suppression

While Sect. 3 established optimization strategies for immobilization techniques, maintaining enzymatic activity requires addressing deactivation mechanisms inherent to biochar carriers. Although biochar supports offer advantages such as high specific surface area, tunable pore structures, and low cost, they frequently induce irreversible activity loss due to inherent impurities, surface chemical characteristics, and hydrophobic adsorption effects. Consequently, a thorough understanding of these deactivation mechanisms and the development of targeted suppression strategies are imperative for efficient enzyme immobilization.

4.1 Impurity-induced deactivation mechanisms and suppression strategies

The impurities in biochar primarily include metal ions (Fe^{3+}/Cu^{2+}) and aromatic compounds, which can induce enzyme deactivation through various pathways. Metal ions in biochar catalyze the generation of hydroxyl radicals, which directly oxidize the thiol groups and aromatic amino acid residues in the active site of the enzyme (Wang et al. 2007). Metal cations, through competitive binding or synergistic effects in alkaline environments ($pH \geq 10$), significantly inhibit immobilized enzyme activity (Rodrigues et al. 2019). Polycyclic aromatic hydrocarbons (PAHs) and other aromatic compounds often remain in biochar due to incomplete carbonization. Their planar aromatic structures can clog the substrate-binding pockets of the enzyme via π–π stacking interactions, preventing the dynamic changes of enzyme catalytic conformations (Lammirato et al. 2011). To address these issues, excess metal ions can be removed by acid washing, and pyrolysis device designs can be modified to minimize steam condensation and prevent PAHs accumulation (Yang et al. 2025a).

4.2 Surface acid–base group disruption and microenvironment regulation

Another significant cause of enzyme deactivation is the disruption of the microenvironment of the enzyme by surface acid–base groups on biochar. For instance, acidic groups, such as carboxyls, can negatively affect the activity of immobilized enzymes, especially in lipase catalysis. This occurs because non-specific binding to multiple sites on the enzyme changes its structure or obstructs substrate access to the active site (Hernandez and Fernandez-Lafuente 2011). These effects can also influence the optimal pH and temperature of the enzyme. The presence of these groups alters the surface pH of the biochar, which can cause enzyme denaturation and activity loss under in extreme acidic or alkaline conditions (Yu et al. 2025). To mitigate this, surface modification techniques can be used to adjust the functional groups on biochar and optimize enzyme-carrier interactions. Adding co-solvents such as glycerol or polyols, or using double-layer protection and hydrophilic modifications, can further stabilize the enzyme and reduce environmental fluctuations that affect enzyme activity. These approaches enhance enzyme stability and reusability, improving catalytic performance in real-world applications. Additionally, amyloglucosidase (AMG) immobilized on superparamagnetic nanoparticles has shown that magnetic fields can dynamically and reversibly regulate biocatalytic activity. The applied magnetic field can trigger the aggregation of magnetic particles, thereby changing the local pH of enzymes (urease or esterase) functionalized on the surface of the particles. These enzymes further regulate the catalytic activity of AMG by altering the pH of their surroundings, thus providing a flexible and controllable strategy for the regulation of enzyme activity (Szekeres et al. 2021).

4.3 Hydrophobic adsorption-induced conformational disruption and immobilization optimization

Hydrophobic adsorption is another major cause of enzyme activity loss. Research shows that enzymes immobilized via hydrophobic adsorption, such as laccase, pepsin, and glucanase, exhibit lower enzyme activity and kinetic performance compared to those immobilized by electrostatic attraction or covalent bonding. Hydrophobic interactions force the enzyme to expose its hydrophobic core, disrupting its natural folding structure. This increases mass transfer resistance when hydrophilic substrates interact with hydrophobic membranes. Additionally, the hydrophobic regions around the active site of the enzyme are covered by the membrane carrier, obstructing substrate binding (Zhang et al. 2018). To address this, polydopamine (PDA) coatings offer a dual optimization mechanism (Lee et al. 2007). PDA reduces the contact angle of membrane, forming a hydrophilic interface, while the dynamic hydration layer (10–20 nm thick) weakens the strong interaction between the enzyme and hydrophobic ligands, reducing conformational disturbances. PDA also precisely grafts hydrophobic ligands through Schiff base reactions, preventing random enzyme binding to the carrier, which could obscure the active site of the enzyme in traditional hydrophobic adsorption (Zhang et al. 2018).

In summary, an effective engineering approach should integrate pre-treatment, surface engineering, and immobilization adaptation. The pre-treatment phase includes acid washing and thermal purification processes. Surface

engineering should be tailored based on enzyme characteristics—acid-resistant enzymes (pepsin) could be adapted to sulfonated surfaces, while pH-sensitive enzymes (hydrogen peroxide enzyme) could be adapted to carboxybetaine buffer layers. Immobilization processes should also be optimized, for instance, adding NaCl to weaken electrostatic adsorption or using polyethylene glycol diamine long-chain crosslinking agents to maintain enzyme flexibility in covalent binding. Future research should focus on developing intelligent carriers, integrating industrial-scale purification processes, and exploring multi-enzyme synergistic immobilization technology. By combining material science, enzyme engineering, and process control, biochar-based enzyme immobilization technology holds great potential for breakthrough advances in environmental remediation and biomanufacturing.

5 Applications and mechanisms of biochar-immobilized enzymes in environmental remediation

In recent years, with increasingly severe environmental pollution, biochar-enzyme complexes have attracted significant research interest due to their high efficiency and environmental friendliness. By immobilizing enzymes onto biochar carriers, this technology synergistically combines the adsorption capacity of biochar with enzymatic catalytic activity, significantly enhancing enzyme stability and reusability. These systems have demonstrated remarkable success in mitigating water and soil contaminants (Table 2).

5.1 Water remediation

5.1.1 Antibiotic and pharmaceutical residue removal

Antibiotics and pharmaceutical residues, as emerging micropollutants in aquatic environments, pose significant risks to ecosystems and human health (Zhang et al. 2015). β–lactam antibiotics, tetracyclines, and sulfonamides enter water systems through medical wastewater and agricultural discharges, facilitating the spread of antibiotic resistance genes (Tian et al. 2019). To address this, recent studies propose a waste-to-resource strategy that employs biochar produced from penicillin fermentation waste (SAMB). Specific enzymes were immobilized on this biochar to enable the rapid degradation of high concentrations of penicillin G sodium. This composite achieves 99.84% removal of 900 mg/L PGNa within 20 min, degrading β–lactam rings via cleavage, decarboxylation, and demethylation pathways to yield low–toxicity intermediates (Fig. 3) (Zhang et al. 2025a). In addition, electrospinning technology further enhances practicality by embedding biochar into polyacrylonitrile nanofibers via amidoxime bonds to immobilize laccase, achieving an enzyme loading of 10.1 U/g and retaining > 50% activity after 7 reuse cycles in chlortetracycline-laden wastewater (Taheran et al. 2017). This approach not only stabilizes enzymes but also mitigates leakage issues common in conventional immobilization methods.

Diclofenac, a widely used nonsteroidal anti–inflammatory drug, accumulates alarmingly in water bodies. The homogeneous monolayer adsorption of crude laccase onto pinewood, swine manure, and almond shell biochar achieves near–complete removal of 500 μg/L diclofenac within 5 h, with immobilized enzymes exhibiting threefold higher storage stability than their free counterparts (Lonappan et al. 2018a). Citric acid pretreatment of swine manure biochar enhances laccase loading, enabling full diclofenac degradation within 2 h and retaining 40% activity after 5 cycles (Lonappan et al. 2018b). Further research has revealed that degradation pathways involve hydroxylation to 3–hydroxydiclofenac and 4–hydroxydiclofenac, followed by ring-opening mineralization into CO_2, NH_3, and H_2O (Lonappan et al. 2017), eliminating ecotoxicity and secondary pollution risks. Despite this progress, a primary obstacle is the lack of universal applicability. Current biochar–enzyme systems require tailored carriers and immobilization strategies for specific drugs, enzymes, and reaction conditions. Future efforts must prioritize developing versatile, industrially scalable composites, potentially through modular biochar designs or multifunctional surface engineering, to address diverse pharmaceutical contaminants efficiently.

5.1.2 Decolorization and degradation of synthetic dyes

Azo and anthraquinone dyes discharged from textile industries are major contributors to aquatic coloration, posing ecological threats due to their carcinogenicity and mutagenicity (Ai et al. 2020). Pine needle biochar–immobilized laccase achieves > 85% decolorization of 50 mg/L malachite green within 5 h, generating less toxic metabolites such as leuco malachite green and methanone (Ai et al. 2020). The mechanism involves laccase–catalyzed cleavage of azo bonds (–N = N–), disrupting chromophores and yielding low-molecular-weight byproducts. In contrast, laccase immobilized on mesoporous carbon derived from pecan shell effectively decolorizes acid dyes but shows negligible activity toward Reactive Black 5 (Ramirez–Montoya et al. 2015). This finding underscores that enzyme-substrate specificity is a critical determinant of efficiency. To enhance versatility, researchers propose developing a biochar–enzyme–dye compatibility database to optimize immobilization strategies for targeted dye removal.

Table 2 Performance metrics of biochar-immobilized enzymes in pollutant removal

Immobilized enzyme	Pollutant	Removal efficiency	Reusability	Reference
Amine-functionalized magnetic biochar–laccase	Bisphenol A (water)	60 min; 20 mg/L; 100%	6 cycles; 90%	Yang et al(2025b)
Bamboo biochar–laccase	Bisphenol A, malachite green, methyl orange (water)	2 h; 50–60 mg/L; 74.72–94.53%	5 cycles; 79.48–86.82%	Zhang et al. (2025b)
Antibiotic fermentation residue immobilized multi–enzyme	Penicillin G sodium (water)	20 min; 900 mg/L; 99.84%	5 cycles; 51.94%	(Zhang et al. 2025a)
Acid-modified cherry stone–laccase	Dyes (water)	4 h; 50 mg/L; 92%	–	Antanaskovic et al. (2024)
Magnetic pine biochar–laccase	Trichloroethylene (water)	48 h; 10 mg/L; 92.1% 48 h; 10 mg/L; 92.1%	10 cycles; 48.5%	(Yang et al. 2024b)
Pistachio shell biochar–laccase	Diclofenac, carbamazepine, ciprofloxacin (water)	60 min; 50 mg/L; > 99%	10 cycles; 35.29%	(Al-sareji et al. 2024)
Mushroom substrate biochar–laccase	Aflatoxin B1 (water)	5 h; 80 μg/kg; > 90%	5 cycles; 85%	Rasheed et al. (2024)
Magnetic biochar–PET hydrolase	Microplastics (soil)	90 d; 200 mM; 71.5%	5 cycles; 58.5%	(Han et al. 2024)
Alginate–biochar–laccase	Chloroform (soil)	5 h; 1000 ppb; 96.32%	5 cycles; > 50%	Zheng et al. (2024)
Chestnut shell biochar–crude enzyme	Polycyclic aromatic hydrocarbons (soil)	10 days; 37%	–	Zhao et al. (2024)
Rapeseed straw–laccase	2,4-Dichlorophenol (water)	6 h; 50 mg/L; 99%	5 cycles; 42%	Xie et al. (2023)
Pine needle biochar–laccase	Mixed dyes (water)	5 h; 50 mg/L; 59.3–88.1%	–	Pandey et al. (2023)
Magnetic biochar–laccase	Quinolones (water)	48 h; 10 mg/L; 65.4–93.7%	7 cycles; 42.1–48.4%	Zou et al. (2023)
Coconut shell biochar–laccase	Pharmaceuticals (water)	120 min; 50 mg/L; 100%	3 cycles; > 90%	Al–sareji et al. (2023c)
Orange peel biochar–laccase	Carbamazepine, diclofenac (water)	24 h; 25 mg/L; 73.34–82.51%	6 cycles; 48.2%	Al-sareji et al. (2023a)
Corn stalk biochar–laccase	Petroleum hydrocarbons (water)	7 days; 3687.44 μg/L; 41.4%	6 cycles; 40.9%	Zhou et al. (2023)
Pomegranate peel biochar–laccase	Pharmaceuticals (water)	2 h; 50 mg/L; 37.2–90.4%	6 cycles; 11.6–47.9%	Al-sareji et al. (2023b)
Rice straw biochar- Horseradish peroxidase	Phenol (water)	1 h; 188 mg/L; 80%	7 cycles; 60%	Liu et al. (2023)
Magnetic biochar–haloalkane dehalogenase	Halogenated hydrocarbons (water)	7 h; 5 mM; 100%	30 cycles; 70%	Jiang et al. (2022)
Pine needle biochar-laccase	Malachite green (water)	5 h; 50 mg/L; 85%	6 cycles; 53%	Pandey et al. (2022)
Modified biochar–laccase	Polycyclic aromatic hydrocarbons (soil)	40 days; 110 ppb; 90%	5 cycles; 80%	Zheng et al. (2023)
Alkali-modified biochar–laccase	Malachite green (water)	300 min; 100 mg/L; > 90%	10 cycles; 85.97%	Wang et al. (2022c)
Magnetic biochar–chromate reductase	Cr(VI) (water)	30 min; 200 μM; 98%	5 cycles; 68.3%	Han et al. (2022)
Halloysite nanotube–laccase	Bisphenol A (water)	12 h; 40 mg/L; 87.31%	8 cycles; 44.24%	Wang et al. (2022a)

5.1.3 Catalytic transformation of phenolic and polycyclic aromatic hydrocarbons

Phenolic wastewater, characterized by high toxicity, recalcitrance, and complex composition, presents significant challenges in water treatment. Bisphenol A (BPA), a representative endocrine-disrupting phenolic pollutant, induces long-term biological harm through hormonal interference (Moradi et al. 2021). Nano-magnetic biochar–immobilized laccase demonstrates exceptional potential for such contaminants. This system achieves complete removal of 25 mg/L BPA within 75 min via synergistic adsorption and enzymatic oxidation, with an enzyme activity reaching 2.251 U/mg (Zhang et al. 2020). The catalytic mechanism involves laccase-mediated oxidation of phenolic hydroxyl groups into phenoxy radicals, which dimerize via ether or carbon–carbon bonds to form diphenyl ether or biphenyl derivatives. Subsequent oxidation generates semiquinone and para-quinone intermediates, ultimately mineralizing into CO_2 and H_2O through ring-opening reactions (Hautphenne et al. 2016).

PAHs, persistent organic pollutants with high stability, carcinogenicity, and bioaccumulation potential,

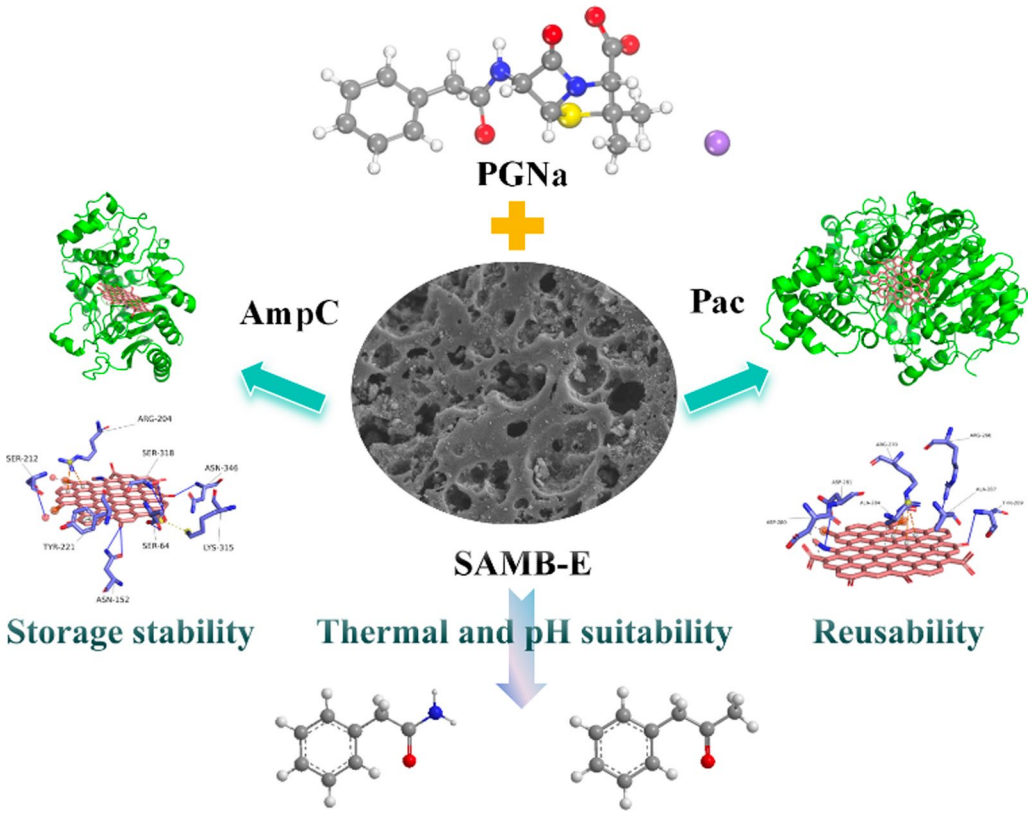


Fig. 3 The synergistic mechanism of multienzyme complex immobilization on antibiotic fermentation waste-derived biochar and its catalytic degradation pathway for penicillin G sodium (Zhang et al. 2025a)

severely threaten ecosystems. Among the 16 US EPA priority PAHs, anthracene and benzo[a]pyrene are the most readily oxidized by laccase due to their low ionization energies. Acid-treated rice straw biochar immobilized with ligninolytic enzymes completely degrades 50 mg/L anthracene within 24 h, retaining 40% activity after 6 cycles (Imam et al. 2021). For high-ring PAHs like benzo[a]pyrene, a nano–biochar–chitosan co–immobilized dual–enzyme system (catechol 1,2–dioxygenase and toluene/o–xylene monooxygenase) achieves 85% degradation of 200 mg/L aromatic hydrocarbons in groundwater through efficient aromatic ring cleavage (Miri et al. 2021). However, current literature lacks detailed mechanistic explanations, which is a key area for future research. Current challenges include enzyme inhibition by high-concentration chlorophenols and steric hindrance limiting degradation of bulky PAHs. Surface sulfonation modifications enhance anti–interference capacity of biochar, while nanoconfined reaction carriers show promise in overcoming spatial constraints, offering pathways to advance practical applications of immobilized enzyme systems in complex pollutant remediation.

5.2 Soil pollution remediation

5.2.1 Enzymatic degradation of pollutants

The enzymatic degradation of recalcitrant soil contaminants—such as chloroform, PAHs, and microplastics—has gained prominence due to its efficiency and environmental compatibility. Immobilizing enzymes on biochar carriers enhances catalytic efficiency and stability while synergizing with soil microbiota to achieve pollutant transformation and ecological restoration. In a case study of contaminated soil at a Taizhou chemical plant, a sodium alginate–biochar–laccase composite (SA–BC–LAC) demonstrated remarkable remediation efficacy through combined chemisorption and enzymatic catalysis. The addition of 5% (w/w) SA–BC–LAC reduced chloroform levels by 88.9% within 1 h, with adsorption and enzymatic degradation contributing 31.3% and 68.7% to total removal, respectively. The multilayered porous architecture of the composite not only enhanced laccase loading but also enriched dechlorinating *Pseudomonas* species, promoting co-metabolic dechlorination pathways. Notably, SA–BC–LAC retained 48% residual activity after 50-days storage, highlighting its long-term remediation

potential (Zheng et al. 2024). For PAHs–contaminated soils, acid-modified chestnut shell biochar immobilized with white-rot fungal enzymes degraded 37% of benzo[a]pyrene over 10 days via hydroxylation and radical-mediated oxidation, culminating in mineralization to CO_2 and H_2O (Hussain et al. 2024). Glutaraldehyde-crosslinked laccase on 30-mesh biochar achieved 64.6% degradation of 2,4-dichlorophenol, with efficiency strongly dependent on biochar particle size and porosity. Smaller particles increased enzyme loading by > 30% due to higher surface area (Wang et al. 2021b). In addition, magnetic biochar further simplifies catalyst recovery via external magnetic fields, reducing operational costs. For instance, magnetic biochar–immobilized polyester hydrolase (MB–LCC–FDS) degraded polyethylene terephthalate microplastics through an "adsorption–hydrolysis–metabolism" cascade, converting them into mono(2-hydroxyethyl) terephthalate (MHET), which soil microbes metabolize into non-toxic byproducts. This approach also modulated microbial communities, increasing *Skermanella* abundance while enhancing nitrogen fixation and phosphorus transport (Han et al. 2024). These biochar–enzyme systems operate via a tripartite mechanism—adsorption, catalysis, and microbial synergy—to degrade chlorinated hydrocarbons, PAHs, and microplastics while restoring soil ecological functions. Implementation requires tailored carrier–enzyme combinations based on pollutant characteristics and careful assessment of intermediate metabolite risks, offering innovative solutions for industrial-scale soil remediation.

5.2.2 Soil amendment and carbon sequestration

Biochar exhibits multidimensional synergies as a soil amendment, enhancing soil health and carbon sequestration while restoring ecological functions through microbial community regulation and enzyme activity modulation. Its porous architecture provides physical refuges for soil microbes, mitigating environmental stressors like drought and extreme pH. Low–temperature pyrolyzed biochar (< 500 °C), rich in labile carbon and nitrogen, significantly increases microbial biomass and diversity. In controlling soil-borne diseases, biochar adsorbs pathogen-secreted enzymes (pectinase and cellulase) and toxins (*Fusarium* mycotoxins), reducing their bioavailability. Immobilization decreases pectinase activity by 57% and reduces tomato wilt incidence by 40%, mediated by electrostatic interactions and steric hindrance between biochar functional groups (–COOH) and enzyme active sites (Yang et al. 2024a). While laboratory models validate these adsorption mechanisms, field dynamics—particularly competitive adsorption with soil organic matter—require further exploration. Biochar–enzyme composites concurrently degrade pollutants and sequester soil organic carbon via physical adsorption and chemical bonding, establishing a dual"remediation–sequestration"mechanism. For example, biochar–immobilized carbonic anhydrase enhances CO_2 absorption in potassium carbonate solutions while leveraging the stable carbon matrix of biochar for long-term sequestration, which merges ecological restoration with climate regulation (Fig. 4) (Shen et al. 2025). This triad mechanism—enzyme immobilization, carbon locking, and pollutant

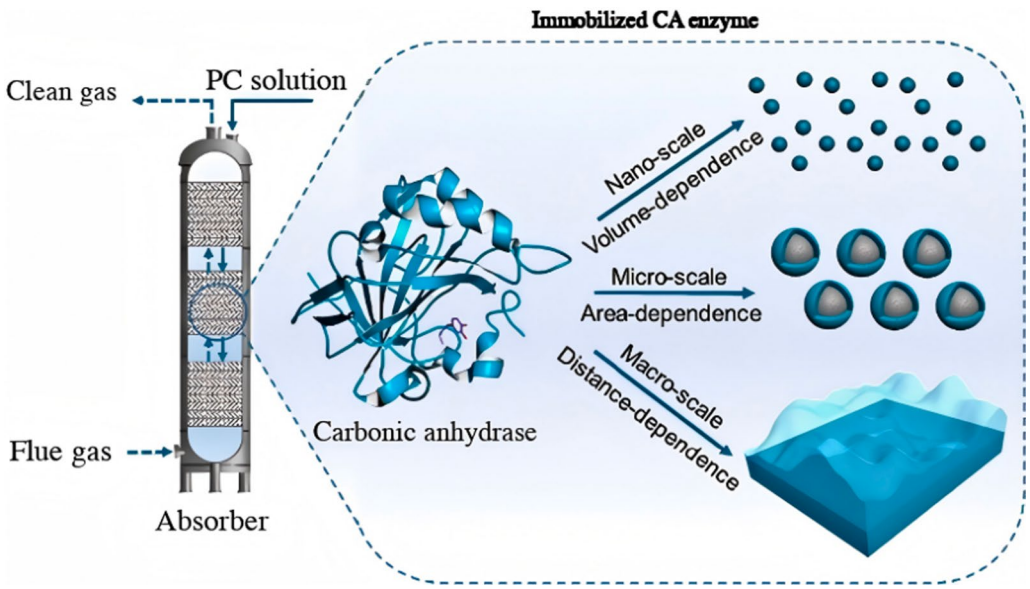


Fig. 4 Synergistic CO_2 capture mechanisms of immobilized enzymes (Shen et al. 2025)

passivation—enables synergistic soil improvement and carbon management. Future research should prioritize multifunctional designs, such as pH-responsive smart biochar coatings, to simultaneously suppress pathogens, stabilize heavy metals, and optimize carbon sequestration efficiency, accelerating the translation from laboratory innovations to large-scale agricultural remediation.

6 Discussion: challenges and future perspectives

6.1 Breakthroughs in the intelligent design paradigm to overcome the carrier–enzyme matching bottleneck

The primary challenge of biochar–immobilized enzyme systems lies in precisely matching the carrier and enzyme molecules, which requires overcoming multi-scale design barriers from molecular recognition to macroscopic structural alignment. Traditional trial-and-error methods are time-consuming, costly, and fail to reveal the underlying mechanisms of carrier–enzyme interactions, leading to issues such as active site shielding or conformational distortion. To enhance design efficiency and overcome these challenges, machine learning, a powerful optimization tool, has been widely applied in parameter optimization, pattern recognition, and predictive model development. By integrating machine learning with molecular simulations, researchers can efficiently predict the interactions between biochar and enzymes and select the best carrier–enzyme pairing from various design options. This approach breaks through the limitations of traditional methods and provides innovative solutions for enzyme immobilization systems.

6.1.1 Molecular simulation–driven functional group matching

By utilizing artificial neural networks (ANN) and random forest algorithms to construct predictive models, it is possible to precisely calculate the binding free energy (ΔG) between biochar surface functional groups and enzyme active sites, thereby enabling the rational selection of physical adsorption and covalent binding sites. For example, research by Sankar and Achary showed that this method increased lipase loading to 255 mg/g of carrier, enhanced enzyme-specific activity to 5808 U/mg, and retained 69% activity after 15 reaction cycles (Sankar and Achary 2020). The random forest model, which integrates soil properties (metal types and clay content), biochar attributes (pyrolysis temperature), and crop parameters, successfully predicted the uptake of heavy metals by crops ($R^2 = 0.73$), revealing that soil features contributed 79.7% to heavy metal absorption. This new paradigm provides insights for tailoring biochar design to enhance heavy metal remediation (Li et al. 2024).

6.1.2 Biomimetic mineralized pore design

Inspired by natural biomineralization processes, a biomimetic method has been used to synthesize MOFs with adjustable mesopores by using bovine serum albumin as a template. This design achieves a synergistic effect of micropores, mesopores, and macropores. Micropores (< 2 nm) serve as enzyme anchoring sites, providing a stable immobilization interface, while mesopores (2–50 nm) accelerate substrate diffusion, improving diffusion efficiency. Macropores (> 50 nm) significantly reduce mass transfer resistance. Proper matching between the carrier pore size and the enzyme molecular dimensions molecule greatly enhances the stability and catalytic efficiency of the immobilized enzyme. Notably, the substrate affinity of the immobilized HRP@UIO-66 is similar to that of the free enzyme, indicating that the optimized pore structure effectively prevents blockage of the substrate mass transfer channel after enzyme adsorption, thereby improving catalytic efficiency (Wu et al. 2022).

6.1.3 Multi–modal intelligent design platform driving industrial transformation

The preparation parameters of biochar, including pyrolysis temperature, feedstock type, and modifying agent concentration, significantly influence its pore structure and surface chemistry. Recently, machine learning models (such as ANN and support vector machines) have been used to predict the optimal preparation conditions for biochar. For example, using a database of pyrolysis temperature (300–700 °C), feedstock ash content (5–30%), and modifier concentration (0.1–5 mol/L), the ANN model can predict the specific surface area with an error rate of less than 10%, significantly shortening the experimental optimization cycle (Luo et al. 2023b). Furthermore, a bibliometric analysis reveals that research on optimizing biochar–enzyme composite systems using machine learning has increased by 3.5 times since 2020. Future research can combine high-throughput characterization techniques (in situ X-ray diffraction and synchrotron radiation spectroscopy) to create a multi–modal data-driven predictive platform (Luo et al. 2023a; Yang et al. 2021).

6.2 Multi–technology synergy for complex functional systems

Single remediation techniques often fail to address complex pollution scenarios effectively, and multifunctional, synergistic systems are emerging as a future development trend. By integrating processes such as adsorption, catalysis, and electrochemical reactions, biochar–immobilized

enzyme systems can simultaneously remove multiple pollutants.

6.2.1 Nanomaterials for enhanced synergy

MOFs combined with biochar carriers enhance catalytic efficiency. For instance, a MOF–biochar composite increased the catalytic efficiency of β–lactamase by six times compared to free enzymes (Yang et al. 2021). Additionally, polymer-assisted nanoflowers loaded with glucose oxidase and horseradish peroxidase improved enzyme activity by 2–3 times with no activity loss after six cycles, while significantly enhancing heat stability and pH tolerance (Pomini et al. 2023). Future research may explore combining biochar with magnetic nanoparticles (Fe_3O_4) or conductive polymers (polyaniline) to create composite systems that enable multi-stage pollutant removal through adsorption, enzyme catalysis, and electrochemical oxidation.

6.2.2 Closed-loop resource recovery process

Biochar–immobilized enzymes extend beyond pollutant degradation to enable resource recovery. In the paper recycling industry, laccase plays a crucial role in removing ink and lignin, contributing to fiber resource regeneration. The enzyme oxidizes lignin to release ink captured on the fiber surface, replacing toxic chemical bleaching agents and significantly reducing wastewater toxicity. Although this process can maintain the quality of recycled fibers under optimized conditions, challenges remain, such as high enzyme production costs and insufficient stability. Immobilization on biodegradable carriers enhances enzyme reusability and resistance to temperature and pH, driving the paper industry towards a "zero chemical input – biobased economy" transition (Pandey and Gupta 2024).

6.3 Key challenges in engineering application conversion

Despite breakthrough progress in laboratory research, the large-scale application of biochar–immobilized enzyme technology still faces multiple challenges.

6.3.1 Innovation in continuous flow reactors

Traditional batch processing methods are inadequate for large-scale remediation needs. Continuous-flow immobilized enzyme membrane reactors are critical for scaling up the application. Research has shown that by using thermal conversion technology to combine wood biochar with polyvinylidene fluoride, a high-throughput multifunctional membrane can be created. The biochar membrane flux reached 6895 ± 72 L/(m^2·h), which is 460 times greater than traditional copper alginate membranes, making it suitable for high-flow wastewater pre-treatment. This membrane demonstrated an adsorption capacity of 187 mg/g for rhodamine B dye, achieving a 93% retention rate for *Escherichia coli* through pore size screening. This design enables sequentially integrated pollutant removal via adsorption, sieving, and enzymatic catalysis (Ghaffar et al. 2018). In another case, after immobilizing laccase on pine and pig manure biochar, a continuous fixed-bed column was constructed with a removal rate of 88% for bisphenol A, and the fixed-bed biochar column was successfully scaled up using the Thomas and Yoon–Nelson models (Lonappan et al. 2019). Future research should focus on optimizing reactor fluid dynamics, such as introducing microchannel structures or 3D-printed porous supports, to reduce mass transfer resistance and improve treatment efficiency.

6.3.2 Breakthroughs in cost control technology

Raw material costs and energy consumption are key constraints for large-scale applications. In-situ conversion technologies for agricultural and forestry waste (straw and rice husks) can reduce raw material costs by 30–50% (Gross et al. 2021). For example, in-field preparation devices for straw biochar have achieved precise control of pyrolysis temperature (± 10 °C), reducing energy consumption for processing per ton of raw material to 200 kWh (Luo et al. 2023a). The reuse of biochar–enzyme composites is economically feasible. Future research should focus on developing low-energy modification technologies, such as photocatalytic activation or plasma treatment, to further reduce costs.

6.4 Development directions for life cycle management

The sustainable development of biochar–immobilized enzyme technology requires a systematic evaluation from a life cycle perspective, covering preparation, application, and regeneration.

6.4.1 Construction of a carbon-neutral closed-loop industry chain

The life cycle assessment (LCA) consensus of biochar derived from waste pyrolysis indicates a net carbon reduction range of –0.2 to –0.9 t CO_2e/t, primarily from carbon sequestration (> 50%), byproduct energy substitution, and the inhibition of N_2O/CH_4 in soil (19–52%) (Zhu et al. 2022). Moving forward, a "waste–biochar–enzyme–carbon credit" high-value chain should be developed. This model efficiently degrades pollutants (petroleum hydrocarbons) through immobilized enzymes, reducing remediation energy consumption, while enhancing soil carbon sequestration to generate carbon benefits from remediation. By integrating biochar carbon sequestration and supply chain emissions, net negative emissions can be systematically achieved (Hussain et al. 2024). The current bottleneck lies in energy

consumption during enzyme fermentation, chemical modification emissions of carriers, and the costs associated with magnetic recovery and carbon regeneration. There is an urgent need to develop low-temperature mineralization modifications and in-situ regeneration technologies for flow-bed systems. Resolving these limitations will enhance the carbon-neutral industry lifecycle efficiency of the chain, advancing waste valorization, environmental remediation, and carbon neutrality goals.

6.4.2 Long-term ecological risk assessment

The widespread use of biochar could pose several risks, including:

① Toxicant leaching: feedstock-derived heavy metals, PAHs, and dioxins may contaminate ecosystems. Sewage sludge-derived biochar leaches PAHs at concentrations up to 11.75 μg/L in leachates (Chen et al. 2019).

② Soil microbiome disruption: biochar amendments reduce abundance and activity of Microbacteriaceae and Aeromicrobium, impairing soil ecosystem functionality (Qiu et al. 2019).

③ Environmental toxicity: environmentally persistent free radicals in biochar exhibit neurotoxicity to soil biota, inhibiting their survival and reproduction (Xiang et al. 2021).

④ Nanoparticle hazards: small biochar particles are more readily ingested by organisms, thereby amplifying the adverse effects of pollutant adsorption on organisms (Peng et al. 2024).

Given these risks, the use of biochar, especially at high concentrations, should be carefully evaluated for its long-term impact on the environment and ecosystem.

7 Conclusions

Biochar–immobilized enzyme technology combines the adsorption capacity of biochar with enzymatic catalysis, offering an efficient and environmentally friendly solution for the remediation of water and soil pollutants. This review synthesizes the application mechanisms, emphasizing enzyme–carrier interactions, the selection of immobilization techniques, and remediation efficacy. Biochar, as a carrier, offers advantages such as low cost, high specific surface area, and abundant surface functional groups. Through pyrolysis and surface modification, biochar can optimize its compatibility with various enzymes, significantly enhancing enzyme stability, activity, and reusability. Biochar–enzyme composites exhibit significant synergy in removing diverse contaminants—including organic pollutants, pharmaceuticals, dyes in water, and organic compounds along with microplastics in soil-enabling versatile remediation. Furthermore, biochar contributes carbon sequestration and soil enhancement, amplifying its dual role in ecological restoration and carbon neutrality. Despite promising lab-scale results, field deployment faces challenges such as feedstock variability, enzyme deactivation, and long-term ecological safety. Future research must prioritize intelligent design platforms for optimized carrier–enzyme compatibility, multifunctional composites, and rigorous ecological risk assessments. The large-scale application of biochar–immobilized enzyme technology requires addressing challenges such as raw material cost, energy consumption, and ecological safety. However, its potential in environmental remediation remains immense. Through interdisciplinary innovation and technological optimization, biochar–immobilized enzymes are poised to provide significant solutions for global environmental pollution control and sustainable industrial applications, contributing to the advancement of green and low-carbon technologies.

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Data availability
The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

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

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