



Review

Immobilization of laccase on biochar for the remediation of organic pollutants: A comprehensive review

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ABSTRACT

The resolution of long-residue, carcinogenic, and mutagenic organic pollutants has become a critical global challenge. This review focuses on recent advances in the understanding of biochar-immobilized laccase systems for the remediation of organic pollutants. The background and significance of research in this field are first introduced, followed by an emphasis on the major challenges and limitations currently encountered. Subsequently, an in-depth analysis of the latest research findings concerning the degradation of organic pollutants is conducted. The characteristics of laccase, along with the advantages and disadvantages of various immobilization techniques such as adsorption, covalent binding, and cross-linking, the properties of different immobilization carriers, and the benefits of using biochar as a carrier for laccase immobilization are discussed. Furthermore, the degradation mechanisms of five types of organic pollutants mediated by biochar-immobilized laccase are explored, along with factors influencing degradation efficiency. Biochar-immobilized laccase enhances enzyme stability and allows for reuse, thereby reducing operational costs. In addition, its simple preparation process significantly improves degradation efficiency while broadening its applicability in real-world pollution control scenarios due to its supportive and protective role. This review aims to provide valuable references to assist in selecting the most suitable biochar-immobilized laccase system for effective remediation of pollutants.

1. Introduction

The global economic upsurge has spurred the rapid production and extensive utilization of industrial and agricultural chemicals, many of which are released into the environment as potentially toxic pollutants, thereby severely disrupting ecological balance. These pollutants typically exhibit characteristics such as semi-volatility, persistence, and high lipophilicity, posing significant risks to human health, including potential genetic mutations, carcinogenicity, and reproductive disorders [1]. The issue of organic pollutants has increasingly drawn attention from both the scientific community and regulatory bodies worldwide, prompting researchers to propose various remediation technologies that integrate physical, chemical, and biological approaches. These methods capitalize on the specific properties of organic pollutants to effectively remove and degrade them, thereby minimizing their adverse impacts on human health and the environment. For instance, Wu et al. developed environmentally friendly biomass aerogels via the moxibustion method,

which can physically adsorb and remove nearly 100 % of environmental pollutants such as mitoxantrone and malachite green [2]. Furthermore, numerous studies have underscored the efficacy of photocatalytic technology in degrading pollutants [3]. However, many chemical and physical methods are economically burdensome, challenging to implement at large scales, and prone to secondary pollution, making them less viable for widespread application [4]. As a result, the development of low-cost, efficient, and environmentally sustainable technologies for organic pollutant remediation remains a critical focus and ongoing pursuit for researchers globally.

Bioremediation processes involve harnessing the metabolic capabilities of diverse microorganisms to restore environmental integrity [5]. Enzymes, which are distinguished by their high catalytic efficiency and specificity toward a wide range of pollutants, demonstrate significant potential for environmental remediation [6]. Enzyme-based bioremediation represents an efficient, rapid, eco-friendly, and socially viable method for degrading persistent xenobiotic compounds within natural

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ecosystems [7]. This approach typically employs microorganisms along with their corresponding enzymes to convert harmful contaminants into less toxic or non-toxic substances. Several classes of enzymes, such as laccases, hydrolases, and peroxidases, play active roles in bioremediation processes [8]. Laccase, a copper-containing polyphenol oxidase enzyme featuring four copper ions at its catalytic core, is capable of oxidizing a broad array of substrates, including phenols, polyphenols, aminophenols, methyl/methoxy-substituted phenols, aryldiamines, and anilines [9]. Laccase-mediated catalysis involves the simultaneous reduction of molecular oxygen to water and the oxidation of the substrate, resulting in the formation of its respective radical species [10]. Due to water being its only byproduct, laccase is often described as a “green catalyst” [11]. These enzymes have found applications across multiple domains, including food processing, dye decolorization, pulp and paper manufacturing, and bioremediation [12–15]. The catalytic performance of laccase is influenced by various factors. Research has shown that the use of free enzymes in conventional applications is associated with several limitations, such as limited reusability, decreased stability, and poor cost-effectiveness. These challenges can be effectively mitigated through enzyme immobilization techniques [16–18]. Enzyme immobilization has emerged as a widely utilized strategy to improve enzymatic stability and enable repeated usage. Through continuous research efforts, laccase has been successfully immobilized onto a wide range of support materials, spanning from traditional matrices to advanced nanomaterials [19–22].

Biochar, a material characterized by multi-layered and fibrous porous structures, is generated as a by-product during the pyrolysis of carbon-rich biomass under oxygen-limited conditions [23]. It is regarded as a promising adsorbent and/or catalyst for the removal of organic compounds due to its extensive surface area, abundant surface functional groups, and rich surface defects [24–26]. Furthermore, biochar has been extensively utilized in metallurgy, chemistry, pharmacy, electronics, and bio-energy sectors, owing to its unique physicochemical properties (e.g., high pH, carbon content, cation exchange capacity, significant specific surface area, and aromatic structure) and sustainable economic advantages [27–29]. In recent years, biochar has emerged as a key material in environmental management, particularly in the adsorptive removal of various organic pollutants from diverse environmental matrices [30–32]. Previous studies have demonstrated the effectiveness of biochar in eliminating contaminants such as pesticides, pharmaceuticals, polychlorinated biphenyls, steroid hormones, dyes, and polycyclic aromatic hydrocarbons (PAHs) [33–38].

Single methods are often insufficiently effective in addressing the wide variability of environmental conditions and pollutant characteristics. Consequently, the integration of multiple approaches has demonstrated a broader scope of application, improved efficiency, and enhanced economic advantages [39]. The high specific surface area, porous structure, and excellent chemical stability render biochar an ideal carrier for laccase immobilization, owing to its significant adsorption capacity [40]. Previous related studies have shown that immobilizing laccase on organic materials or biochar-based composites can improve enzyme stability, reusability, and resistance to fluctuations in ionic conditions during the degradation process [41–43].

Recent advances in the degradation of organic pollutants have significantly deepened our understanding of laccase immobilization. The combination of biochar with laccase represents a promising strategy. However, there are still limited studies exploring the synergistic use of biochar's adsorption capacity and laccase's enzymatic activity for the degradation of organic pollutants. This review provides a comprehensive synthesis of the distinctive properties of biochar-immobilized laccase in the context of organic pollutant degradation, thereby addressing the existing knowledge gap. The objectives of this review are to: (1) systematically summarize the characteristics and advantages of biochar as a carrier for immobilized laccase; (2) present an overview of the methodologies employed for laccase immobilization on biochar; (3) comprehensively analyze the remediation mechanisms of biochar-

immobilized laccase in the cooperative degradation of organic pollutants; and (4) evaluate the key factors influencing degradation efficiency when utilizing biochar-based laccase immobilization technologies. This review offers a synthesis of recent developments in biochar-immobilized laccase systems, with a focus on their application in organic pollutant degradation. Its primary aim is to provide valuable foundational insights for the selection of optimal biochar-immobilized laccase systems in the remediation of organic pollutants and environmental protection.

2. Laccase: source, diversity and mechanism

2.1. Laccase source and diversity

Laccase was first discovered by Yoshida in 1883 from the exudates of Japanese lacquer trees (*Rhus verniciflua*) [44]. Subsequent research has demonstrated that it is produced by a diverse range of cellular organisms [45]. According to the UniProtKB database, laccase is present in 16 halobacteria (archaea), 6258 eukaryotes, and 1026 bacteria [46]. Laccase-producing organisms are classified into four major groups: fungal laccases, bacterial laccases, insect laccases, and plant laccases.

2.1.1. Fungi

Fungal laccases are highly efficient and widely utilized in industrial wastewater treatment due to their ability to degrade complex organic pollutants. The characteristic of fungal laccases as metal-containing oxidases was first identified by Bertrand in 1985 [47]. These enzymes are categorized into two groups: true laccases, which lack the ability to oxidize tyrosine, and false laccases, which possess this capability. Over 60 fungal species have been reported to produce laccase, particularly within the Ascomycetes, Deuteromycetes, and Basidiomycetes phyla. Notably, Basidiomycetes such as *Phanerochaete chrysosporium*, *Agaricus bisporus*, *Cerrena unicolor*, and *Trametes versicolor* are renowned for their lignin-degrading capabilities and efficient degradation of phenolic compounds and quinones. For instance, *Hexagonia hirta* MSF2 exhibits laccase production at 56,713.3 U/mL [48]. Species such as *Trichoderma atroviride*, *Aspergillus niger*, and *Aspergillus oryzae* demonstrate rapid growth and high laccase production. Laccase activity is influenced by pH and temperature, with peak activity observed at pH 5.5 and yeast extract concentrations of 1.3 g/L [49].

2.1.2. Bacteria

Bacterial laccase was first isolated from *Azospirillum lipoferum* in 1993, originating from the rice rhizosphere [50]. Since then, laccase has been gradually identified in many bacterial genera, including *Azospirillum lipoferum*, *Streptomyces lavendulae*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. These bacterial laccases have demonstrated high thermostability, stability across various pH levels, and effectiveness in pollutant degradation [46,51]. For instance, bacterial laccases generally exhibit optimal activity at pH 4–6 and temperatures ranging from 45 to 60 °C. Notably, some recombinant laccases, such as those from *Geobacillus yunchangensis*, remain stable up to 70 °C and pH 5 [52]. *Bacillus tequilensis* SN4 exhibits maximum activity at 85 °C and pH 8, while the coat protein A (CotA) produced by *Bacillus subtilis* shows excellent thermal stability and alkaline tolerance [53]. PAHs are considered serious environmental pollutants in soil and marine ecosystems, can be directly oxidized by laccase when their electrochemical potentials are close to or lower than that of the enzyme [54]. In contrast, laccases derived from *Campylobacter jejuni* and *Escherichia coli* exhibit relatively lower catalytic activity. Notably, *Pseudomonas aeruginosa* ADN04 produces 46 U/mL of laccase following optimization of the culture medium. Other laccase-producing species include *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Pseudomonas extremorientalis* BU118, and *Bacillus subtilis* MTCC2414 [45].

2.1.3. Insects and plants

Insects, particularly beetles and termites, are also known to produce

laccase. Key laccase-producing insect species include various beetles (*Anoplophora glabripennis*, *Tribolium castaneum*, *Monochamus alternatus*), termites (*Coptotermes formosanus*, *Reticulitermes flavipes*), and flies (*Drosophila melanogaster*, *Musca domestica*). Other notable laccase-producing insects include bees (*Apis mellifera*, *Bombus* spp.) and moths (*Bombyx mori*, *Manduca sexta*). Among these, *Rhynchophorus ferrugineus* possesses a laccase gene with high amino acid sequence identity, suggesting significant potential for enzymatic activity [55]. Laccase genes have also been identified in a variety of plants, including dicotyledonous species such as *Arabidopsis thaliana* [56], *Prunus avium* L [57], *Populus trichocarpa* [58], *Pyrus bretschneideri* [59], *Citrus sinensis* [60], lychee [61], and cotton [62], as well as monocotyledonous species [63] like corn [64] and rice [65].

2.2. Degradation properties and catalytic mechanisms of laccases

Organic pollutants introduced into the environment, whether intentionally or unintentionally, are primarily defined by their persistence, potential for bioaccumulation, and toxic effects. Prominent examples of such pollutants include organochlorine pesticides (OCPs) [66], polychlorinated biphenyls (PCBs) [67], dioxin-related compounds (DRCs) [68], and PAHs [69]. Laccase is utilized due to its broad substrate spectrum and the critical property of molecular oxygen serving as the final electron acceptor. The mechanisms of laccase action in the degradation of organic pollutants include oxidation, epoxidation, hydroxylation, dechlorination, and free radical-mediated degradation. Laccase is a glycosylated monomeric or homodimeric protein belonging to the family of blue multicopper oxidases (MCOs), and its action on substrates is mediated by a copper cluster located in the active site. The catalytic core of most laccases consists of four copper atoms involved in redox processes, which are classified into three structural types: type 1 (T1), paramagnetic “blue” copper; type 2 (T2), paramagnetic “non-blue” copper; and two type 3 (T3) centers, forming a diamagnetic spin-coupled center. Laccases catalyze the oxidation of substrates (phenolic and aromatic compounds, aliphatic amines, and inorganic cations), coupled with the reduction of one oxygen molecule to two water molecules. This process occurs in three stages (as shown in Fig. 1): (1) T1 copper is reduced upon accepting an electron from the oxidized substrate; (2) the electron received by T1 copper is transferred to the T2–T3 center; and (3) molecular oxygen is reduced to form two water molecules [70]. The initial electron acceptor in laccase-catalyzed oxidation is T1 copper, located in a cavity near the enzyme surface. Reduction of T1 copper represents the rate-limiting step in laccase-catalyzed reactions. The most typical substrates for laccase are phenolic compounds with relatively low T1 redox potentials, which are oxidized by laccase to phenoxyl

radicals. Depending on the redox reversibility of phenoxyl radicals, radical-based coupling or redox recycling of phenolic substrates as mediators expands the range of laccase substrates [71]. Based on their redox potentials, laccases are categorized into two groups: low-redox-potential and high-redox-potential enzymes. Low-redox-potential enzymes are predominantly found in bacteria, plants, and insects, whereas high-redox-potential laccases are widely distributed in fungi [72]. Laccases catalyze both anabolic and catabolic reactions. Representative catabolic processes include lignin degradation and humus breakdown mediated by fungal laccases. Laccase-catalyzed anabolic reactions are involved in morphogenesis, such as polymeric pigment synthesis, cuticle sclerotization, polyflavonoid synthesis, lignification, and soil organic matter stabilization [73].

Laccase degradation efficiency is closely associated with enzyme activity, substrate concentration, and reaction conditions. Laccase exhibits greater stability under acidic conditions, demonstrating high activity and effective degradation performance [74]. Furthermore, the degradation properties of laccase are also characterized by its non-specific oxidation of pollutants, meaning that laccase can act on a broad spectrum of structurally diverse organic pollutants, thereby showcasing extensive potential applications in environmental remediation.

3. Advantages of biochar as an immobilized carrier

Free laccase is characterized by its limited stability and the challenges associated with recovery and reuse in various applications. The selection of appropriate support materials is critical for successful laccase immobilization. Ideal support materials not only stabilize the enzyme's structure and preserve its activity over multiple cycles but also exhibit several essential properties. Firstly, they must demonstrate superior chemical, thermal, and mechanical stability. Secondly, they should be renewable or cost-effective. Lastly, they need to be environmentally friendly and minimize potential pollution. Furthermore, an effective immobilization material should facilitate stable interactions between the enzyme and its substrate, ensuring secure immobilization while maintaining the structural integrity of the enzyme and its active site. In light of these requirements, biochar stands out as a promising carrier for laccase immobilization in the treatment of organic pollutants. Fig. 2 illustrates the advantages of biochar as an immobilized carrier. Its high surface area, abundant pore structure, and excellent physical and chemical properties render it highly suitable for this purpose. Biochar's unique structure not only enhances enzyme loading and stabilization but also improves the catalytic efficiency of immobilized laccase, showcasing significant potential for applications in environmental

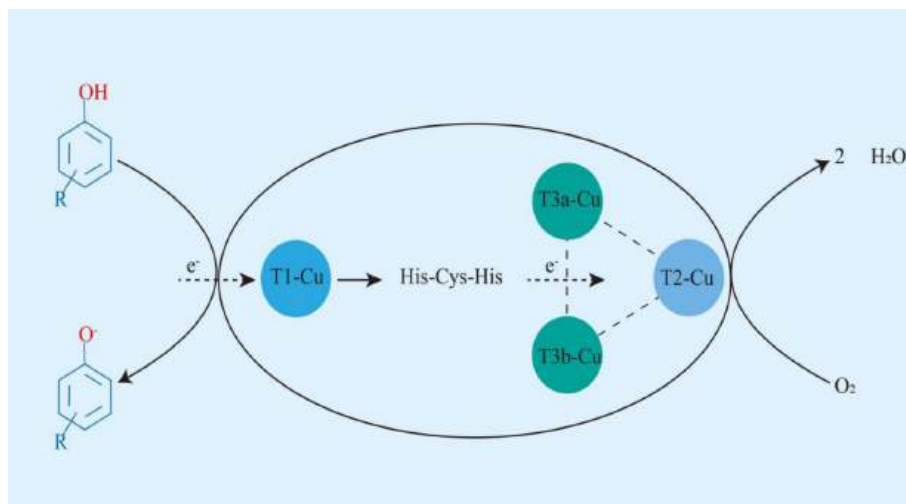


Fig. 1. Mode of action of laccase.

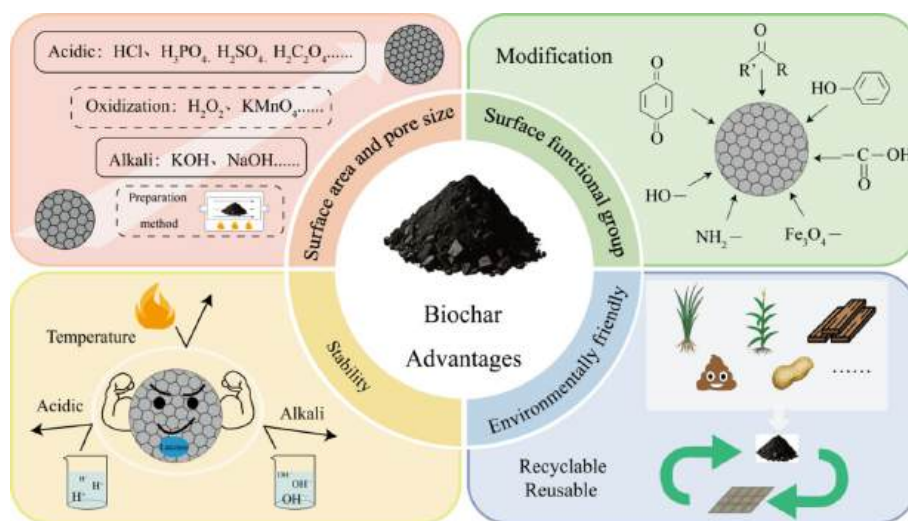


Fig. 2. Advantages of biochar as an immobilized carrier.

remediation and other domains. Some original or modified biochar-based laccase immobilization applications for treating organic pollutants are summarized in Table 1.

3.1. Surface area and pore size

Biochar is an insoluble, stable, highly aromatic, carbon-rich solid generated through the slow pyrolysis of biological residues at elevated

temperatures under oxygen-limited conditions [88]. During pyrolysis, increasing temperatures drive volatiles out of the biomass, leading to the formation of a porous structure and an enhanced specific surface area [89,90]. The porous architecture and large specific surface area of biochar provide abundant active sites for the adsorption and immobilization of laccase. This facilitates stronger interactions between laccase and its substrate, thereby improving the catalytic efficiency of the enzyme [91].

Table 1

Some original or modified biochar-immobilized laccase applications for organic pollutants degradation.

Biochar source	Laccase species	Optimal conditions	Enzymatic activity	Organic pollutants	Degradation efficiency	References
Corn straw (Acidic treatments)	<i>Aspergillus</i> (Purified)	pH 3, 35 °C	Not provide	Polycyclic aromatic hydrocarbons (PAHs), BTEX, kerosene, and petroleum distillates	PAHs: 12.0 %–41.4 % Phenanthrene: 91.2 %	[75]
Maple wood	<i>Coprinus comatus</i> (Purified)	pH 3, 25 °C	800 U/g	Polychlorinated biphenyls (PCBs), such as 4-hydroxy-3,5-dichlorobiphenyl(HO-DiCB)	HO-DiCB: 71.4 %	[76]
Bagasse (Magnetic biochar)	<i>Trametes versicolor</i> (Purified)	pH 3.5, 25 °C	2.251 U/mg	Bisphenol A (BPA)	BPA: 100 %	[77]
Pine sawdust (Magnetic biochar)	<i>Trametes versicolor</i> (Purified)	pH 4, 25 °C	0.5 U/mg	Trichloroethylene (TCE)	TCE: 92.1 %	[78]
Rice straw (alkali-modified biochar)	<i>Trametes versicolor</i> (Purified)	pH 4.1, 25 °C	0.99 U/mg	Malachite green (MG)	MG: 89.68 %–98.77 %	[19]
Wheat straw (alkali and magnetic composite modified)	<i>Trametes versicolor</i> (Purified)	pH 5, 25 °C	120 U/g	Polycyclic aromatic hydrocarbons(PAHs)	PAHs: 77.25 %–91.36 %	[79]
Rice straw (acidic treatments)	<i>Trametes maxima</i> (Crude)	pH 3.1, 25 °C	500 U/g	Polycyclic aromatic hydrocarbons(PAHs), Anthracene	Anthracene: 99 %	[80]
Pinewood (acidic treatments)	<i>Trametes versicolor</i> (Crude)	pH 3.5, 25 °C	1.48 U/g	Carbamazepine	Carbamazepine: 86 %	[81]
Green coconut husk biomass	<i>Trametes versicolor</i> (Purified)	pH 4, 45 °C	51.5 U/g	2,4-Dichlorophenol (2,4-DCP)	2,4-DCP: 72.49 %–84.64 %	[82]
Bamboo	Purchase	pH 4, 40 °C	1404.17 U/g	Bisphenol A (BPA), malachite green (MG) and methyl orange (MO)	BPA: 74.72 % MG: 85.88 % MO: 94.53 %	[83]
Mushroom	<i>Pleurotus ostreatus</i> (Crude)	pH 4.5, 40 °C	16.9 U/mg	Bisphenol A (BPA)	BPA: 90.87 %	[84]
Pine needle	Donated	pH 4, 30 °C	38.8 U/g	Congo red and Malachite green	Congo red: 88.1 % Malachite green: 73.8 %	[85]
Apple branch powder	<i>Trametes versicolor</i> (Purified)	pH 4, 15 °C	≥0.5 U/mg	Quinolone antibiotics	Quinolone antibiotics: 65.4 %–93.7 %	[86]
Spent coffee ground	<i>Megasporeporia</i> sp. (Crude)	pH 4, 28 °C	16.39 U/g	Dye decolorization (azo dye)	Azo dye: 95.00 %	[87]

The type of feedstock significantly influences the specific surface area of the resulting biochar. While the specific surface area and pore structure of biochar vary depending on the feedstock and preparation conditions, they generally offer suitable environments for laccase immobilization [76]. Biomass with higher cellulose and hemicellulose content tends to produce biochar with a greater abundance of oxygen-containing functional groups, which introduces novel and diverse sites for enzyme-support interactions [92].

Different activation methods can effectively enhance the surface area of biochar. For instance, carbon dioxide activation increased the surface area of barley malt residue biochar by 80 % [93]. Similarly, steam activation resulted in a fivefold increase in the specific surface area of biochar prepared from discarded mushroom substrate [94]. Physical activation of biochar directly influences its structural properties, such as pore size and pore volume, which subsequently affects enzyme immobilization. In one study, cotton straw biochar purged with N₂ was subjected to gas activation using carbon dioxide or ammonia after reaching the maximum temperature, leading to an increase in microporous volume. Oak biochar produced in a CO₂ environment exhibited enhanced release of volatile organic carbon and nearly double the total pore volume compared to biochar produced in an N₂ environment [95]. Biochar can also undergo chemical modification, including acid-base modification [96], oxidative modification, and metal oxide-loaded modification [97], to further increase its specific surface area, optimize pore size distribution, and improve laccase immobilization efficiency. For example, treatment with sulfuric and oxalic acids increased the specific surface area of un-activated biochar by up to 250 times [98].

3.2. Stability

Biochar exhibits high thermal stability and can maintain relatively stable structural integrity across a wide temperature range, thereby providing a stable microenvironment for laccase and enhancing its stability. The rigid structure of biochar effectively shields laccase from denaturants or extreme conditions, significantly extending the enzyme's lifespan and enabling its multiple reuse [99]. Additionally, biochar demonstrates excellent chemical stability in acidic, alkaline, and organic solvent environments, ensuring its stability under diverse reaction conditions and preserving the activity of immobilized laccase in complex scenarios [100,101]. The composition of the feedstock also plays a critical role in determining the properties of biochar. During pyrolysis, the thermochemical decomposition of biomass releases water, carbon dioxide, and carbon monoxide, leading to a reduction in the O/C and H/C ratios. This effect is particularly pronounced at higher temperatures, indicating an increase in aromaticity and thus enhanced chemical stability. Biomass with high lignin content produces biochar with higher yields and superior thermal stability compared to materials rich in cellulose and hemicellulose. The degree of aromatization is the most significant factor influencing biochar stability, alongside feedstock composition and pyrolysis process conditions. The aromatic carbon content in biochar increases markedly with rising pyrolysis temperatures [92], resulting in biochar obtained via high-temperature pyrolysis exhibiting greater thermal stability. Similarly, biochar derived from feedstock with high lignin content demonstrates higher stability [90]. Biochar prepared from animal manure is nutrient-rich, possesses high cation exchange capacity, and exhibits enhanced stability. Modified biochar further improves stability. For example, iron-modified biochar forms synergistic effects through iron oxides on its surface, enhancing its antioxidant and corrosion resistance capabilities [102]. This provides a more stable carrier for laccase, thereby prolonging the enzyme's service life.

3.3. Surface functional groups

The surface of biochar is abundant in various oxygen-containing functional groups, such as carboxyl, phenolic hydroxyl, and carbonyl

groups [103]. These functional groups can engage in electrostatic interactions, hydrogen bonding, or covalent bonding with the amino and carboxyl groups present in the laccase molecule. This enhances the interaction force between laccase and biochar, thereby improving the immobilization efficiency and stability of laccase [104]. Biochar can be chemically modified by introducing specific functional groups to optimize its chemical properties as a carrier material [75], which further improves the immobilization efficiency and stability of enzymes [77]. The presence of favorable functional groups on the biochar surface ensures strong binding affinity and reduces enzyme efflux, thus enhancing the overall effectiveness of enzyme immobilization on carriers [105]. For instance, acid modification increases the density of oxygen-containing functional groups, such as carboxyl groups, on the biochar surface, while alkaline modification introduces basic functional groups such as amino groups; thereby enhancing the binding capacity with laccase. It has also been observed that the affinity of modified biochar for laccase is significantly improved, leading to enhanced activity and stability of immobilized laccase [106].

3.4. Environmentally friendly

Biochar is also environmentally friendly and sustainable [107]. The production of biochar is considered a carbon-negative process, contributing to a reduction in atmospheric carbon dioxide levels. Biochar can be derived from a wide variety of raw materials, including agricultural waste, chicken manure, pig manure, wood chips, straw, industrial organic waste, and municipal sludge [108], making it highly suitable for large-scale industrial applications [109]. As a renewable, low-cost, and green material [91], biochar aligns with the principles of green chemistry and sustainable development. Furthermore, biochar effectively enhances microbial growth and activity by providing habitat for soil microbial communities, delivering nutrients and facilitating nutrient transfer, which in turn improves soil ecological health. These properties make it an important application in environmental management and sustainable development.

4. Immobilization methods of laccase

Although laccase has demonstrated significant efficacy in bioremediation, its large-scale application remains constrained by several critical challenges, such as high production costs, poor reusability, and limited operational stability. Enzyme immobilization presents a promising strategy to address these limitations by enhancing reusability, reducing operational costs, and improving enzyme stability under practical conditions. Currently established methods for laccase immobilization on biochar include adsorption, covalent bonding, cross-linking, and encapsulation (Fig. 3).

4.1.1. Adsorption

The adsorption method is a technique for immobilizing laccase through the interaction between the enzyme and various carrier materials, primarily relying on intermolecular forces such as van der Waals forces, ionic bonds, hydrogen bonds, and electrostatic interactions [110]. This approach preserves the native conformation of the enzyme and offers several advantages, including a simple operational procedure, broad applicability, mild reaction conditions, minimal impact on enzyme structure, and reversibility of the immobilization process. Based on the nature of the binding forces between laccase and the adsorbent carriers, the method can be further classified into physical adsorption and ion exchange adsorption.

The physical adsorption method relies on relatively weak intermolecular forces, such as hydrogen bonding, hydrophobic interactions, and van der Waals forces, to immobilize enzymes onto the surface of water-

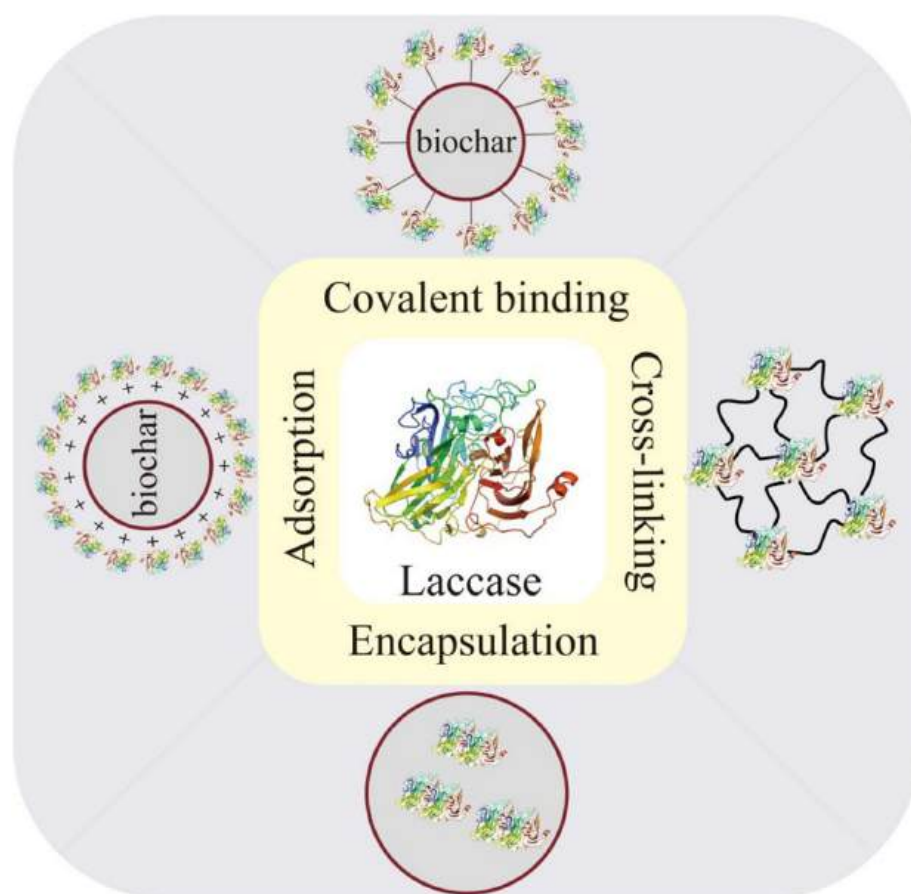


Fig. 3. Four immobilization methods of laccase.

insoluble carriers [111]. Wen et al. [112] immobilized laccase using kaolinite as a carrier, achieving a laccase loading of 12.25 mg/g and an enzyme activity of 839.01 U/g. The reusability of laccase was also enhanced following immobilization. For example, researchers immobilized laccase onto a mesoporous metal-organic framework material, MIL-53(Al), via physical adsorption. The immobilized laccase exhibited high activity recovery and stability, although a slight decrease in laccase activity was observed, the high removal efficiency of triclosan demonstrated a synergistic effect between the adsorption capacity of mesoporous MIL-53(Al) and the catalytic degradation ability of laccase [113]. Nguyen et al. immobilized laccase on granular biochar for the degradation of carbamazepine and reported that the physical adsorption method significantly improved laccase reusability and stability while maintaining its catalytic activity over a wide pH and temperature range [114].

The ion adsorption method relies on electrostatic interactions between laccase and water-insoluble carriers containing ion-exchange groups to achieve enzyme immobilization. Anion-exchange materials are the most commonly used types of such carriers [115]. For example, Brugnari et al. [116] immobilized laccase onto monoaminoethyl-n-aminoethyl-agarose, which significantly improved the enzyme's stability at pH 8.0 and retained approximately 80 % of its activity after 40 days of storage. Other researchers have immobilized laccase on copper (II) or manganese(II) ion-chelating magnetic Fe_3O_4 @CTS microspheres, achieving a laccase loading of about 100 mg/g. The immobilized enzyme retained more than 60 % of its activity after exposure to 55 °C for 14 days or after five reuse cycles, demonstrating excellent thermal and operational stability [117]. Temperature and pH [106] also significantly influence the adsorption stability and efficiency. Typically, the adsorption process is conducted under acidic conditions at temperatures ranging from 25 to 35 °C, under which conditions more than 75 % of

laccase activity can be retained. The selection of appropriate ions in ion adsorption is crucial for carrier-based immobilization. For instance, multi-point coordination between Fe(III) ions and nanofiber carriers can increase the specific surface area of the support and effectively inhibit enzyme desorption [118]. Wang et al. [119] investigated the immobilization of laccase using CTAB-KOH-modified rice straw biochar (CKMB) as a carrier. Their results showed that laccase could be effectively immobilized on CKMB, with a maximum enzyme loading capacity of 57.5 mg/g. Compared to free laccase, laccase@CKMB exhibited significantly enhanced storage stability and pH tolerance. Specifically, it retained approximately 40 % relative activity after 30 days of storage at 4 °C and maintained over 50 % relative activity across a pH range of 2.0–6.0. However, since the adsorption method primarily depends on physical or electrostatic forces to immobilize enzyme molecules, the binding strength is relatively weak. Consequently, the adsorption capacity gradually diminishes over time, leading to potential enzyme leakage and a progressive decline in immobilization efficiency with repeated use [120].

4.1.2. Covalent binding

The covalent binding method involves immobilizing laccase onto the surface of a carrier through the formation of covalent bonds resulting from chemical reactions between the enzyme's side-chain amino acids, such as lysine, cysteine, or aspartic acid, and functional groups on the carrier surface (e.g., amino, carboxyl, or hydrophobic groups) [121,122]. This method ensures strong attachment between the enzyme and the carrier, which significantly reduces conformational changes in laccase, effectively prevents enzyme leaching, and eliminates issues related to enzyme leakage or desorption. For example, Costa et al. [123] immobilized laccase on surface-modified multi-walled carbon nanotubes. Yang et al. [78] successfully immobilized laccase on biochar

enriched with amino-functional groups. Under optimal immobilization conditions, the laccase loading reached up to 782 mg/g. Compared with free laccase, the immobilized enzyme exhibited improved pH and thermal stability. It retained 48.5 % of its initial activity after ten reuse cycles and 80.8 % after 30 days of storage at 4 °C. The immobilized laccase also demonstrated excellent degradation performance toward trichloroethylene (TCE). Under conditions of 25 °C, pH 4.0, an immobilized laccase concentration of 0.35 g/L, and an initial TCE concentration of 10 mg/L, the degradation efficiency reached as high as 92.1 % within 48 h. Diao et al. [124] immobilized laccase in an N-hydroxysuccinimide-activated carboxylated poly(vinyl alcohol) aqueous solution under optimal conditions of pH 3.0 and 40 °C, using an amine-exchange reaction between the activated carrier and the amine groups of the enzyme. This approach improved the pH stability and shifted the optimal pH of laccase activity. Additionally, covalent immobilization of laccase on modified polyimide aerogels has been reported [121], with results showing that the immobilized enzyme exhibited significantly enhanced stability compared to the free form. It retained 22 % of its initial activity after seven reuse cycles and demonstrated improved storage stability, a broader pH stability range, and enhanced thermal stability. However, this method also presents notable disadvantages, such as a complex preparation process, harsh immobilization conditions, and potential loss of enzymatic activity during immobilization. For instance, Costa et al. [123] found that repeated linkage between the active site moiety of laccase and the carrier could lead to partial activity loss, thereby reducing the catalytic efficiency of the enzyme. Therefore, in practical applications of covalent immobilization, it is essential to determine the optimal mass ratio of laccase to carrier and identify the most favorable immobilization conditions.

4.1.3. Cross-linking

The cross-linking method immobilizes laccase on biochar by employing a cross-linking agent to form a stable three-dimensional network structure between laccase molecules and the biochar surface or among the enzyme molecules themselves [125]. Due to the covalent bonding forces, the enzyme protein binds strongly to the carrier, is not easily detached, and typically exhibits higher activity retention compared to other immobilization methods. Bifunctional cross-linkers, such as bis-aldehydes, diamines, and diesters, are commonly used in the preparation of immobilized enzymes. For example, Wang et al. [126] prepared an alkali-modified immobilized laccase using this method. The stability of the immobilized product was significantly improved compared to that of free laccase, with an immobilization efficiency as high as 67.40 % and an enzyme loading capacity reaching 180.81 mg/g. Wang et al. [127] immobilized laccase using the adsorption-crosslinking method with biochar as the carrier and glutaraldehyde as the cross-linking agent. They found that under optimal immobilization conditions, a low volume fraction of glutaraldehyde was insufficient to effectively bind the laccase molecules. As the volume fraction increased, the “mesh-like” structure formed by laccase molecules became more complete, resulting in enhanced stability of the immobilized enzyme. However, when the volume fraction was too high, excessive glutaraldehyde could undergo intramolecular reactions, potentially disrupting the mesh structure. Sadeghzaden et al. [128] synthesized Fe₃O₄ nanoparticles via co-precipitation and immobilized *Trichoderma reesei* laccase using 0.25 % glutaraldehyde as a cross-linking agent after surface amination. The resulting magnetic cross-linked laccase aggregates retained more than 30 % of their initial activity after six consecutive reuse cycles. However, the cross-linking method involves relatively harsh reaction conditions, which may lead to laccase polymerization in solution, potentially reducing the exposure of the active site and lowering catalytic efficiency [129]. Moreover, laccases from different sources require tailored cross-linking conditions. If the cross-linking time is too short or the concentration of the cross-linking agent is too low, insufficient cross-linking may occur, resulting in loss of enzyme activity [130]. Additionally, environmental pollution caused by the leakage or improper

disposal of cross-linking agents remains a concern [131].

4.1.4. Encapsulation

Encapsulation involves the immobilization of laccase within a material possessing a three-dimensional network structure, such as a gel, polymer, or porous matrix, through spatial confinement. This method protects the enzyme's native structure from degradation and enhances its stability during the remediation of pollutants. The encapsulation can be classified into two main types: reticulation encapsulation and microencapsulation based on the type of carrier used. Although both methods operate on similar principles, they differ in terms of carrier characteristics and structural properties. In the reticulation encapsulation method, laccase is entrapped within the mesh-like structure formed by the carrier. This approach offers advantages such as simple operation, mild reaction conditions, and high mechanical stability. However, it also presents certain drawbacks, including potential enzyme leakage and pore diffusion resistance during immobilization. Commonly used encapsulation materials for this method include natural gels such as chitosan beads, alginate, and gelatin, as well as synthetic gels like polyacrylamide. Among these, alginate is one of the most widely utilized carrier materials due to its excellent gel-forming properties. For example, researchers immobilized laccase within calcium alginate beads for the decolorization of various synthetic dyes, and the resulting immobilized enzyme demonstrated efficient dye removal across multiple consecutive batches [132]. In a study by Naghdi et al. [133], laccase from *Trametes versicolor* was encapsulated within a chitosan-nano biochar matrix for the first time. The encapsulated enzyme exhibited significantly improved stability under varying pH and temperature conditions compared to the free form.

Microencapsulation is an immobilization technique in which the enzyme is encapsulated within a membrane device (e.g., hollow fiber or microcapsule) through physical or chemical means, enabling the simultaneous immobilization of multiple enzymes [134]. This method is generally easy to operate, effectively preserves the native structure and long-term stability of laccase, and offers a cost-effective approach for enzyme immobilization. For example, Dai et al. [135] prepared multi-walled carbon nanotube (MWCNT)-modified electrostatically spun fibrous membrane-immobilized laccase (MWCNTS-LCEFM) via emulsion electrostatic spinning. Laccase and MWCNTs were encapsulated into fibers, resulting in an activity recovery of 85.3 %, with the specific surface area and tensile strength increased by two to three times, along with improved tolerance to environmental stressors. Piao et al. [136] encapsulated laccase (Lac) into a poly(ethylene glycol) hydrogel using UV-assisted emulsion polymerization, followed by cross-linking with glutaraldehyde to form Lac/particles. The resulting particles were spherical and micrometer-sized (137–535 μm), exhibiting a 100 % enzyme encapsulation efficiency and an 18.9 % activity recovery rate. Compared to free laccase, the immobilized enzyme demonstrated enhanced stability across a pH range of 3–7. However, the microencapsulation method also presents several limitations, including relatively low laccase concentration, limited loading capacity, and pore size constraints. Overall, while the embedding method can be performed under mild conditions and helps maintain the enzyme's structural integrity and stability, it is associated with challenges such as enzyme leakage, restricted pore diffusion, susceptibility to microbial contamination, and low immobilization efficiency, all of which may hinder its practical application.

In summary, each laccase immobilization method possesses distinct advantages and limitations. A comprehensive overview of various laccase immobilization techniques, including their corresponding strengths and drawbacks, is provided in Table 2.

4.2. Comparison of biochar with other laccase immobilization carriers

In addition to biochar, numerous laccase immobilization carriers have been thoroughly investigated and documented in scientific

Table 2
Comprehensive comparison of four laccase immobilization methods.

Laccase immobilization method	Advantage	Disadvantage	Stability	Reusability	Activity retention
Adsorption	Simple, cheap, no additional reagents required, mild reaction conditions.	Easy enzyme shedding, low immobilization efficiency, and reduced enzyme activity.	>50 % (2 months)	50 %–60 % (10 cycle)	40 %–70 %
Covalent binding	Strong bonding, no leakage or desorption, long life cycle.	Complex preparation process, harsh immobilization conditions, and low enzyme activity.	50 %–70 % (50 days)	>40 % (6 cycle)	30 %–60 %
Cross-linking	Long service life and strong bond.	Intense reaction conditions and difficulty in use with small fixed carrier particles.	>70 % (7 h)	>78 % (10 cycle)	40 %–80 %
Encapsulation	Strong binding and high enzyme immobilization rate.	Strict conditions, pore size limitations, leakage, and practical application limitations.	>70 % (24 h)	>80 % (8 cycle)	50 %–70 %

literature. These carriers encompass traditional inorganic materials such as silica, alumina, and zeolites, as well as advanced materials including nanomaterials such as carbon nanotubes, graphene oxide, and metal oxide nanoparticles, and highly porous structures like metal-organic frameworks (MOFs). Each carrier type demonstrates distinct physico-chemical characteristics that have a significant impact on the performance of immobilized laccase. For example, inorganic materials are recognized for their excellent thermal stability and mechanical durability, whereas nanomaterials provide a large surface area and improved electron transfer properties. MOFs, in contrast, are characterized by their exceptional porosity and adaptable structural features, which can be engineered to maximize enzyme loading and activity.

While these carriers offer various benefits, they also present specific limitations. Inorganic materials may exhibit limited surface area and weak enzyme binding affinity, while nanomaterials can be expensive and challenging to scale up for industrial applications. MOFs, despite their potential, may demonstrate structural instability under harsh environmental conditions. These factors directly affect both the immobilization efficiency and the enzymatic activity, which governs the catalytic effectiveness of the immobilized enzyme. Therefore, the selection of a suitable carrier is a crucial aspect of the immobilization process. An appropriate support material can substantially improve not only the stability and reusability of laccase but also its catalytic performance in breaking down diverse substrates, such as organic pollutants in wastewater. To aid in this selection process, Table 3 presents a detailed comparison of biochar with inorganic materials, nanomaterials, and

MOFs. The table summarizes key attributes, including the origin of each material, their respective advantages and limitations, interaction mechanisms with laccase during immobilization, and their overall feasibility for practical applications. This comparative analysis serves as a valuable reference for researchers and industry professionals aiming to optimize carrier selection for laccase immobilization in different applications.

5. Mechanism of biochar immobilized laccase for the remediation of organic pollutants

Organic contaminants are one of the primary causes of environmental pollution, largely attributed to their aromatic ring structures [152,153]. Biochar-immobilized laccase can degrade pollutants either by directly oxidizing organic compounds such as phenols and aromatic amines or by indirectly oxidizing non-phenolic substrates with the assistance of redox mediators (Fig. 4). The synergistic interplay between biochar's adsorption capacity and laccase's enzymatic degradation significantly enhances the efficiency of organic pollutant treatment.

5.1. Olefin pollutants

Olefin-based organic pollutants are widely encountered in various industrial processes, such as petrochemical production, plastic manufacturing, and fuel combustion. Although these compounds have significant industrial applications, they pose considerable risks to

Table 3
Comparison between biochar and other carriers for laccase immobilization.

Carrier	Source	Advantages	Disadvantages	Properties for immobilized laccase	Practicality	References
Biochar	Agricultural waste; forestry residues; industrial by-products etc.	Abundant oxygenated functional group; Simple preparation; Low cost; Environmentally friendly; Easy to recycle.	Uneven pore distribution; Low enzyme loading.	Wide pH range; High enzyme activity; Low reusability.	Wastewater treatment; Soil remediation; biosensor.	[88–109]
Inorganic material	Natural minerals and rocks; synthetic.	High mechanical strength; Good thermal/chemical stability; Low cost.	Low specific surface area; Limited surface functional groups.	Low enzyme activity; Good reusability.	Wastewater treatment, soil remediation.	[137–140]
Nanomaterials	Natural minerals; synthetic.	Ultra-high specific surface area; High mechanical strength; High enzyme loading; Adjustable surface functional groups.	Easy to agglomerate; High cost of synthesis; Material not easily recycled; High cost; Environmental risks.	High laccase stability; High enzyme activity; Higher reusability.	Wastewater treatment, soil remediation, biocatalyst.	[141–146]
Metal Organic Frameworks (MOFs)	Metals, organic ligands.	Ultra-high specific surface area; Aperture adjustment; High enzyme loading; Extremely high enzyme stability	Complex preparation; High cost; Framework disintegration; Instability in aqueous environment.	Good thermal/pH stability; High enzyme activity; Excellent reusability.	Wastewater treatment, soil remediation, biosensor.	[147–151]

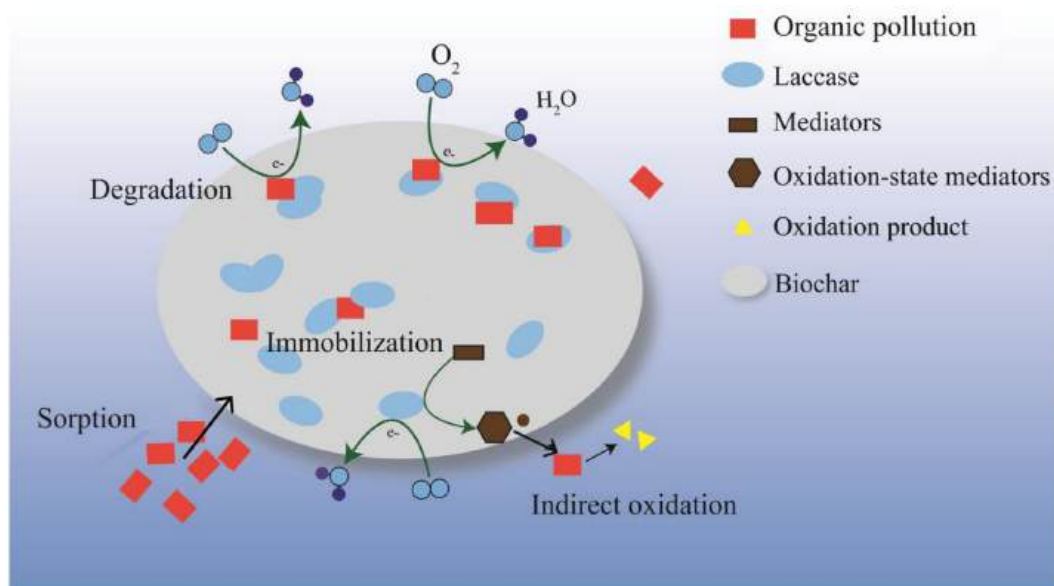


Fig. 4. Schematic illustration for degradation of organic compounds by biochar-immobilized laccase.

ecosystems and human health when released into the environment. Biochar-immobilized laccase has been shown to effectively degrade trichloroethylene (TCE) into low-toxicity products through epoxidation and hydroxylation reactions, which facilitate dechlorination. The oxidation of TCE by immobilized laccase can be attributed to two primary mechanisms: epoxidation and hydroxylation. In both cases, laccase enables efficient and simultaneous dechlorination (Fig. 5). On one hand, TCE is oxidized by laccase into a double carbon single bond epoxide, which is subsequently converted into ethylene oxide through dechlorination. Ethylene oxide then undergoes ring-opening to form intermediate products such as acetaldehyde, which ultimately decompose into methane. On the other hand, the hydrogen atoms in the TCE molecule are progressively substituted by hydroxyl groups through oxidation, potentially leading to complete mineralization into CO_2 and H_2O , thereby achieving efficient degradation of TCE. Under conditions of 25.2°C , pH 4.0, an immobilized laccase concentration of 0.35 g/L, and an initial TCE concentration of 10 mg/L, the degradation efficiency reached as high as 92.1 % within 48 h. During the TCE degradation process, a limited number of intermediates harmful to aquatic organisms may be generated. However, the majority of the final products are non-toxic [78].

5.2. Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are widely used in the electronics industry as components of adhesives and plastic materials. Due to their thermal and chemical stability, PCBs persist in the environment despite regulatory restrictions. PCBs are classified as persistent organic pollutants (POPs) owing to their toxicity and resistance to degradation in soil and aquatic environments. The laccase-catalyzed oxidation of chlorinated contaminants such as biphenyl requires the involvement of a mediator to facilitate the reaction. This mechanism is referred to as the laccase-mediated system (LMS). 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), a synthetic compound, effectively mediates the oxidation of non-phenolic substrates and was the first substance recognized as a laccase mediator. The oxidation of ABTS by laccase occurs in two stages: the formation of ABTS^{2+} followed by the generation of ABTS^{2+} [154].

A laccase mediator system (LMS) employing mediators such as 1-hydroxybenzotriazole (HBT), dimethyl sulfoxide (DMSO), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) can significantly enhance the degradation of contaminants [155]. Laccase is capable of oxidizing nonphenolic compounds in the presence of mediator molecules like ABTS and HBT. Laccase mediators can interact with

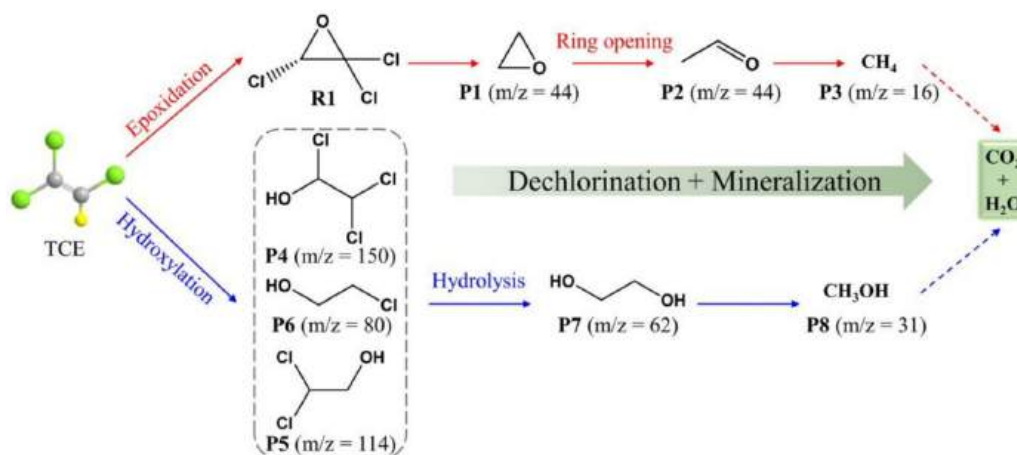


Fig. 5. Degradation pathway of TCE by laccase [78].

specific residues within the laccase structure to modify factors such as steric hindrance, charge distribution, and hydrogen bonding between the substrate and the enzyme [151,156]. According to Li et al., the optimal laccase immobilization conditions identified from an orthogonal experiment were pH = 3, a laccase concentration of 16 g/L, and a contact time of 8 h. Moreover, when ABTS was used as a medium, laccase achieved a degradation efficiency of 71.4 % for 4-hydroxy-3,5-dichlorobiphenyl (HO-DiCB) after 5 h. In contrast, under the same conditions, the removal efficiency in the control experiment was only 28.3 % [76].

5.3. Phenolic and chlorophenolic pollutants

Chlorophenols exert severe adverse effects on the human nervous and respiratory systems, thereby posing significant health risks. These compounds exhibit persistence in the environment, emit a strong odor, and demonstrate poor biodegradability, in addition to their toxic and carcinogenic properties [157]. As a result of their highly toxic impacts on the environment, chlorophenols have been categorized as priority-controlled persistent pollutants by both the United States Environmental Protection Agency (USEPA) and the European Union [158].

The removal of phenolic and chlorophenolic pollutants by biochar-immobilized laccase primarily involves the adsorption capacity of biochar and enzymatic degradation mediated by laccase. Xie et al. demonstrated that the degradation pathway of 2,4-dichlorophenol (2,4-DCP) proceeds as follows: under the catalytic action of laccase, 2,4-DCP is initially dechlorinated into 2-chlorophenol, 4-chlorophenol, and phenol; subsequently, 2-chlorophenol undergoes further dechlorination to produce phenol, while 4-chlorophenol is dechlorinated into p-cresol. Thereafter, under the continuous action of laccase immobilized on alkali-modified biochar, both phenol and p-cresol are oxidized into p-benzoquinone, which is subsequently ring-opened into maleic acid. Maleic acid continues to degrade and is ultimately mineralized into CO₂ and H₂O. After 30 days of storage, the relative enzyme activity remained at 83 %. The removal of 2,4-DCP was 99 %. The intermediates of 2,4-DCP degradation by immobilized laccase included p-phenol, p-benzoquinone and maleic acid [90]. The degradation process is illustrated in Fig. 6.

5.4. Polycyclic aromatic hydrocarbon pollutants

Polycyclic aromatic hydrocarbons (PAHs) are widely present in fossil fuel combustion byproducts, industrial emissions, and urban smog. Due to their persistence, toxicity, and bioaccumulation potential, PAHs constitute a significant threat to environmental quality and human health. Recent studies have highlighted the efficacy of laccase in degrading diverse PAH micro-pollutants, including anthracene, phenanthrene, and benzo(a)pyrene.

Laccase can directly oxidize PAHs pollutants. Furthermore, the degradation of PAHs by laccase is significantly enhanced with the

participation of mediators [79]. Biochar has been utilized as an effective sorbent for selected PAHs such as phenanthrene, fluoranthene, and benzo(a)pyrene, due to its high aromatic surface content resembling that of black carbon. Its structure primarily consists of disordered stacks of highly polarizable graphene sheets that provide high-affinity adsorption sites for PAHs [159]. Acid treatment enhances the suitability of biochar as a convenient carrier for laccase immobilization; functionalized biochar exhibits approximately a twofold increase in surface area compared to untreated biochar. Laccase was successfully immobilized on functionalized biochar, achieving an immobilization yield of 66 %. The immobilized enzyme demonstrated operational stability over 6 cycles while retaining 40 % of its initial activity. Acid treatments generally remove amorphous aggregates and minerals from the pores of biochar, leading to oxidation of surface carbon and formation of carboxylic acid (–COOH) functional groups on the biochar surface. These functional groups enhance anchorage between the adsorbent and adsorbate. The laccase-mediated PAH oxidation pathway requires six distinct electron transfers (ETs) to oxidize PAHs into their corresponding quinones (Fig. 7). For instance, in the case of anthracene, the degradation mechanism involves initial oxidation of anthracene to 9-anthracenol or 10-anthracenol followed by dehydrogenation to produce the final product: 9,10-anthraquinone. This immobilized system was applied for anthracene degradation in aqueous batch mode resulting in complete degradation of 50 mg/L anthracene within 24 h exposure [80,160].

5.5. Dye contaminants

Dye pollutants vary in composition depending on their source and are primarily categorized into azo dyes, indigo dyes, anthraquinone dyes, and triphenylmethane dyes. The mechanism of laccase action differs significantly among various dye types. Laccase facilitates the degradation of dyes via a multistep oxidative process that involves the cleavage of chemical bonds within the dye molecule, leading to the formation of smaller, less complex compounds (Fig. 8). The rate of decolorization is predominantly influenced by factors such as electron distribution, charge density, and steric hindrance. Laccase can directly oxidatively decompose anthraquinone dyes and azo dyes. During the degradation of azo dyes, laccase induces structural alterations in the dye molecule, generating phenoxy radicals by disrupting chromophore assembly. Specifically, one electron occupies the phenol or naphthalene ring to form the phenoxy radical, while the second electron is extracted to produce an aromatic cation. Azo dyes containing hydroxyl groups, particularly in the ortho and para positions relative to the azo bond, exhibit enhanced decolorization efficiency due to their strong electron-donating properties. For example, under optimized conditions (pH 4 and 45 °C), more than 85 % removal of malachite dye was achieved after 5 h. In addition, the immobilization system was applied to degrade Brilliant green, where nearly 93 % of the dye was removed within 4 h at pH 5 and 30 °C [85,99,162].

6. Factors affecting pollutant degradation using biochar-immobilized laccase

6.1. Acid/base influence

Acid/alkali modification represents the most widely adopted approach for biochar modification. Acid treatment effectively removes impurities from biochar and introduces acidic functional groups onto its surface. It has been observed that weak acids may serve as a more suitable choice compared to strong acids, as the latter can potentially disrupt the structural integrity of biochar, thereby negatively impacting both biochar modification and laccase immobilization. Numerous studies have demonstrated that alkali modification enhances the specific surface area of biochar, enlarges its pore size, and improves its adsorption capacity. However, under conditions of high alkalinity and high

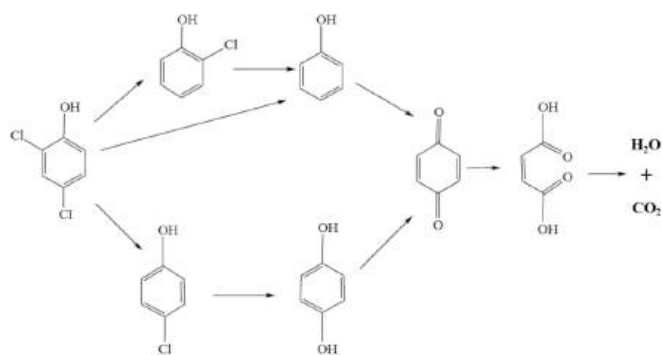


Fig. 6. Degradation pathways of 2,4-DCP by laccase [96].

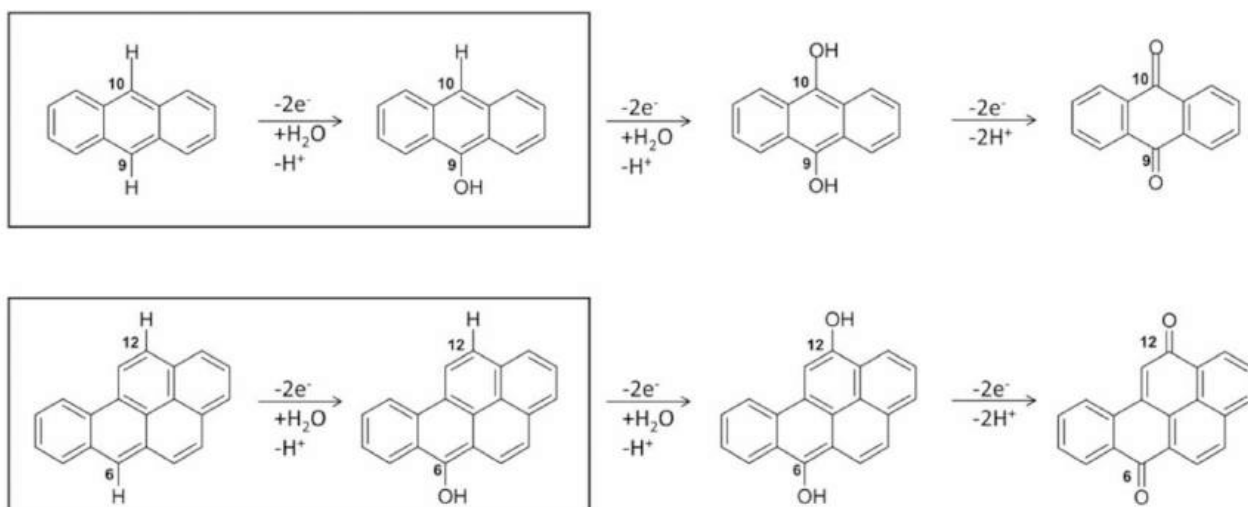


Fig. 7. The six-electrons oxidation of anthracene and benzo[a]pyrene to quinones species catalyzed by laccases [161].

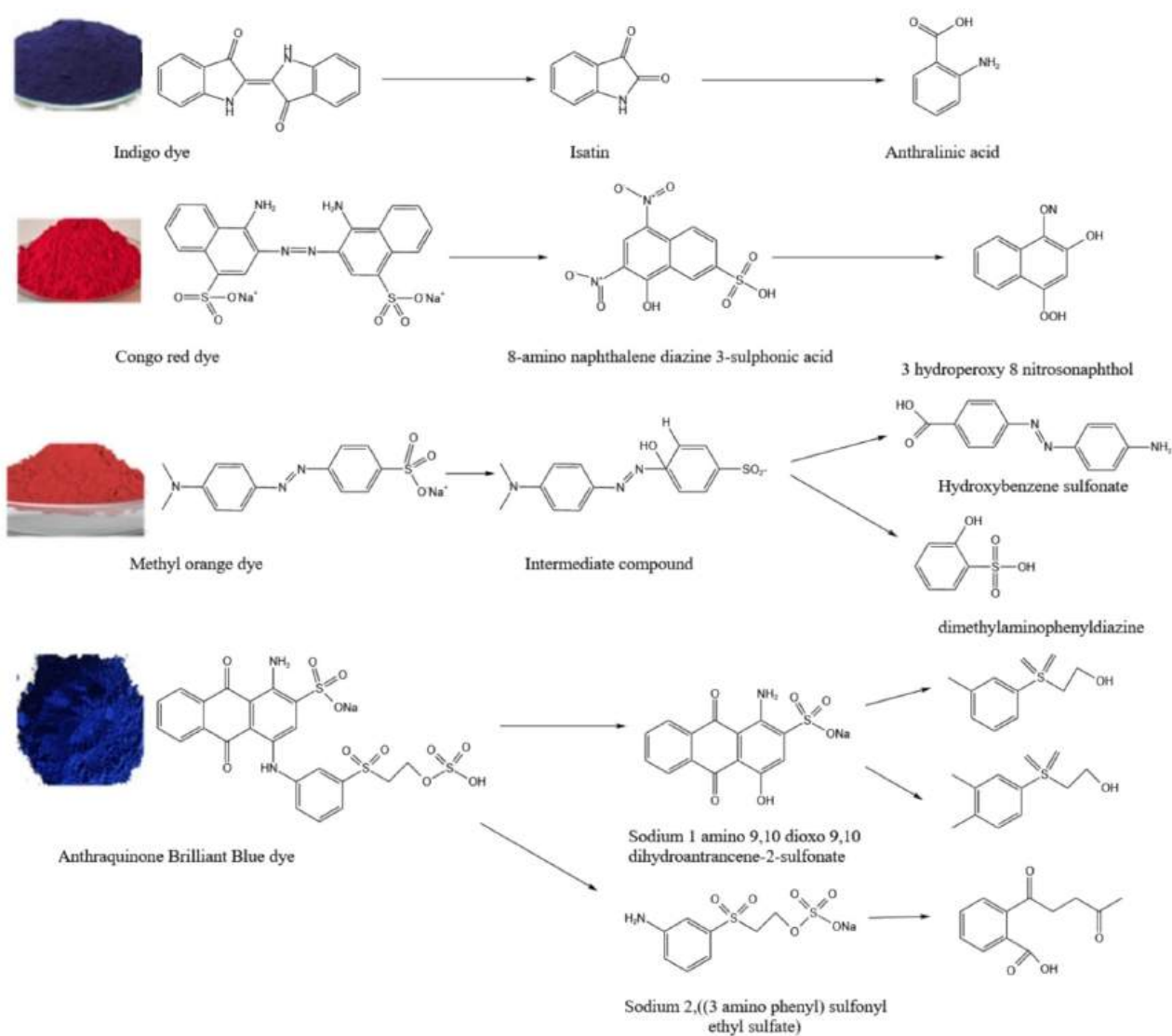


Fig. 8. Degradation pathway of dyes by laccase [45].

concentration, the structural stability of biochar may be compromised, leading to a reduction in the relative enzymatic activity of laccase. An optimal NaOH concentration of 6 mol/L has been identified for alkali modification [163].

6.2. Crosslinker concentration

The primary role of crosslinkers in the immobilization process is to aggregate laccase molecules to form cross-linked enzyme aggregates, thereby enhancing the loading capacity of biochar for laccase, improving the efficiency of biochar immobilization with respect to laccase, and enabling a higher laccase loading on biochar. The relationship between crosslinker concentration and relative enzyme activity exhibits a complex pattern. At low concentrations, the relative enzyme activity increases with an increase in crosslinker concentration, as low concentrations are insufficient to fully utilize the adsorption sites on biochar. During this phase, an increase in crosslinker concentration promotes the formation of more cross-linked enzyme aggregates, which facilitates greater laccase loading on biochar and enhances relative enzyme activity. However, excessively high crosslinker concentrations can exert toxic effects on laccase activity. High concentrations of crosslinkers may alter the spatial structure of laccase and induce conformational changes in its three-dimensional structure during the crosslinking process, ultimately leading to enzyme inactivation [126].

6.3. The storage stability of laccase

Storage stability primarily reflects the variation in laccase activity over storage time. The storage duration of both free and immobilized laccase plays a critical role in laccase applications and cannot be overlooked. Low storage stability significantly constrains the industrial application of laccase. Despite multiple washings of the modified biochar, acidic functional groups remain present. During long-term storage, these acidic groups are gradually released, thereby influencing the pH value of the solution. Laccase exhibits resistance to inactivation under acidic conditions. Conversely, alkaline groups released during prolonged storage may disrupt the structural integrity of laccase, resulting in a rapid decline in its enzymatic activity [126].

6.4. The concentration of laccase

The enzyme activity of immobilized laccase increased with increasing enzyme concentration, beyond which the enzyme activity became independent of enzyme concentration. This indicates that up to 14 mg/mL of enzyme molecules were covalently bonded in a monolayer on the surface of the nanoparticles during the experiment. Subsequently, the surface of FNBC was saturated with enzyme molecules; despite further increases in enzyme concentration, no additional enzyme molecules could bind. Similarly, Silva et al. [164] immobilized laccase on functionalized spent grains and reported a rapid increase in enzyme activity upon increasing the enzyme concentration up to 5 mg/mL, beyond which there was no significant difference in enzyme activity. In a related study, Salis et al. demonstrated that enzyme activity increased linearly with enzyme concentration; however, higher loading resulted in reduced laccase activity. They attributed this phenomenon to limitations in substrate diffusion within the support pores before reaching the active site of the enzyme [165]. Conversely, Cristovao et al. observed two distinct slopes in the trend of enzymatic activity for laccase immobilized on coconut fibers when the enzyme concentration was increased from 8 to 67 mg/mL and then to 260 mg/mL. They concluded that enzymatic adsorption was not restricted to a single layer on the carrier and that secondary-layer adsorption was possible [166]. Furthermore, Tastan et al. observed a gradual increase in the activity of the immobilized enzymes with increasing initial enzyme concentrations [167].

6.5. Properties of biochar

For biomass-based materials, the pyrolysis process facilitates the fracture of the organic structure of the material and provides favorable conditions for laccase immobilization. The thermochemical decomposition process is significantly influenced by the type of biomass material, its composition, and process parameters such as heating rate, temperature, and residence time. Structurally, biochar with a larger surface area can immobilize a greater amount of enzyme. However, a larger surface area typically corresponds to a smaller pore size, which may limit the amount of immobilized enzyme. Ideally, the pore size should be sufficiently large to allow the enzyme to diffuse into it; it is recommended that the pore size be four to five times larger than the molecular size of the enzyme. Pore sizes smaller than 10 nm result in reduced enzyme loading, likely due to difficulties encountered by enzymes entering these pores. Pore diameters ranging from 10 to 100 nm (mesoporous and macroporous materials) enable consistent enzyme loading because protein-protein interactions can hinder access to smaller diameter pores. Conversely, pore diameters exceeding 100 nm lead to a reduced specific surface area available for immobilization, thereby decreasing enzyme loading.

The presence of functional groups on the surface of biochar influences enzymatic degradation processes. Wang et al. highlighted that biochar containing redox-active organic functional groups on its surface can serve as an electronic mediator. This distinctive capability enables it to donate electrons to one molecule while accepting electrons from another, thereby promoting efficient electron transfer between the enzyme and substrate. Consequently, this phenomenon enhances catalytic activity in immobilized enzymes [168].

6.6. Properties of pollutant

The inhibition mechanism of laccase activity by pollutants is closely associated with the structural characteristics of laccase, such as its copper-containing active center and protein conformation, as well as its catalytic mechanisms involving electron transfer and substrate binding. Heavy metals present in pollutants can inactivate laccase by either replacing copper ions in its active center or inducing conformational changes in the protein structure. According to a study by Lorenzo et al., when syringaldazine was used as the substrate, all copper-chelating agents except EDTA exhibited strong inhibitory effects on laccase activity (approximately 100 %) at inhibitor concentrations below 20 mM [169].

The catalytic process of laccase involves an electron transfer chain. When the redox potential of certain pollutants does not match that of laccase, the electron transfer cannot be completed. Instead, these pollutants may occupy the electron transfer channel, thereby inhibiting the catalytic cycle. For example, a study by Peng et al. developed a MIL-68 (Al)/PVA/Lac system and found that it increased the removal efficiency for anthraquinone compared to free laccase and MIL-68(Al)/PVA. Moreover, as the concentration increased from 10 to 30 mg/L, the removal efficiency of MIL-68(Al)/PVA/Lac remained above 93 %, whereas that of free laccase decreased from 94.67 % to 82.31 %. However, excess amounts of benzoquinone intermediate alizarin green interfered with the active center of laccase and reduced removal efficiency [170].

The distinct chemical structures of pollutants result in varying degrees of oxidative sensitivity. For instance, azoxystrobin (a strobilurin fungicide) contains conjugated double bonds and aromatic rings that are highly susceptible to oxidation by laccase [171,172]. These functional groups facilitate the electron transfer process during laccase-mediated oxidation, thereby promoting efficient degradation [173]. In contrast, phoxim, an organophosphate insecticide, lacks such readily oxidizable groups, and its degradation primarily depends on the hydrolysis of phosphate ester bonds, which is less conducive to laccase-catalyzed reactions [174].

7. Conclusion and prospect

This paper reviews the current research progress in the remediation of organic pollutants using biochar-immobilized laccase degradation. It focuses on the properties of laccase, the introduction of four immobilized laccase technologies and immobilization supports, the removal mechanism of pollutants by biochar-immobilized laccase, and the role of different influencing factors. The remediation performance of immobilized laccase on pollutants can be significantly improved compared to free laccase. Specifically, this immobilization technology improves the environmental stability of laccase and expands the range of degradation thresholds, thus broadening its scope of action and improving the degradation efficiency of organic pollutants.

Although biochar-immobilized laccase (BIL) is an attractive method, it still encounters challenges such as operational complexity, biochar stability, and environmental variability in both theoretical research and practical applications. Modified biochar (e.g., oxidative modification; acid-base modification; metal salt or metal oxide modification; magnetic modification) has garnered increasing attention. However, modified biochar (e.g., oxidized; acid-base; metal salt or metal oxide; magnetic modification) increases operational difficulty and experimental costs despite its excellent performance. Sustainable and efficient utilization of resources and materials is essential for green development. Therefore, further development and comparison of improved methods to produce optimal carriers are important research directions. In practical applications, BIL technology's effectiveness is limited by complex environmental conditions and localized microorganisms that may interfere with the degradation process. Future studies should optimize biochar combined with advanced modification techniques while enhancing nano-scale biochar materials' modification and immobilization technology to improve loading capacity, storage stability, catalytic oxidation performance, and recycling efficiency for laccase. Additionally, the scope for application in industrial fields can also be expanded to enhance practical application effects in energy sectors as well as others like soil remediation, food processing, and biosensors which require thorough study & development. In addition, in practical scenarios, the synergistic effect between biochar-immobilized laccase with other substances or processes needs consideration to improve its efficacy under complex environments. For example, investigating interactions between microbial combinations along with enzymes/chemicals could lead toward developing more effective pollution control strategies or industrial production processes.

CRedit authorship contribution statement

Yameng Chen: Writing – original draft, Visualization, Investigation, Data curation. **Li Li:** Investigation, Data curation. **Yang Yong:** Methodology. **Caixia Wang:** Funding acquisition. **Qingming Zhang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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