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Biochar production from waste biomass applied in enzyme immobilization for removal of emerging contaminants in an approach of treating waste-by-waste – a literature review

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Abstract

To meet the demands of exponential population growth, industrial and agricultural activities have intensified, resulting in the release of numerous hazardous substances, including emerging contaminants (ECs). Such chemicals include pharmaceuticals, emerging pathogens, pesticides, industrial chemicals, and microplastics. ECs are persistent in various environments, difficult to remove during wastewater treatment, and their elimination has become of global concern. In fact, the mitigation of ECs aligns with some of the United Nations Sustainable Development Goals (SDGs), such as SDG 6, SDG 11, SDG 12, SDG 13, and SDG 14 which are related to minimizing hazardous chemicals in water bodies, management of waste through its life cycle and the conservation of water resources for sustainable development. One promising approach is the "waste-by-waste" strategy, which adopts a circular economy perspective by repurposing residues from industrial, agricultural, and domestic sources to remove ECs. Such compounds are usually degraded by oxireductases, especially laccases, which oxidize ECs, reducing the toxicity of the pollutants and their intermediates. These enzymes can be immobilized in waste-derived biochar, enhancing catalytic performance and system reusability in environmental remediation, representing a sustainable and cost-effective alternative for ECs degradation. This review investigates the potential of waste-derived biochar for enzyme immobilization and its application in ECs mitigation. It highlights the principles of waste-by-waste treatment and the circular bioeconomy, outlines methods of biochar production and enzymatic immobilization, and critically discusses recent advances as well as the main challenges of this emerging approach.

Keywords: Biochar; Circular Economy; Enzyme; Immobilization; Antibiotics; Pesticides; Microplastics.

1. Introduction

In 2023, 2.1 billion tons of industrial waste were generated throughout the year, of which less than 2% were recycled and more than 70% were discharged without treatment [1]. Not only industries are contributing to global pollution, but also domestic activity. Municipal waste is expected to increase by 73% from 2020 (2.24 billion tons) to 2050, reaching a total of 3.88 billion tons [2]. In this context, it is crucial to find solutions to the growing pollution. One alternative is to use the waste that is currently being disposed to develop new green technologies capable of replacing current ones.

A prime example of that would be the production of biochar from waste biomass, as this would reduce greenhouse gas emissions from biomass decay, providing a sustainable strategy for waste management, directly impacting global warming [3]. Another relevant contribution of biochar in climate change mitigation is its use in carbon dioxide removal (CDR), as this material can lower CO_2 levels in the atmosphere [4]. The strategy to produce biochar from waste-biomass can be seen as means to achieve the UN Sustainable Development Goals (SDGs) such as Clean Water and Sanitation, Sustainable Cities and Communities, and Climate Action UN Goals 6, 11–14, respectively [5–7].

Biochar can be defined as a stable, black, porous carbonaceous, lightweight, solid product obtained from biomass thermal decomposition [8]. This material is similar to activated carbon, but produced under milder conditions and presenting lower costs. In fact, it is possible to consider that activated carbon is a biochar that went through chemical or physical activation [9]. The differences between biochar and activated carbon will be further discussed in Section 3.2. It is possible to highlight agricultural, municipal (e.g. wastewater) and culinary waste as potential raw materials for biochar production [10, 11]. Applications of biochar include soil decontamination, water treatment, wastewater treatment, as food additive, in biosensors, in petroleum-based filter substitutes and in capacitors [3, 11]. The wide applicability of biochar can be attributed to its properties, such as biodegradability, stability, conductivity and nutritional value. In bioremediation of soil and water, biochar's performance stands out due to its adsorption capacity [11]. Physicochemical properties impact its adsorption capacity, such as its large surface area, porosity, high cation exchange capacity and surface functional groups, allowing the removal of heavy metals or organic contaminants from the environment [11].

In recent years, there has been a growing interest in the use of biochar as a support for enzyme immobilization. Its large surface area allows better loading on the immobilizing matrix, while the homogeneous distribution of different-sized pores leads to better diffusion of enzymes in its internal structure [4]. Also, the use of biochar-immobilized enzymes overcomes most disadvantages of using free enzymes – decrease in catalytic activity and stability, and higher operational costs linked to poor reusability [12]. Immobilization improves enzyme stability allowing for more cycles of biocatalysis, facilitating enzyme recovery, thus, reducing operational costs [13]. The support matrix utilized for enzyme immobilization needs to be inert, stable and should not interact with the catalytic site of the molecule [13]. From an industrial point of view, it is desirable that the support used for enzyme immobilization is cost-effective – biochar fits such interests [12]. The main methods for enzyme immobilization are adsorption, covalent bonding, entrapment and encapsulation. Specifically for biochar, covalent bonding and adsorption are the preferred methods [4].

After immobilization in biochar, the enzymes can be applied in environmental remediation, as they can degrade a variety of pollutants and remove water contaminants [12]. Oxidoreductases, specifically laccases, are used for degradation of pesticides and pharmaceuticals, pollutants considered emerging contaminants (ECs) [12]. ECs are harmful compounds of growing concern, which are not monitored in the environment and have no current regulations on toxicity, treatment, and disposal [14]. ECs include pharmaceuticals, personal care products, emerging pathogens, cyanotoxins, pesticides, industrial chemicals, micro/nano plastics, nanomaterials, and antibiotic resistance genes [15].

As previously exposed, the use of waste biomass to produce biochar offers a sustainable solution for waste management, in a circular bioeconomy approach. And its further use in enzyme immobilization is a promising alternative to reduce environmental pollution caused by ECs, within a strategy of treating waste-by-waste (Figure 1). The use of biochar for enzyme immobilization and/or environmental bioremediation has been partially addressed in previous review articles [4, 8, 9, 13]. However, to the best of the authors' knowledge, there are no literature reviews that specifically discuss the use of waste-derived biochar as an enzymatic support for EC degradation within a waste-by-waste approach. Therefore, this review summarizes and discusses the most up-to-date strategies to obtain biochar from waste biomass, the main techniques for enzymatic immobilization, the use of biochar-immobilized enzymes in removal of ECs, together with its main advantages and drawbacks.

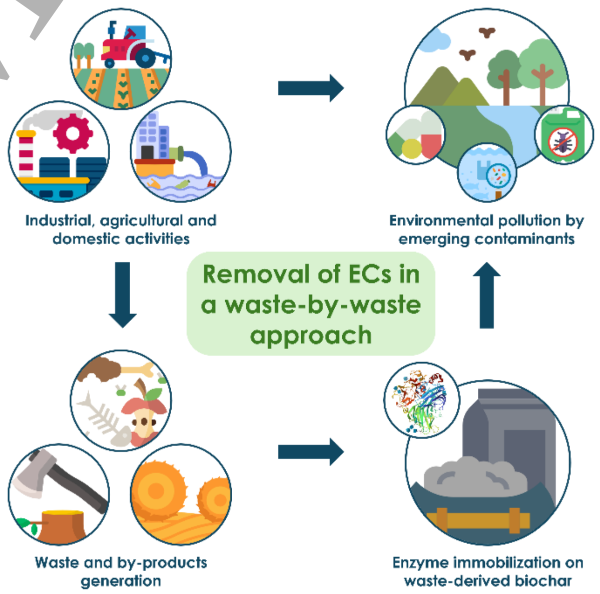

Figure 1 – Removal of ECs with waste-derived biochar-immobilized enzymes in a waste-by-waste approach.

For that, the concept of circular economy and the use of waste biomass in this context will be introduced and discussed (Section 2). Further, techniques for biochar obtention, modification and activation will be presented in Section 3, as well as examples of biochar production from waste materials. In the following sections, biochar-based enzyme immobilization techniques (Section 4) and their application in ECs removal (Section 5) will be detailed and discussed. Finally, advantges, constraints and perspectives of using immobilized enzymes in waste biochar for ECs degradation will be explored (Section 6).

This literature review addresses important issues regarding climate change and environmental pollution reduction, presenting a novel approach of treating waste-by-waste. The use of biochar as a support matrix for enzyme immobilization in such strategy is relatively new, and this article will present its current gaps and challenges – such as lack of biochar structure modelling, simulation of real-life ecosystems and economic analysis to guarantee their commercial application. Different approaches to overcome these drawbacks will also be presented and discussed.

2. Waste biomass and circular economy

The traditional linear economy model that follows the take-make-use-dispose approach [16] has led to perduring challenges of resource scarcity, GHG emissions, and waste management [17]. However, the demands for energy, materials, food, and other products are constantly rising with populational growth. Therefore, there is an imperative necessity of transitioning to a more sustainable alternative to meet these demands. In this context, the Circular Economy (CE) concept arises, with the proposal of closing production cycles by reducing, reusing, and recycling materials [18]. The main goal of CE is to minimize waste, while maintaining and/or maximizing the value of products and resources [19]. The no-waste technology is directly linked to CE as it comprises processes that do not generate waste. In addition to CE, the bioeconomy concept has also gained attention. In this case, the raw materials for production of energy and/or products are bio-based, alleviating the dependence on fossil sources [20].

The concepts of CE, no-waste technology, and bioeconomy harmonize with biochar production, as this material can be obtained from different types of waste applying low-cost techniques [21]. Agriculture generates high amounts of waste biomass that can be applied to produce biochar. For example, the processing of 1 ton of sugarcane usually leads to the production of 250 kilograms of sugarcane bagasse [22]. When processing soybeans, up to 80 kg of hulls can be generated from each ton of crop processed [23]. In the case of corn, it is estimated that 1 ton of corn stover is produced for each ton of corn kernels obtained [24]. During rice milling process, 200 kg of rice husks are produced from 1 ton of paddy rice [25]. These feedstocks usually present a low-added value and are considered process residues with limited applications. However, they can be directed for biochar obtention, generating more value and closing production cycles (Figure 1).

The application of biochar for enzyme immobilization and further removal of ECs in a waste-by-waste approach is in sync with the concepts of CE, no-waste technology, and bioeconomy. Immobilized enzymes have the main advantage of reutilization, as they can be applied in sequential processes while retaining their activity [26]. Enzymatic processes are usually considered expensive due to the cost of enzymes, and the possibility of reusing these molecules can relieve these associated costs [27]. For enzyme immobilization, the supporting material needs to be inert, regenerable, economical, and to maintain enzymatic activity [28]. Therefore, biochar derived from low-cost technology and waste biomass is a potential material for supporting enzymes in immobilization processes. The final price of biochar is highly dependable on the initial feedstock cost, on the logistics involved, and on the process employed for biochar obtention [29, 30]. Thus, integrating the production of biochar in biorefineries can significantly reduce the costs involved with feedstock price and logistics. Patel and Panwar estimated the production cost of $232.87 per ton of biochar, also projecting that production of 1 ton of biochar could mitigate 6.22 tons of CO_2 [31]. Besides, biochar characteristics of high surface area, presence of functional groups, and possible modulations of properties are also vital for enzymatic immobilization [32]. After immobilization in biochar, the enzymes can be applied in ECs removal from the environment, closing the cycle in a approach of treating waste by waste.

3. Biochar

Biochar can be produced from a wide range of different thermochemical methodologies of biomass degradation in controlled environments and with a specific atmosphere. From the application of this thermochemical process on basically any lignocellulosic resource, its components are broken down and devolatilized into their fundamental constituents [33]. The methods of the reaction to these various products partially compete, and the distribution of the output products can be affected by the conditions and the type of the process [34].

3.1 Techniques for biochar obtention

Pyrolysis is the thermochemical degradation of organic compounds, at temperatures ranging from 300 to 1000 °C, in an inert atmosphere in the absence of oxygen, resulting in the breaking of chemical bonds and the formation of three main fractions: bio-oil, syngas, and biochar [34]. The different configurations of the pyrolysis process are commonly grouped into three categories: slow, fast, and flash pyrolysis, and are defined based on the temperatures, the retention time, and the heating rate applied in the process, which determines different products in terms of mass yield and compositions [35]. In the comparison between different methods, slow pyrolysis is considered the one that converts the largest amount of biomass into biochar (30-60%), generally operating at temperatures of 400-600 °C, with a low heating rate of 0.1-1 °C/s, for long retention times (ranging from hours to days) [36]. Fast pyrolysis is commonly conducted at temperatures between 400 and 800 °C, with a higher heating rate (200 ◦C/min) and a shorter residence time (a few seconds). Although it presents higher scalability potential, this is due to the added value of the process from other products, given its production of substantial amounts of bio-oil and syngas along with solid biochar, whose yield (10-20%) is usually lower compared to slow pyrolysis (30-60%) [36]. On the other hand, the flash pyrolysis process is similar to fast pyrolysis but typically applies to even higher temperatures (800-1000 °C), with retention times shorter than 1 second, resulting in even lower biochar mass yields (10-15%), but producing a fuel with higher energy density [37]. Also, microwave-assisted pyrolysis offers an alternative process for biochar production. Yong et al. (2022) [38] evaluated a microwave-assisted pyrolysis at 600 °C with 1000 W for converting wood-based panels bonded with isocyanate adhesive into bio-oil and biochar, resulting in biochar yield of 27%. The resulting nitrogen-doped biochar features a porous structure suited for adsorption and electrochemical applications [38].

Factors such as atmosphere composition (Ar, H_2, N_2, their mixture, among others), feedstock choice, type of thermal treatment, their mineral composition, and the rate of heating govern the properties of pyrolytic biochar. However, Ippolito et al. 2020 [39] claimed that the main parameters influencing the characteristics of biochar produced by pyrolysis are the feedstock characteristics and the reaction temperature. Regarding the type of feedstock, they showed that biochars derived from wood (hardwoods, such as bamboo and oak; and softwoods, such as pine and spruce) present an average specific surface area of 184 m^2/g and a pore volume of 7.01 m^3/ton. In contrast, biochar produced from crop waste, grasses, and manures exhibits average specific surface areas ranging from 52 to 98 m^2/g and pore volumes between 0.8 and 3.4 m^3/ton. This difference may be attributed to the importance of lignin content in influencing key biochar properties. Feedstocks with high lignin content can produce biochar with high fixed carbon content, high specific surface area, and a well-developed aromatic structure [40, 41]. This is probably because lignin helps the biomass to retain its original macroscopic architecture through the pyrolysis process, resulting in a biochar that maintains a morphology closely resembling that of protolignin, with distinct vertical microchannels and fibrous ridge patterns [41]. Regarding pyrolysis temperature, the authors show that biochar produced at temperatures between 700-800 °C exhibits higher specific surface areas, ranging from 204 to 208 m^2/g, compared to 27-97 m^2/g for temperatures between 300-600°C; they also present greater total pore volumes, around 8.87 m^3/ton, in contrast to a maximum of 4.68 m^3/ton for the 300-700 °C range [39]. Moreover, temperatures above 700°C, when combined with molar ratios of H/C and O/C below 0.5 and 0.2, enhance the stability of biochar in soil, leading to half-lives exceeding even a thousand years [39].

Just like the fast pyrolysis process, the gasification method turns carbonaceous material into hydrocarbons and gaseous fuels (CH_4, CO_2, H_2, CO), along with a low generation of biochar (5-10%) [37]. This method is an autothermal strategy wherein heat is generated through exothermic reactions at high temperatures (above 700°C) in an oxygen-poor environment, involving the partial oxidation of the feedstocks in a gasifier [42]. This eliminates the need for an inert atmosphere like nitrogen or helium, and thus, along with the fact that the process focuses on the production of energy and other compounds, it enhances the economic viability of this process and its scalability [43]. James R. et al. 2020 [44] reported that increasing the airflow rate from 8 to 20 L/min promoted an increase in the specific surface area of the biochar, as well as its enrichment in alkaline functional groups. These characteristics improve the thermal stability of biochar and its potential for soil acidity correction. However, the oxidative conditions applied during the gasification process led to the degradation of acidic functional groups, such as carboxylic and phenolic groups [44]. This limitation directly impacts the cation exchange capacity (CEC) of the biochar, making it less efficient in retaining essential nutrients, such as Ca^{2+}, Mg^{2+}, and K^+, especially in degraded soils and in soils with low fertility [45]. Furthermore, the scarcity of acidic functional groups reduces the affinity of biochar for cationic heavy metals like Pb^{2+}, Zn^{2+} and Cd^{2+}, compromising its performance in environmental remediation applications [46, 47].

The most applied and studied methods for biochar production, as described above, involve the thermochemical conversion of dry biomasses, after the application of a drying method [48]. However, recently, the hydrothermal carbonization method has been investigated for its application to biomass with high moisture content (above 60%) [49], using the water present in the feedstock as both reactant and solvent, and presenting, due to a simpler process, a potentially higher cost-effectiveness [48]. The formation of the so-called hydrochar usually occurs at temperatures of 180-300°C and under pressures of 1-6 MPa, if the formation of hydrochar alone is required [37], while temperatures between 200-400°C result in greater liquefaction and the formation of biofuels, along with the hydrochar [50]. Harisankar et al. 2022 [51] evaluated different water sources on biochar yield using hydrothermal carbonization and reported a hydrochar yield of 74% from rice straw using sea water.

3.2 Techniques for biochar activation/modification

Normally, the properties of biochar obtained from biomass pyrolysis are not fully efficient to achieve the expected results in environmental remediation processes, due to limitations in surface functional groups, poor porosity, and low specific surface area [43]. For further improvements in the quality and characteristics of biochar, an extra process for the its activation and functionalization can be applied and are usually grouped into physical activation (steam and gas activation) and chemical modification (acids, bases, and oxidants). As a matter of comparison, many of the modifications applied to biochar are simplified adaptations of industrial activated carbon methods [9]. Activated carbon is produced through more rigorous processes, involving high temperatures (≥800°C) and intensive physical or chemical activation, resulting in materials with high surface area (500-2000 m²/g) and controlled porosity, intended for industrial applications in purification, adsorption, and catalysis, with significantly higher costs and process control requirements [52, 53]. While for biochar activation, the process aims to confer greater selectivity, reactivity, and stability to the biochar, in addition to specific functionalities for certain types of contaminants [54].

For gaseous activation of biochar, air, ozone, or CO_2 are used as reactants. The reaction between the gas and carbon occurs at 800-900°C, for 30 min to 3 hours, promoting increase in surface area, and functionalization, while increasing the total carbon content [55]. The dehydrogenation and aromatization reactions are the major processes that help the formation of large polynuclear aromatic hydrocarbon rings and increase the carbonization yield. Additionally, the use of ozone (O_3) has been explored to increase the presence of acidic surface groups and oxides, as well as to enhance the cation exchange capacity, thereby improving the adsorption properties of biochar [56].

Chemical modification of biochar has also been applied for the enhancement of its properties, aiming to make it a promising material for specific environmental applications. Phosphoric acid, for instance, catalyzes micropore formation through acid-driven crosslinking, improving adsorption. For example, a mushroom substrate-based biochar activated with phosphoric acid showed an acetaminophen adsorption capacity of 236.8 mg/g and 84.7% removal efficiency [57]. The biochar was impregnated with phosphoric acid (1:2 w/w), left overnight, and then pyrolyzed at 700-900 °C, under a N_2 atmosphere. Nitric acid (HNO_3), and hydrogen peroxide (H_2O_2) are other examples of catalysts that can be used for biochar activation, enhancing the surface functionality of biochar through distinct mechanisms. Nitric acid introduces nitro and nitrate groups and, at higher concentrations, increases carbonyl, carboxyl, and hydroxyl functional groups, facilitating the enrichment of nitrogen binding active sites [58]. Hydrogen peroxide, in its turn, promotes the formation of oxygen-containing functional groups in biochar, and can be used in combination with metallic catalysts, forming Fenton or Fenton-like systems that catalyze the generation of hydroxyl radicals [59]. These types of systems, such as FeMn/biochar/H_2O_2, already effectively degraded organic pollutants, such as naphthalene [60], and diethyl phthalate [61]. However, it is important to note that chemical agents may also cause decomposition of organic matter, producing effluents and by-products, such as salts, tars, heavy oils, and metal residues, depending on biomass type and process conditions [62].

Despite these advancements, the commercial-scale application of chemically modified biochar remains limited due to challenges in reproducibility, scalability, biodegradability, and long-term performance [63]. Most developments remain at the research or pilot scale. Comprehensive assessments are needed to overcome commercialization barriers and ensure the sustainability and cost-effectiveness of biochar technologies.

3.3 Biochar production from waste biomass

One important initiative to overcome barriers to biochar commercialization is the legal standardization of the product. Due to the high dependency of biochar properties on feedstock type, pyrolysis conditions, and post-treatment modifications, international organizations, such as the International Biochar Initiative (IBI) and European Biochar Certificate (EBC), have proposed legal definitions and standardization efforts [64]. Among the technical requirements set by the EBC, particular attention is given to a minimum total carbon content of 50% on a dry weight basis, alongside a hydrogen-to-carbon molar ratio (H/C) below 0.7, which reflects high aromaticity and chemical stability. The standards also define the acceptable feedstocks to be used, such as crop residues, food and animal by-products, sludges from wastewater treatment, and algae, along with a maximum of 10% (dry basis) of additive materials, like lime or bentonite.

As production technologies mature and certified quality guarantees are widespread, biochar produced from waste biomass is being adopted at larger scales. The global biochar market was valued at $542 million in 2023 and is projected to reach $6.3 billion by 2033, with an estimated Compound Annual Growth Rate (CAGR) of 10.9% from 2025 to 2033 [65]. A complementary report by the International Biochar Initiative (IBI) and the US Biochar Initiative (USBI) highlights that revenues across the sector, including producers, distributors, value-added manufacturers, and equipment suppliers, exceeded \$600 million in 2023, following a remarkable CAGR of 97% between 2021 and 2023, with revenues projected to reach \$3.3 billion by 2026 [66]. To illustrate this scenario, Carbonity, the first industrial-scale biochar plant in Canada, has started operations in a plant with an initial annual production capacity of 10,000 metric tons of biochar, by the auto-thermal pyrolysis of forestry residues, which is expected to triple by 2026 [67]. These recent commercial reports highlight the rising demand for high-quality biochar produced from waste biomass that fulfil strict legal and environmental criteria. In Table 1, some relevant recent research in biochar production from waste biomass are summarized.

Table 1 – Overview of biochar production from various waste biomass feedstocks

Waste Biomass	Technique	Process Conditions	Biochar Yield	Main Finding(s)	Reference
Rubber seed shell	Slow pyrolysis	600 °C, 3 h, N_2, 10 °C/min	-	In-house reactor produces biochar with better properties and lower cost	[68]
Luffa cylindrica fiber	Slow pyrolysis	300-500 °C, 2-20 °C/min, N_2	-	A lab-scale solar energy-based pyrolizer was built, resulting in better adsorption properties of biochar	[69]
Grape marc	Slow pyrolysis	450 °C, 5 °C/min, N_2	40%	High minerals lower decomposition T; lignin raises yield	[70]
Spent coffee ground	Slow pyrolysis	600 °C, 30 min, N_2	30%	High C content improves fuel quality; low activation energy aids combustion	[71]
Mustard straw	Slow pyrolysis	400-1000 °C, 30-180 min, N_2/CO_2, 3-7 °C/min	13-21%	CO_2 boosts porosity and surface area	[72]
Canola and oat hulls	Slow pyrolysis	600 °C, 60 min, N_2, 5 °C/min	28%	Higher T raises carbon content and stability	[73]
Pomegranate peel	Slow pyrolysis	300 °C, 20 min, N_2, 15 °C/min	55%	Low temperature and particle size improve yield	[74]
Pine needles	Slow pyrolysis	350-700 °C, N_2, 10-50 °C/min	27-56%	Rich in nutrients that enhance soil fertility	[75]
Rice husk	Slow pyrolysis	300 °C, 5400 s, N_2, 20 °C/min	38%	Higher temperature improves HHV and fixed carbon; slow pyrolysis yields energy-rich biochar	[76]
Olive pomace and mill wastewater	Co-pyrolysis	600 °C, 1 h, inert, 15 °C/min	31%	Biochar is nutrient-rich and suitable as biofertilizer	[77]
Rice husk, gasification tar, municipal solid waste	Co-pyrolysis	400-800 °C, 5-30 min, N_2	17-24%	Synergistic effects enhance yield and energy content	[78]
Rice husk and HDPE	Co-pyrolysis	400 °C, 15-45 min, N_2, 10 °C/min	54%	Plastic boosts energy value; affects fixed C and HHV	[79]
Maize-cob waste	Fast pyrolysis	463-593 °C, 10-19 min, inert	32-37%	Faster conditions offer higher bio-oil production efficiency	[80]
Pine chips, olive husk, hazelnut shell	Fast pyrolysis	500-525 °C, fluidized bed, inert	11.2%	Higher N_2 velocity boosts oil yield; steep T gradients reduce products	[81]
Cherry pulp	Slow and fast pyrolysis	400-700 °C, 10-200 °C/min, N_2	31%	Higher temperatures increase aromaticity; fast heating lowers yield, but raises porosity	[82]
Rice husk	Two-step pyrolysis	Step 1: 300 °C, 2 °C/min, 0.5 h; Step 2: 600 °C, 10 °C/min, 1 h	39%	Lower switch T boosts yield; two-step enhances carbon and calorific value	[83]
Corn and rice straws	Hydrothermal carbonization	190-250 °C, 3 h	41-54%	Citric acid increases calorific value and lowers activation energy	[84]
Rice husk	Hydrothermal carbonization	632-1023 °C, 21-100% O_2	14-33%	O_2-limited combustion yields highly porous biochar	[85]
Miscanthus biomass	Gasification	1200 °C, 5-20 °C/min, N_2/CO_2	-	Lignin biochar has high reactivity and low activation energy	[86]

Pinewood sawdust	Gasification	700-900 °C, 0.2-0.4 of air equivalent coefficient, 0.5-1.5 of steam/biomass ratio	-	Temperature and air equivalent coefficient are key factors influencing biochar yields	[87]

4. Biochar-based enzyme immobilization techniques

The immobilization of enzymes on solid support enhances cost-efficiency by enabling enzyme reuse, improving experimental control, and producing enzyme-free final products [4]. This technique is considered one of the most promising strategies to overcome poor operational stability and enzyme recovery, as it has been shown to improve enzyme stability, resistance to extreme conditions, and reusability [8, 88].

The support matrix must preserve the catalytic activity of the enzyme, ensure chemical inertness, allow regeneration, prevent enzyme leaching, and minimize nonspecific adsorption [4]. However, immobilization processes can sometimes increase overall production costs. Therefore, it is crucial to develop more economical carriers based on waste-derived materials such as biochar [12].

Several methods exist for immobilizing enzymes on solid supports, including adsorption, entrapment and encapsulation, covalent binding, ionic binding, and cross-linking (Figure 2). For bioremediation applications, the most used methods are adsorption, covalent bonding, cross-linking and their combinations [12].

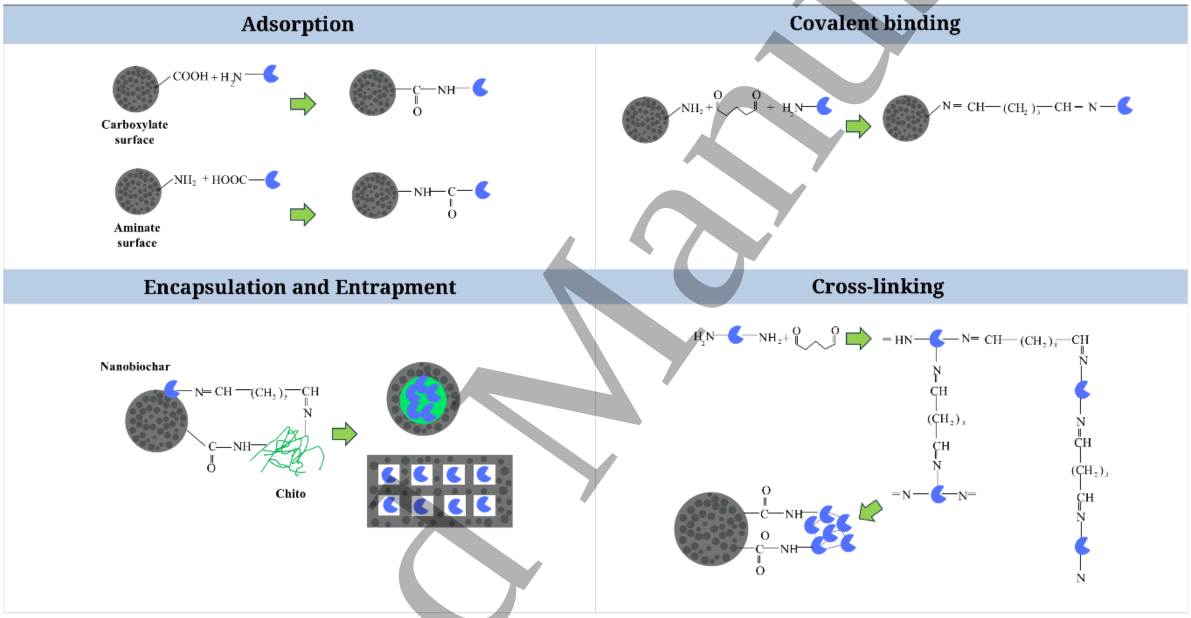

Figure 2 – Methods for enzyme immobilization on biochar

4.1 Adsorption

The presence of surface oxygen-containing functional groups on the biochar surface, such as carboxyl and hydroxyl groups, enhances its adsorption capacity through hydrogen bonding and π-π interactions, thereby facilitating the adsorptive immobilization of enzymes [11, 87].

Enzyme adsorption onto biochar is mainly governed by electrostatic interactions which depend on the correspondence between the isoelectric points of enzymes and the biochar, influencing the overall adsorption capacity. Specifically, negatively charged functional groups on the biochar surface interact with positively charged moieties on the enzyme's molecules [88].

Additionally, certain strategies, such as nitrogen doping, can enhance adsorption by promoting the formation of polar functional groups on the biochar surface and etching it into more porous structure. The incorporated nitrogen introduces nitrogen-containing functional groups into layered porous carbon, thereby improving surface reactivity and adsorption potential. The amine groups on the biochar surface can bind to the carboxyl groups present on the enzyme's protein surface (Figure 2) [87].

Structural properties of the biochar, particularly porosity, also play a crucial role in enzyme adsorption. The size of the enzyme determines the accessibility of surface pores and thus its contribution to adsorption efficiency: smaller enzymes can penetrate and access a larger portion of the biochar surface, whereas those with higher molecular weight and larger size are more restricted. In addition, hydrophobic interactions may contribute to enzyme adsorption, since biochar exhibits varying degrees of hydrophobicity and many enzymes possess hydrophobic surface regions as well [88].

An advantage of this method is that it does not require the use of chemical reagents, making it ideal for environmental applications, as it aligns with the principles of green chemistry and sustainability [89]. However, enzyme leaching may occur due to the weak nature of these interactions, which can lead to desorption. Nevertheless, the reversibility of binding can be advantageous, as it allows the support to be regenerated and reloaded with fresh new enzymes once enzymatic activity declines [89]. The adsorption of enzymes onto biochar surfaces depends on several factors, including the type and size of the biochar, the functional groups present on its surface, the type of enzyme used, and the pH of the solution [13].

One strategy to enhance the adsorption capacity of biochar is the development of nano biochar (nano-BC). Compared with bulk biochar (bulk-BC), nano-BC exhibits significantly superior adsorption performance, primarily due to its distinct physicochemical properties. The nanoscale particle size gives nano-BC a specific surface area typically 2 to 5 times greater than that of bulk-BC, thereby providing substantially more active sites for contaminant adsorption. Furthermore, the milling process used in nano-BC production exposes additional functional groups (e.g., -COOH, -OH) on its surface, which further increases its affinity for both organic and inorganic pollutants. These properties give nano-BC a potential advantage in enhancing water and fertilizer retention and reducing heavy metal pollution and providing soil structure [13].

4.2 Entrapment and encapsulation

The entrapment or encapsulation of enzymes is typically carried out within polymer networks or microcapsules that allow the diffusion of substrates and products while retaining the enzymes. This method is simple, cost-effective, and irreversible. It prevents enzyme aggregation and leaching, while improving enzymatic stability. Since there is no direct covalent interaction between the enzyme and the support, the enzyme's conformation is preserved [8].

Among the various immobilization techniques, enzyme encapsulation in polymers has proven particularly effective, as it provides protection under harsh conditions. Natural polymers such as biochar are often used due to their biocompatibility, non-toxic nature, and favorable surface interactions [90]. However, the success of encapsulation depends on several factors, including the chemical nature of the polymer, pore size and volume, and the compatibility between the enzyme and the matrix. These factors are critical to ensure efficient diffusion of substrates and products in and out the encapsulation structure [91].

For example, in the encapsulation of *Trametes versicolor* laccase with a chitosan-nanobiochar matrix, the pendant aldehyde groups on the chitosan (Cs) surface react with the amino groups of the enzyme, forming imine bonds (—CH = N—). Under acidic conditions, the amino groups in Cs can be protonated, generating polycations. At the same time, carboxylic acid groups are introduced onto the nanobiochar surface through acidic treatment. Consequently, the positively charged Cs molecules can be adsorbed onto the negatively charged functionalized nanobiochar (FNBC) surface via electrostatic interactions (Figure 2) [90].

This method offers two main advantages for enzyme immobilization. First, its small particle size reduces mass transfer resistance between the enzyme and substrate. Second, it allows for the efficient separation of the immobilized enzyme from the reaction medium, enabling enzyme reuse and consequently reducing both capital and operational costs [90].

Moreover, entrapment is widely used in bioremediation applications because it is rapid, inexpensive, non-toxic, and highly versatile [92]. In this method, enzymes are physically caged within a matrix through covalent or non-covalent interactions. These matrices minimize conformational changes and help retain the native activity of the biocatalyst. Furthermore, entrapment usually causes minimal interference with the natural properties of the enzyme [91]. Common entrapment methods are fiber entrapping, gel entrapping, microencapsulation, among others [92].

4.3 Covalent binding

Covalent binding involves the formation of stable complexes through covalent bonds between enzymes and solid support via functional groups and amino acid side chains. These functional groups of the enzyme react with the reactive sites of support material, resulting in strong, irreversible covalent linkages. [4, 13]. The efficiency of this immobilization method depends on the availability and reactivity of various enzyme functional groups, such as carboxyl, amino, indole, sulfhydryl, thiol and phenolic groups [4].

Covalent binding occurs through chemical reactions between amino acid side chains in the enzyme, such as lysine, cysteine, or aspartic acid residues, and functional groups present on the carrier surface (e.g., amino, carboxyl, or hydrophobic groups). This process forms strong and irreversible covalent linkages between the enzyme and the biochar, which minimizes conformational changes, prevents enzyme leaching, and eliminates issues related to enzyme desorption or instability (Figure 2) [11, 87, 93].

The surface of biochar contains multiple functional groups that can participate in these reactions. The number and reactivity of these functional sites can be enhanced by several techniques, such as chemical modification (e.g., glutaraldehyde addition) or surface coating, to increase the availability of binding sites for enzyme immobilization [94].

Glutaraldehyde (GA) is one of the most widely used cross-linking agents for covalent immobilization. It possesses two aldehyde groups that can react covalently with other functional groups via aldol condensation or Schiff base reaction. Therefore, enzyme immobilization using GA depends on the abundance of –OH and $–NH_2$ groups on the biochar surface [95]. During enzyme immobilization, covalent bonds are formed between the amino groups of the enzyme and the glutaraldehyde groups of GA, generating imine (Schiff bases) linkages [96].

Functionalization of solid supports with cross-linking agents prevents enzyme loss during operation, as this process is irreversible. Moreover, covalent attachment can help preserve the active conformation and correct orientation of the enzyme, enabling a more controlled and specific immobilization compared to non-covalent methods [96].

Additionally, the efficient covalent immobilization on biochar depends on factors such as the amount of biochar, enzyme concentration, and the concentration of the coupling reagent. However, it is important to note that these reagents may reduce the conformational flexibility of the enzyme, potentially lowering its catalytic activity [4].

4.3 Ionic binding

The ionic binding is a non-covalent immobilization in which ionic bonds are formed between enzymes and water-insoluble solid support materials containing ion-exchange residues. This binding can be reversed by modifying factors such as ionic strength, polarity, and temperature. Support materials commonly used in this method include synthetic polymers and polysaccharides, as they possess functional groups capable of participating in ion exchange [92, 93].

This method is relatively simple in comparison with covalent binding and can even enhance enzyme activity due to slight alterations in the enzyme's active site and overall conformation. However, a major limitation of ionic binding is the relatively weak interaction between the enzyme and the support matrix, which may affect stability of immobilized enzyme [92].

4.4 Cross-linking

The cross-linking method involves the reaction of a multifunctional reagent with amino or carboxyl groups in the enzyme, causing the enzyme molecules to link together and form water-insoluble aggregates [94]. This method is commonly applied in solid support-free immobilization systems, such as cross-linked enzyme aggregates (CLEAs) and cross-linked enzyme crystals (CLECs). This method is a relatively simple technique that allows for potential long-term reusability and can also result in the formation of covalent bonds [8].

In biochar-based systems, cross-linking agents form a stable three-dimensional network structures between the enzyme molecules and the biochar surface, or among the enzyme molecules themselves (Figure 2) [93]. Amino functionalization of the biochar further enhances binding interactions by increasing hydrogen bonding and electrostatic attraction between enzyme and the support. This modification increases the positive charge density of the biochar surface, which promotes the attachment of negatively charged regions of the enzyme molecules through electrostatic adsorption, thereby enhancing immobilization efficiency [86].

However, one of the main drawbacks is the possibility of conformational changes in the enzyme structure, which may lead to a loss of enzymatic activity. To mitigate this issue, cross-linking is often combined with other immobilization strategies [8]. In fact, it is generally not used as a standalone method, but rather in combination with adsorption techniques, which can increase enzyme loading capacity to a certain extent [94].

For example, in the immobilization of a laccase in a nanosized magnetic biochar (L-MBC), a combination of adsorption and cross-linking was employed. First, laccase was incubated in a phosphate buffer solution containing magnetic biochar nanoparticles, followed by the addition of ammonium sulfate to induce enzyme precipitation. Subsequently, a glutaraldehyde solution was added to cross-link the enzymes. This immobilization strategy improved storage stability, pH tolerance, and thermal stability. Additionally, the presence of magnetic biochar enabled easy magnetic separation and enhanced reusability [95].

5. Biochar-based enzymatic immobilization for removal of emerging contaminants

5.1 Emerging contaminants (ECs)

Emerging contaminants (ECs) are newly identified synthetic or natural chemicals or biological agents that are detected in the environment with potential hazardous effects to humans and ecosystems [15]. ECs include pharmaceuticals, personal care products (PPCPs), per-and poly-fluoroalkyl substances (PFAS), emerging pathogens, toxins, pesticides, industrial chemicals, micro and nanoplastics, nanomaterials, antibiotic resistance genes (ARGs), hormones, plasticizers, antimicrobials, among others [14, 15]. Such substances are an environmental threat, as they are persistent, difficult to remove from ecosystems, and they are not eliminated during wastewater treatment [96].

Furthermore, during their degradation, intermediates compounds are formed, which are also resistant to degradation and can be even more toxic than their parent compound [14]. With the lack of regulatory framework to reduce the pollution caused by these contaminants, ECs accumulate in the food chain, representing potential risks to the environment and mankind [97]. It is needless to state that the removal of ECs is imperative for human and global health.

5.2 Enzymes for ECs degradation

Enzymes can be applied in a bioremediation strategy to remove ECs from the environment (Table 2). Catalytic bioremediation is advantageous compared to microorganisms or phytoremediation due to better activity for pollutant degradation with lower waste generation [14]. Usually, oxidoreductases are used in ECs bioremediation, especially in the removal of pharmaceutical compounds [8].

These enzymes can catalyze the oxidation of a variety of contaminants, such as phenols, herbicides, pesticides, dyes, and pharmaceuticals [12]. Among oxidoreductases, laccases play a significant role in degrading ECs, by oxidizing the compounds, reducing the toxicity of the pollutants and their intermediates [14]. Additionally, laccases present a wide substrate range (from phenols to polycyclic aromatic hydrocarbons), simple reaction requirements, high stability and low inhibition [98]. Since laccases only require oxygen as substrate and produce water as by-product, they are considered green catalysis [12]. These characteristics make them excellent enzymes for ECs removal from the environment in a bioremediation strategy.

Table 2 – Different waste biomass used in biochar production and its utilization in enzyme immobilization for ECs removal

Enzyme	Waste biomass for biochar obtention	Biochar production technique	Enzyme immobilization method	EC removal	Reference
Laccase	Avocado seeds	Slow pyrolysis	Covalent immobilization	Acetaminophen 99% removal after 24h, at pH 4 – 5 and 25°C	[99]
Laccase	Sour cherry stones	Slow pyrolysis	Adsorption	Brilliant Green 93% removal after 4h, pH 5 and 30°C	[12]
Laccase	Holm oak tree prunings	Slow pyrolysis	Covalent immobilization	Tetracycline and sulphonamides 100% removal after 20h, at 40°C	[100]
Laccase	Wheat straw	Slow pyrolysis	Adsorption–crosslinking	2,4–dichlorophenol 64.6% removal in soil with 40% of water content, pH 4, 35°C and 5 days	[101]
Laccase	Pine needle	Slow pyrolysis	Adsorption	Malachite dye 85% removal after 5h, at 30°C	[4]
Laccase	Corn straw	Slow pyrolysis	Adsorption	Petroleum contaminants Up to 91.2% of phenanthrene removal at 30°C, 180 rpm, for 7 days	[102]
Lacasse	Bagasse	Slow pyrolysis	Cross-linking	100% bisphenol A after 60 minutes of treatment	[88]
Laccase	Coconut husk	Slow pyrolysis	Adsorption	72.49% - 84.64% removal of 2,4-dichlorophenol	[103]
Laccase	Mushroom residue	Slow pyrolysis	Covalent immobilization	Degradation of endocrine-disrupting chemicals: of bisphenol A (90.87%), estradiol (92.95%), and	[104]

				ethinylestradiol (80.87%) after 24 hours of treatment	
Laccase	Pig manure	Slow pyrolysis	Covalent immobilization	98.9% removal of diclofenac after 2h	[105]
Laccase	Pine sawdust	Slow pyrolysis	Adsorption-crosslinking-covalent binding	92.1% degradation of trichloroethylene (TCE) after 48h	[106]
Laccase	Pine white wood	Slow pyrolysis	Covalent immobilization	58.3% degradation of chlortetracycline in continuous mode at 1 mL/h.cm^2	[107]
Peroxidase	Sawdust of beech and oak wood mixture	Slow pyrolysis	Cross-linking	90% removal of phenol from wastewater	[108]
PET hydrolase	Laden pine sawdust	Slow pyrolysis	Covalent immobilization	29.6% PET-MPs reduced, converting PET into mono(2-hydroxyethyl) terephthalate (MHET) in soil microcosm	[109]

5.3 ECs degradation with enzymes immobilized in waste-derived biochar

To overcome the main disadvantages of using free enzymes in bioremediation, such as lowering of catalytic activity and stability, higher operational costs, and poor reusability they can be immobilized in biochar [12]. The use of immobilized enzymes in waste-biochar has been studied in recent years (Table 2). This strategy has an intrinsic advantage as biochar can be produced using waste biomass such as agricultural by-products, forestry residues, municipal and domestic scrap, and different types of wood [13]. This concept fits in with the perspective of treating waste-by-waste, as waste materials are repurposed to produce enzymatic support matrices which are further applied to remove wasteful contaminants from the environment. Additionally, due to high surface area and porosity, the biochar itself can be used to remove a range of contaminants, such as metal ions, dyes, and pharmaceuticals [9]. These characteristics can be further explored with oxidoreductases immobilized in waste-biochar – especially by adsorption technique – which enhance ECs degradation, with promising environmental applications [8, 13].

Such approach was studied by Da Silva and co-workers (2022) [99], where a laccase was immobilized in biochar from avocado seeds to remove the pharmaceutical acetaminophen. The authors found that the enzyme assumed a more stable conformation at pH 4 and 5, favoring the catalysis of the target compound. After 24 hours, the concentration of acetaminophen was decreased by 99% indicating that laccase immobilized in avocado seed biochar has potential to be used for remediation of emerging pollutants.

Comparably, Antanasković and collaborators (2024) [12] also studied the immobilization of laccases in biochar from waste materials. The authors used sour cherry stones to produce biochar, which was further utilized as a support for brilliant green degradation. At 30°C, pH 5 and 4 hours of reaction, 93% of dye was removed. The developed system showed potential for removal of ECs and a sustainable solution for wastewater treatment.

Laccases can also be immobilized via covalent binding in mushroom residue biochar, as studied by Yu and co-workers (2021) [104]. The biochar-enzymatic complex could degrade endocrine-disrupting chemicals such as bisphenol A (90.87%), estradiol (92.95%), and ethinylestradiol (80.87%) after 24 h of reaction. Due to the biochar-immobilization, the enzyme presented enhanced stability at pH 2–5, could retain 86.4% of its activity after 30 days at 4°C, and after eight cycles maintained 65.2% of its initial activity. The catalyst facilitated oxidation of endocrine-disrupting substances, generating reactive radicals that further degraded into less harmful substances. The authors concluded that the biochar-immobilized enzyme achieved pollutant removal through synergistic catalysis and adsorption, suggesting that this system is a promising strategy for ECs degradation from the environment.

Another type of oxidoreductase that can be used in ECs degradation is the peroxidase enzyme. The potential of this catalysis in removing phenolic compounds from wastewater was studied by Petronijević and colleagues (2021) [108]. The peroxidase was immobilized via cross-linking in biochar produced from sawdust of beech and oak wood in slow pyrolysis. In the best condition (pH 7, 2 h), 90% of phenol could be removed. The immobilized enzyme retained 79% of its activity after 4 washings, with 64% of phenol removal. The authors concluded that enzyme immobilization in waste-biochar improves its characteristics, increases stability and reusability.

Other than oxidoreductases, some hydrolytic enzymes can be applied to remove ECs. That's the case of the research developed by Han and co-workers (2024) [109], in which PET hydrolase was immobilized in biochar to remove microplastics. The authors produced magnetic biochar from laden pine sawdust via slow pyrolysis, which was then used to immobilize the chosen enzyme. When this system was applied in a PET-contaminated soil, a 29.6% microplastic weight loss was observed. Additionally, the biochar-immobilized enzyme could be recycled for five consecutive cycles, maintaining 58.5% of residual activity. Furthermore, the authors noticed that the soil microbiota composition changed after application of the catalytic complex, also enhancing nitrogen fixation, phosphorous uptake and transport.

6. Advantages, constraints and perspectives of using enzymes immobilized in waste-derived biochar for ECs degradation

The use of waste-derived biochar for enzyme immobilization and its further use in ECs removal has the intrinsic advantage of fitting in the context of treating waste-by-waste. Additionally, the use of agricultural and domestic waste for biochar production offers a strategy to lower its production costs, facilitating its large-scale production and application. For example, in May 2025, CHAR Technologies Ltd. (Canada) announced a partnership with Synagro Technologies (US) and the Baltimore City Department of Public Works to launch a commercial-scale pyrolysis plant that will simultaneously remove PFAS from biosolids, produce syngas and biochar [110].

Even though this is exciting news regarding biochar production from waste materials, there are no reports of waste-derived biochar being used for enzyme immobilization and further ECs removal at a commercial scale. Such strategy is restricted to university-level and academic reports (e.g. Table 2). To boost the application of these systems at a commercial level, partnerships between universities and companies are required.

This would not only be a solution for waste management and environment pollution, but it would also be aligned with some of the UN SDGs, such as SDG 6, 11, 12, 13 and 14. For example, the SDG 6 is related to guaranteeing the availability and sustainable management of water and sanitation, with focus on minimizing release of hazardous chemicals and materials in water bodies [5]. SDG 12 (ensure sustainable consumption and production patterns) has the goal to achieve the management of chemicals and waste throughout their life cycle [111]. Lastly, SDG 14 – related to conservation and sustainably use the oceans, seas and marine resources for sustainable development – is also related to the topic, since it is focused on the prevention and reduction of marine pollution, from land-based activities [112]. Therefore, the removal of ECs using this strategy is not only of significance from the environmental perspective, but also from the public health and government management perspectives.

Even with all the advantages discussed above, it is necessary to consider some key-points about the removal of ECs with enzymes immobilized in biochar. For example, it is important to confirm that the contaminants elimination is due to the catalytic reaction and not associated with the biochar itself. This is because biochar can adsorb environmental pollutants, organic contaminants and cations [8]. To overcome this issue, Pandey and colleagues (2022) [113] analyzed toxic malachite green dye removal with two systems: (i) laccase immobilized in pine needle biochar and (ii) deactivated laccase (80°C, 2h) immobilized in the same support. The authors found that after 5 hours at 30°C, 57% of dye was removed when the deactivated enzymatic system was used. With the same conditions, but using the non-denatured laccase, 85% of the initial dye content was removed. The authors attributed this result to the catalytic enzyme action causing dye degradation, combined with the adsorption of the degraded products on biochar.

Another aspect that must be taken into consideration is the application of these enzymes systems in real environmental conditions. Most studies evaluate EC removal in controlled laboratory settings, often overlooking key variables present in practical field applications. Contrarily, the study developed by Wang and collaborators (2021) [101] analyzed the removal of 2,4–dichlorophenol in soil with free laccase and with the enzyme immobilized in wheat straw biochar. After 5 days, 44.4% of the target compound was removed with the free enzyme, compared with 64.6% removal when laccase immobilized in biochar was used. The authors concluded that the immobilized enzyme had better activity, higher stability, better catalytic degradation towards 2,4–dichlorophenol, and that the biochar carrier could improve the physical and chemical properties of the soil, playing a positive role in the improvement of the soil ecological environment.

Overall, the use of biochar produced by waste biomass to remove ECs from different ecosystems is a promising research field. As discussed previously, one must consider the effects of biochar alone and in conjunction with enzymes to properly analyze the advantages of these systems. Future research should focus on the development of a molecular structural model for biochar, as it can provide information about its reactivity and help further functionalization for different applications [8]. More studies focusing on simulating environmental conditions are necessary to apply these systems in real-life ecosystems. Also, partnerships between industries and universities are necessary to apply the developed technology at larger, commercial scale.

5. Conclusion

The growing concern about environmental pollution with ECs boosted a new research pipeline focusing on treating waste-by-waste. Using different types of residues to produce biochar, immobilizing enzymes in this waste-derived matrix and further applying this system to remove ECs from the environment is a promising strategy. Other than removing toxic compounds, such approach has the advantage of providing a solution for waste management, as biochar can be obtained from industrial, agricultural and domestic residues, fitting in the circular economy context. This waste-by-waste strategy is also aligned with the UN SDGs 6, 11–14 of the 2030 Agenda. The biochar can be produced via a range of technologies, but slow pyrolysis is the one that converts the largest amount of biomass into the desired product. Also, the most suitable techniques for enzyme immobilization in biochar are adsorption, covalent bonding, cross-linking and their combinations, due to their eco-friendliness and non-toxic nature. ECs are usually degradaded by oxireductases, especially by laccases, which oxidize these compounds, reducing the toxicity of the pollutants and their intermediates. Even though this approach has shown significant advances, future studies should focus on simulating real-life ecosystems, biochar structure modelling, and economic analysis to truly make these systems cost-effective, guaranteeing their application in ECs bioremediation. Finally, collaboration between companies, industries and universities are imperative to make this technology available at a commercial scale.

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