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Review

Biochar: A Sustainable Alternative in the Development of Electrochemical Printed Platforms

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Abstract: Biochar is a pyrolytic material with several environmental benefits such as reducing greenhouse gas emissions, sequestering atmospheric carbon and contrasting global warming. However, nowadays, it has moved to the forefront for its conductivity and electron transfer properties, finding applications in the fabrication of electrochemical platforms. In this field, researchers have focused on low-cost biomass capable of replacing more popular and expensive carbonaceous nanomaterials (i.e., graphene, nanotubes and quantum dots) in the realization of sensitive cost-effectiveness and eco-friendly electrochemical tools. This review discusses recent developments of biochar-modified screen-printed electrodes (SPEs). Special attention has been paid to biochar's manufacturing processes, electron-donating capabilities and sensing applications. Examples of representative works are introduced to explain the distinct roles of biochar in several electro-bioanalytical strategies.

Keywords: biochar-modified screen-printed devices; sustainable sensors; eco-friendly materials; electrochemical enhancer; green chemistry

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Citation: Cancelliere, R.; Cianciaruso, M.; Carbone, K.; Micheli, L. Biochar: A Sustainable Alternative in the Development of Electrochemical Printed Platforms. *Chemosensors* **2022**, *10*, 344. https://doi.org/10.3390/chemosensors10080344

Academic Editors: Damiano Monticelli, Gilberto Binda and Davide Spanu

Received: 20 June 2022
Accepted: 18 August 2022
Published: 22 August 2022

1. Introduction

With the continuous progress in technology, understanding carbon nanomaterials (CNMs) has reached a new level of interest. Their excellent electrical conductivity, stability, potential high-throughput screening and easy-to-use assay procedure make these compounds suitable for the fabrication of realizing sensors and energy storage devices [1,2]. The production of carbon nanostructures has greatly increased the use of these devices, creating a wide range of applications based on their use, ranging from the creation of miniaturized printed electrodes to the development of flexible electronic devices, humidity sensors or passive sampling systems [3–5]. Until now, several electronic and electrochemical tools are developed and applied at the lab scale. Nevertheless, to allow the deployment of these technologies on a large scale, it is necessary for a cost-production reduction and their actual health safety. In addition, CNMs are rather energy-intensive and expensive and require long syntheses, and studies on their toxicity are not yet concluded [6]. For example, activated carbon is conventionally derived from coal, while CNMs such as carbon nanotubes and graphene can be produced through specific techniques (i.e., chemical vapor deposition, electric arc discharge, etc.) using gaseous petrochemicals (i.e., methane, acetylene, ethylene, hydrogen, etc.) at high temperatures (>800 °C) [7]. These high-temperature and resource-intensive processes can be suitable for large-scale production in industrial settings but are not compliable with a fully sustainable solution or production in smaller installations. Alternative eco-friendly carbon material, produced at low cost, competes to replace CNMs, widely used at the industrial level [8]. Recently, attention is being focalized on green recycling carbon material, called biochar. The latter is an inexpensive, solid, recycling carbonaceous material obtained by pyrolysis of renewable sources (i.e., wood chips and pellets, bark, straw, walnut shells and rice husks, bagasse, sewage sludge, etc.) [9–16], and its production requires a lower overall energy input than activated carbon, resulting in lower net energy consumption with a low net cost. Its morphological and physicochemical properties can strongly vary with the feedstock source choice and the conditions of the pyrolysis treatment [17–23]. In addition, the high porosity combined with its surface being rich in functional groups on one side and its excellent electrical conductivity and biocompatibility on the other expand its applications in different fields. A schematic representation of biochar's applications and its most exploited qualities is reported in Figure 1.

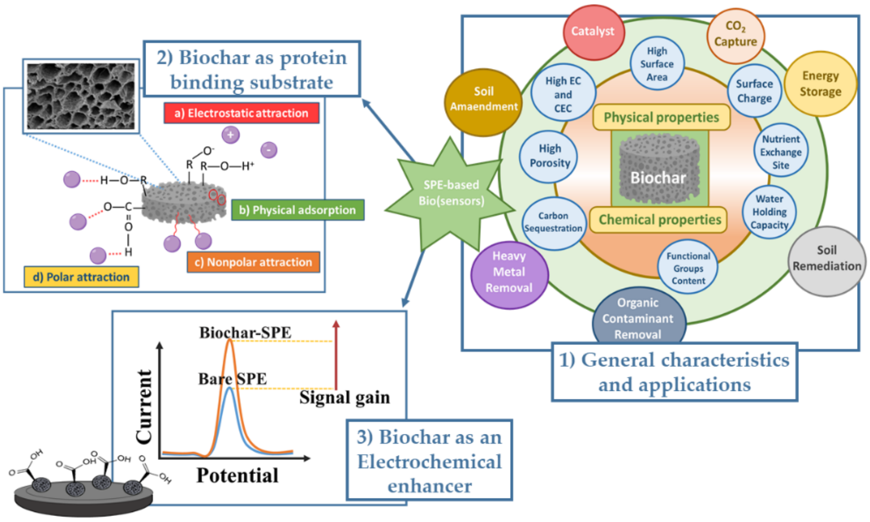

Figure 1. Chemical–physical properties of biochar and its application in the development of electrochemical SPEs-based biosensors.

Until a few years ago, biochar has been used in a large number of applications such as adsorbent for wastewater treatment and agronomic applications (i.e., immobilizing organic and inorganic pollutants), or as an amendment in agricultural soils (effects on soil, agricultural yields, and nitrogen leaching and emissions) [18,20–26]. However, recently, its potential as an eco-friendly and smart nanomaterial for several electrochemical applications is increasingly exploited [27]. In 2015, Joseph et al. reported the redox properties of different biochar, which are rich in carbon (amorphous and graphitic C, labile organic compounds, and minerals) in this regard [28]. In particular, they underlined that the electrochemical properties of biochar are a function of the concentration and composition of the various redox-active minerals and organic compounds and, at the same time, of their production method [29,30]. Many electrochemical studies, present in the literature, reported the use of biochar as a surface modifier of electrodes (i.e., carbon paste, glassy carbon, etc.) [31–33], but only a few of them concern SPEs [34–36]. Precisely with that in mind, the present work aims to illustrate the recent applications of biochar as a sustainable nanomaterial for electrochemical applications in the field of SPEs. Before all else, its capabilities to act as an electrochemical enhancer (improving the electron transfer process when used for the modification of SPEs), as a booster of the active surface area of the electrodes and as a protein binding substrate (i.e., enzyme, antibody, etc.) in the production of biosensors were carefully studied and discussed [37].

2. Chemical and Physical Characteristics of Biochar

2.1. Correlation between Biochar Characteristics and Its Production Processes

Biochar can be considered one of the ways to recover the residual biomass supply chain. There are different renewable energy options available today, but biomass is the only renewable source of carbon. Biomass enhancement would make it possible for the waste management industry to lessen enormous quantities of residue every year, thus guaranteeing considerable savings. In general, the conversion of this wasting material can be achieved by two different types of processes: biochemical and thermochemical conversion [38]. During these processes, the structural woody parts of biomasses (i.e., lignin, cellulose, etc.) are converted into a variety of condensed aromatic matters (cracking of side-chain C–C bonds, with the transition from unsaturated to saturated alkyls), inorganic salts (i.e., P, K, Ca, etc.) and polycyclic aromatic hydrocarbons (PAHs) belonging to ash division [39]. A general summary is reported in Table 1.

Biochemical processes are based on the use of enzymes, fungi and microorganisms that cause chemical reactions, which originate in the biomass under particular conditions. These processes are particularly suitable for those biomasses with less than a 30 organic carbon/nitrogen ratio (C/N) and greater than 30% humidity at harvesting. Conversely, thermochemical conversion processes are suitable for those biomasses with greater than 30 C/N ratios and less than 30% humidity content, such as wood and all its derivatives (sawdust, shavings, etc.), lignocellulose crop by-products (cereals straw, residues from vines and fruit trees pruning, etc.) and some processing waste (husk, chaff, shells, carpel, etc.) [40]. As for the biochemical conversion processes, different technologies such as carbonization, gasification, or pyrolysis are usually used for the thermal decomposition of feedstock, thus producing biochar, gases and oil [41,42].

Table 1. Thermochemical and biochemical conversion of biomass and their specific products.

Process	Treatment	Main Product	Reference
Thermochemical	Slow pyrolysis	Biochar	[43]
	Fast pyrolysis	Bio-oil	[44]
	Flash pyrolysis	Bio-oil	[45]
	Liquefaction	Bio-oil	[46]
	Gasification	Syngas	[47]
Biochemical	Transesterification	Biodiesel	[48]
	Photobiological H_2 production	Hydrogen	[49]
	Alcoholic fermentation	Ethanol	[50]
	Anaerobic digestion	Methane	[51]

Concerning biochar, it is produced from the pyrolysis (200–900 °C) of waste feedstock in the absence of oxygen and its chemical (i.e., adsorption capacity, etc.) and physical properties (i.e., morphology, surface structure, etc.) depend mainly on the raw material used and the specific conditions under which it is produced and treated [39,52–54]. Notably, there are two types of pyrolysis: fast and slow pyrolysis [55]. The first one, which occurs in a very brief period (several seconds), is usually used for the production of bio-oil (yielding 75%) [56]. However, most biochar is produced through slow pyrolysis processes, a process with longer production and treatment time (from hours to several days) [57]. Indeed, a decrease in the amount of biochar produced was assessed when increasing heating rates are used, as reported in previous work [58]. Precisely, an increased surface area using higher pyrolysis temperatures was generally obtained [59]. This is ascribable to the aliphatic alkyl and ester groups and the exposed aromatic lignin component, which, once destroyed, contributes tremendously to the increase in the surface area [60]. In particular, a direct proportionality between micropore volume and surface area is founded and, in this way, the increase in surface area is primarily due to the increased pore size distribution of the biochar [57]. Furthermore, it was observed that biochar produced at higher temperatures is more effective in remedying soil and water contamination (because of the increased surface area and the biochar's microporosity) [52], while biochar produced by a low heating rate presents a smaller surface area (due to deformation, cracking, or micropore blockage) [61]. Moreover, it was observed that uncontrolled decomposition conditions of biomass lead to the emission of copious quantities of greenhouse gases into the atmosphere, such as CO_2 and CH_4. The latter, if properly stored, could be used for energy purposes [40]. Examples of biochar production processes are reported in the literature and schematically summarized in Table 2.

Table 2. General ratio data of different pyrolysis product distribution.

Process	Temperature	Time	Solid (Biochar)	Liquid (Bio-Oil)	Gas (Syngas)
Fast pyrolysis	Mild (≈500 °C)	<2 s	12%	75% (25% H_2O)	13%
Intermediate Pyrolysis	Mild	Moderate	25%	50% (50% H_2O)	25%
Slow Pyrolysis	Mild/low	Long	35%	30% (70% H_2O)	35%
Gasification	High (>800 °C)	Long	10%	5% (tar + H_2O)	85%

Klupfel et al. (2014) carefully studied the production of biochar. In particular, they explained that this process and its correlated release of gases cause a separation between the sheets of condensed poly-aromatic creating void spaces, fissures and pores that enhance their surface area [62]. Furthermore, Saifullah et al. (2018) demonstrated that the chemical composition of the biomasses and temperature used in the production of biochar incredibly affect its properties; indeed, different examples of raw stuff applied, and the modality of temperature were discussed [63]. For instance, Rafiq et al. (2016) observed that biochar produced at low temperatures displays a high number of volatile substances and a lower amount of ash and fixed carbon contents than that produced by the flash temperature approach [64]. Wang et al. (2018) reported that both enhancing the temperature of pyrolysis and varying other pyrolysis input elements, a different ratio between carbon, hydrogen, oxygen and nitrogen was noted [65]. Compared to gasification technologies, pyrolysis is characterized by a higher yield of solid fractions [66–68]. A general representative scheme of pyrolysis is reported in Figure 2.

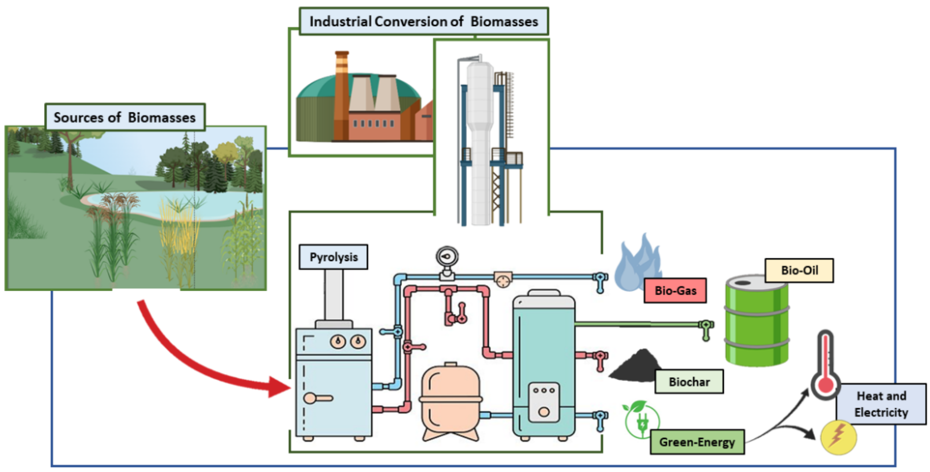

Figure 2. Schematic representation of the industrial conversion of biomasses by pyrolysis.

2.2. Chemical Properties of Biochar

Biomass feedstock involves processes and operating conditions (i.e., temperature, humidity, presence of gases, etc.) that are factors that strongly influence the chemical properties of biochar, and sometimes causing the accumulation of poisonous substances in the final product [14]. For these reasons, it is critical to optimise the conditions of thermochemical conversion to avoid the presence of toxic gaseous emissions. Table 3 summarizes some of the analytical parameters used for the characterization of biochar, comparing the quality standards proposed by the two major associations that promote the safe use of biochar, namely the European Biochar Certificate (EBC) and the International Biochar Initiative (IBI) [69]. The EBC recognizes two qualities of biochar, namely "basic" and "premium", with different limit values for pollutants and heavy metals. EBC uses the term “biochar” only for products with a total carbon value greater than 50%, while all those below that value are classified as carbonaceous materials (PCM) [70]. On the other hand, the IBI classifies biochar into three classes based on the different percentages of organic carbon content: Class 1 is above 60%, Class 2 is between 30% and 60% and Class 3 is between 30% and 10% [71]. Both associations require a maximum hydrogen/carbon (H/C) ratio of 0.7, as an indicator of the carbonization degree and, thus, the stability of the biochar. Only the EBC further indicates the oxygen/carbon (O/C) ratio, which must be less than 0.4. In both analyses, the total amount of ashes and nitrogen must be declared; for the EBC the residual amount of phosphorus, potassium, magnesium and calcium are also required, while phosphorus and potassium are optional for IBI. Concerning the presence of heavy metals, the EBC indicates two sets of limit values for lead, cadmium, copper, nickel, mercury, zinc and chromium, which differ for basic biochar and premium biochar. Values for cobalt, molybdenum, arsenic, selenium, boron, chlorine and sodium are present in the IBI analysis. The two standards have some differences in the maximum limits of polycyclic aromatic hydrocarbons (PAHs), where the EBC indicates two threshold limits for the quality of basic biochar (<12 mg kg^{-1}) and premium (<4 mg kg^{-1}), while the IBI indicates a single wider range of 6–300 mg kg^{-1}. The limit values for polychlorinated biphenyls (PCB) are slightly higher for the IBI, which accepts values between 0.2 and 0.5 mg kg^{-1}, while, according to the EBC, they must be below 0.2 mg kg^{-1}. Regarding polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), if considered together, the IBI requires them to be below 17 ng kg^{-1}, while the EBC indicates a maximum limit of 20 ng kg^{-1}. Moreover, the amount of volatile organic compounds (VOCs) is an important indicator to evaluate the pyrolysis process, given the fact that VOCs are mainly due to the presence of pyrolysis gases condensed on the surface or in the pores of the biochar. This parameter is required only for EBC, while it is optional for IBI. Some characteristics, such as electrical conductivity, pH, or water content, are given by both agencies, while other characteristics, such as the bulk density, surface area, or particle size distribution, are reported by only one or the other agency (see Table 3). Lastly, it is possible to carry out tests to evaluate the inhibition of germination in the case of biochar used as a soil conditioner [41,69–71].

2.3. Activation Processes

For scientific applications, it is possible to modify biochar using activating agents, which can be physical or chemical. These processes used to modulate the properties of biochar and to obtain an activated substrate, suitable for multiple purposes, can be performed after pyrolysis or take place simultaneously [72,73]. The main methods for the physical activation of biochar include the use of steam, ball mills and sonication processes. Specifically, in steam-based activation, the partial gasification of biochar with the consequent release of CO_2 and H_2 cause pore formation with a huge increase in the internal surface area [74,75]. In this respect, ball mills and sonication are usually used: the first to enlarge the volume and size of the pores, the second (at low frequencies) to involve the exfoliation and disruption of the regular structures of the graphite oxide planes, respectively [76–78]. Contrastively, chemical activations are based on the use of acids, bases, oxidizing agents or inorganic salts. These can alter some physical–chemical properties and introduce specific functional groups on the surface. Treatment with acids can be used to remove impurities or to introduce acidic functional groups, while, with the addition of oxidizing agents, oxygen-containing functional groups can be introduced and increase the hydrophilic profile [72,79–82]. The use of alkali can decrease surface polarity and alter porosity, thus affecting the adsorptive capacities of biochar. In addition to chemical and physical treatments, biochar can be activated by other methods, such as the use of UV radiation or magnetic treatments, which do not require toxic substances or environmentally harmful processes. UV radiation, particularly UV-A, can oxidize the surface and introduce oxygen-containing functional groups, while magnetic modifications can impart ferromagnetic properties to biochar, allowing better separation of particles in various treatments [68,69].

Table 3. Comparison between European Biochar Certificate version 4.8 and IBI Biochar Standard version 2.0 [10].

Parameter	European Biochar Certificate V4.8	IBI Biochar Standards V2.0
C content	Required (total C) Biochar $\geq$ 50% PCM < 50%	Required (organic C) 10% Minimum Class 1: $\geq$60% Class 2: $\geq$30% and <60% Class 3: $\geq$10% and <30%
Molar H/C$_{org}$ ratio	Required 0.7 maximum (molar ratio)	Required 0.7 maximum (molar ratio)
Total Ash	Required	Required
Molar O/C ratio	Required 0.4 maximum	Not required
Macronutrients (NPK)	Required (Total N) Required (Total P, K, Mg, Ca)	Required (Total N) Optional (Total P and K)
Heavy metals, metalloids and other elements	Required Metals: Pb, Cd, Cu, Ni, Hg, Zn, Cr Basic grade: Pb < 150 mg kg^{-1} Cd < 1.5 mg kg^{-1} Cu < 100 mg kg^{-1} Ni < 50 mg kg^{-1} Hg < mg kg^{-1} Zn < 400 mg kg^{-1} Cr < 90 mg kg^{-1} Premium grade: Pb < 120 mg kg^{-1} Cd < 1 mg kg^{-1} Cu < 100 mg kg^{-1} Ni < 30 mg kg^{-1} Hg < 1 mg kg^{-1} Zn < 400 mg kg^{-1} Cr < 80 mg kg^{-1}	Required Metals: Pb, Cd, Cu, Ni, Hg, Zn, Cr, Co, Mo Metalloids: B, As, Se Others: Cl, Na Maximum Allowed Thresholds: As 12–100 mg kg^{-1} Cd 1.4–39 mg kg^{-1} Cr 64–1200 mg kg^{-1} Co 40–150 mg kg^{-1} Cu 63–1500 mg kg^{-1} Pb 70–500 mg kg^{-1} Hg 1–17 mg kg^{-1} Mo 5–20 mg kg^{-1} Ni 47–600 mg kg^{-1} Se 2–36 mg kg^{-1} Zn 200–7000 mg kg^{-1} B Declaration Cl Declaration Na Declaration
PAHs	Required Basic grade: <12 mg kg^{-1} Premium grade: <4 mg kg^{-1}	Required 6–300 mg kg^{-1}
PCBs	Required <0.2 mg kg^{-1}	Required 0.2–0.5 mg kg^{-1}
PCDD/Fs	Required <20 ng kg^{-1}	Required <17 ng kg^{-1}
Volatile Matter	Required (Volatile Organic Compounds (VOCs))	Optional (Volatile matter)
Electrical conductivity	Required	Required
pH	Required	Required
Bulk density	Required	Not required
Particle size distribution	Not required	Required
Water content	Required (water content)	Required (Moisture content)
Surface area	Required (Specific surface area)	Optional (Total surface area and external surface area)
Germination inhibition	Not required	Required

2.4. Morphological and Physicochemical Properties of Biochar

Biochar has been proven to be a promising option in producing a relatively high specific surface area upon proper activation due to readily available pores in the microstructures [83]. The tenability in porosity and surface chemistry are undoubtedly the salient features of biochar. In fact, due to its versatility, biochar has been widely studied as a low-cost adsorbent, soil conditioner and catalyst for various environmental applications. When added to soils, biochar interacts with plants (i.e., roots, root hairs, etc.), micro-organisms, soil organic matter, proteins and nutrient-rich soil to form organo-mineral–biochar complexes [84]. This is ascribable to the redox reactions of the complexes formed and to significant changes in the original matrix it creates [28,85]. These characteristics and properties are influenced by specific conditions (i.e., different feedstocks, additives, pyrolysis conditions, etc.) and thermal treatments. In the function of the feedstock provenience and the thermal treatment of biomass, the produced biochar showed a different heterogeneous grade of the material, with various levels of oxidation and compositions. The effect of the pyrolysis temperature on the morphology and surface area of biochar has been studied. By using Brunauer–Emmet–Teller (BET) and Fourier transform infrared spectroscopy (FTIR), the increase in the surface area correlated to the development of aromatic compounds with a decrease in aliphatic groups was assessed. Moreover, an important improvement in carbon biomass sequestrant with a reduction in gas emissions was found when heteroatoms (such as nitrogen and phosphorous) are present in the charcoal structure [86]. In 2017, Chacòn et al. published an interesting review explaining the electrochemical properties of biochar for environmental applications. Among others, they underlined how the production and modification methods of this material were able to alter its electrochemical performances [87]. In addition, they also pointed out how biochar is involved in soil remediation including contaminant degradation, direct interspecies electron transfer and microbial electron shuttling. Concerning the applications, Chacòn and coworkers [87] focalized their attention on the reduction/oxidation properties of biochar related to its energy storage ability. Peculiarities are also reported in other works, related to the faradic electron conductivity due to the π-electron delocalization and the presence of graphite-like sheet structures [88–91]. Another interesting work is that of D. Zhang's group, in which an in-depth electrochemical and morphological characterization of biochar obtained from four different raw materials (i.e., rice husk, bamboo, caragana, garbage, etc.) was reported [92]. This study is helpful to understand the electron transfer process of microbial redox reactions mediated by biochar and the impact of the raw material, from which it is produced, on the bioremediation of environmental contaminants. However, in general, there are several examples reported in the literature, in which morphological and chemical characterizations were made to understand the better applications of biochar [71,81]. For example, with the analysis of the adsorption isotherms, obtained by the BET method, the porosity and specific surface area of biochar can be evaluated [93]. Furthermore, Fourier transform infrared (FT-IR) and solid-phase carbon nuclear magnetic resonance (NMR) spectroscopy can be employed to check the change in functional groups on the surface [34,71]. However, the electrical and conductivity characterizations of this carbonaceous material have become more common in recent years. In this regard, Rahman et al. (2020) reported advances in the development of a biochar-based catalyst for sustainable electrochemical application [24]. In this review, the authors underlined the performances shown by biochar in electrochemical applications, proposing it as a viable alternative material to commercial functional carbon materials.

2.5. Application of Biochar in Sensing

Since the application of biochar as a soil conditioner, catalyst and carbon sequester has been successfully reported, other strategies based on this recycling material to promote sensors with improved analytical performances continue to be widely studied today. In 2020, an inspiring review about sustainable materials for the design of sensors, reporting how the pyrolysis and activation process can affect biochar features, was published [94].

Here, different applications of biochar underlining the potentiality and the promising features of these carbonaceous and recycled materials for electrochemical applications have been reported (Figure 3). In Figure 3, a general scheme for the modification of screen-printed and bulk electrodes using biochar is reported. Moreover, a few examples of works reported in the literature are discussed below.

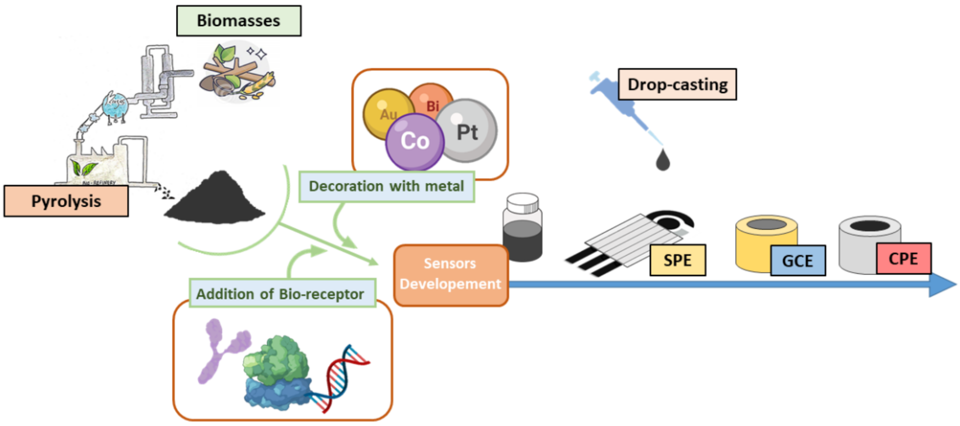

Figure 3. Schematic representation of the steps involved in the fabrication of biochar-modified electrodes.

2.5.1. Carbon-Paste and Glassy-Carbon Electrodes

In recent years, an increasing number of publications were centered on biochar-modified glassy-carbon electrodes (GCEs) and carbon paste electrodes (CPEs) [95–100]. In these papers, biochar was used for its adsorbent properties and as a highly effective electrode modifier for the pre-concentration of organic contaminants. In these studies, the porous surface of biochar, rich in chemical groups (i.e., carboxyl, hydroxyl, phenolic groups, etc.), which can interact in several ways with the target analytes, was exploited for the pre-concentration and voltammetric determinations of inorganic ions and organic species [98,101–103]. Another interesting use of biochar is that in which it was deposited alone or decorated with metallic nanoparticles (i.e., mercury, bismuth, gold, etc.) or electro-catalytic potential nanostructures (i.e., nickel hydroxide, copper hexacyanoferrate, etc.) for the construction of a powerful electrochemical sensor. These methods, based on voltammetric procedures (i.e., stripping, differential pulse, square wave voltammetry, etc.), have provided significant improvement in the performances (sensitivity and selectivity) of the sensor developed. Table 4 reports significant works in which biochar-modified CPE and GCE were used. In these works, biochar was exploited to electrochemically determine heavy metal ions and organic compounds, including hormones, insecticides, herbicides or antibiotics, in different matrices.

Table 4. Main source, preparation, application and limit of detection (LOD) obtained from biochar-modified CPE and GCE.

Feedstock	Electrode	Analyte	LOD (nM)	Linear Range (M)	Real Sample Matrix	Reference
Castor oil cake	Biochar-CPE	Cd Pb	69 (Cd) 9.8 (Pb)	$(0.03–5.0) \times 10^{-5}$ $(0.001–5.0) \times 10^{-5}$	wastewater	[95]
Castor oil cake	HgND-Biochar-CPE	Zn	170	$(0.07–3) \times 10^{-5}$	collyrium and ointment	[96]
Castor oil cake	Bi-nano-Biochar-CPE	Pb	1.41	$(0.005–1) \times 10^{-3}$	Overglaze decorated ceramic dishes	[97]
Castor oil cake	Biochar-CPE	Cu	400	$(0.1–3.1) \times 10^{-5}$	Vodka, cachaça, gin and tequila	[98]

Table 4. *Cont.*

Feedstock	Electrode	Analyte	LOD (nM)	Linear Range (M)	Real Sample Matrix	Reference
Castor oil cake	Sbmicro-Biochar-CPE	Paraquat	34	$(0.03–1.0) \times 10^{-6}$	natural water coconut water	[99]
Castor oil cake	Biochar-CPE	Isoniazid	63	$(0.1–1.0) \times 10^{-5}$	Synthetic human urine	[100]
Castor oil cake	Biochar-CPE	Methyl Parathion	39	$(0.01–170) \times 10^{-6}$	Drinking water and fruit juices	[101]
Sugarcane bagasse	BioNano-GCE	17 β-estradiol	11.3	$(0.05–20) \times 10^{-6}$	Groundwater	[102]
Kiwi skin	$ZnCl_2$/Biochar GCE	AA Dopamine UA	20 160 110	$(0.05–200) \times 10^{-6}$ $(2–2000) \times 10^{-6}$ $(1–2500) \times 10^{-6}$	Human urine	[103]
Dandelion pappus	Npc/GCE	Try	30	$(1–103) \times 10^{-6}$	Commercial amino acid injection and Fetal calf serum	[104]
Bamboo	BioNC-GCE	Tmx	220	$(0.5–35) \times 10^{-7}$	Spinach, Rice, Pear, Red soil, Tap water, Farmland water, River water, Lake water	[105]
Water Hyacinth	Biochar-rGO-CPE	Cbz	2.3	$(30–900) \times 10^{-8}$	Orange juice, Lettuce leaves, Drinking water, Wastewater	[106]
Castor oil cake	Ac-biochar-CPE	Caffeic acid	30.9	$(1.0–3000) \times 10^{-6}$	White, Rose and Red wine	[107]
Castor oil cake	Ni/biochar-CPE	Glucose	137	$(5–100) \times 10^{-6}$	Human saliva Blood serum	[108]
Pumpkin stems	Biochar-GCE	AA Dopamine UA	2300 30 510	$(30–95) \times 10^{-6}$ $(1–65) \times 10^{-6}$ $(2–230) \times 10^{-6}$	SH and Human blood serum	[109]
Bamboo fungus	Biochar-GCE	Bis-A	1068	$(0.02–10) \times 10^{-6}$	Groundwater	[110]
Kelp	Act-biochar-GCE	Acp	4	$(2.5–2000) \times 10^{-6}$	Human serum And Medical tablet	[111]

Acronyms: Biochar-modified (Biochar-CPE), Hg nanodroplets (HgND), Bi-nanostructures (Bi-nano), Sb-microparticles (Sb-micro), Biochar nanoparticle (BioNano), Ascorbic and Uric acid (AA and UA), Nanoporous carbon (Npc), Tryptophan (Try), Biochar nanocomposite (BioNC), Thiometoxan (Tmx), reduced graphene oxide nanocomposite (rGO), Carbendazim (Cbz), activated biochar (Ac-Biochar), Snail hemolymph (SH), Bisphenol A (Bis-A), Acetaminophen (Acp).

Several papers, present in the literature, reported the use of Castor oil biochar to develop voltammetric sensors for the detection of heavy metals in water or food [95–101]. In this regard, one of the first applications of biochar was reported by Suguhiro et al. in 2013 [95]. In this work, the authors exploited both the high affinity for heavy metals [99,100] and the electrocatalytic capacity of biochar to develop a voltammetric sensor for the determination of lead (II) and cadmium (II). Chiefly, they proposed, for the first time, the characterization of adsorptive properties of biochar, typical of bio-carbon obtained under low-temperature pyrolysis conditions [112], using differential pulse adsorptive voltammetry (DPAdSV). The sensors showed excellent analytical performances with a low LOD (nanomolar range), excellent sensitivity, stability and recovery (>94%), with the absence of significant interference by common cations present in waters. Comparable results were obtained by Olivera et al. (2015) for the determination of copper using biochar (form the same feedstock)-modified CPE. Moreover, for organic compounds, interesting results were obtained when biochar by bamboo was modified with inorganic salts such as $ZnCl_2$ [113] or carbonate minerals dolomite (DM) [114] or used to modify the electrodic surface of GCE. The electrochemical performance of these modified electrodes improved in terms of sensitivity (lower LOD) and stability, especially when used in clinical and food matrices. Another remarkable application is that of L. He and coworkers (2020). They developed a sensitive and reusable electrochemical biosensor for bisphenol A (BPA) based on newly synthesized biochar nanoparticles (BCNPs) [115]. In this work (the general scheme is reported in Figure 4), a stable immobilization of the tyrosinase (Tyr) enzyme using the carboxyl functionality of the highly conductive magnetic biochar nanoparticles was proposed. This biosensor, based on GCEs, presented very promising analytical performances with a detection limit (LOD) of 2.78 nM with linear ranges from 0.01 to 1.01 µM [115].

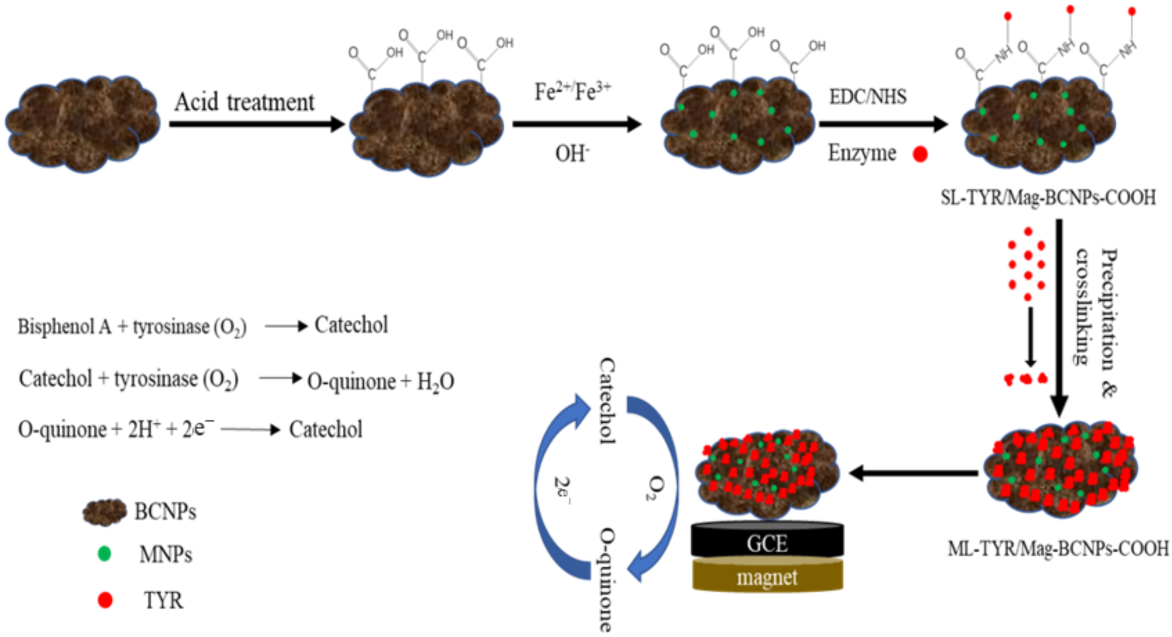

Figure 4. General scheme for the sensor fabrication proposed by He and coworkers [110]. Permission request.

Moreover, this is not the only biochar-based biosensor for the detection of bisphenol A. Indeed, Y. Liu and coworkers (2019) presented an electrochemical tyrosinase enzyme (Tyr)-based biosensor using a highly conductive sugarcane-derived biochar nanoparticle (BCNP) for the detection of BPA [110]. In this biosensor (BCNPs/Tyr/Nafion/GCE), a higher sensing signal (improved amperometric current responses) due to the conductivity property of biochar nanoparticles was observed. Precisely, a decreased charge transfer resistance and lowered reduction potential were obtained if compared with the biosensor based on bare GCE (Tyr/Nafion/GCE). Moreover, biochar-based biosensors showed robust analytical performances with a sensitivity of 3.18 nM and a linear range from 0.2 to 10 µM. These results were confirmed when the device was employed in real water detection, as evidenced by the high accuracy compared to that of high-performance liquid chromatography. Another attention-grabbing application of biochar is that proposed by A. Sobhan and coworkers (2021). In this work, they employed activated biochar (activated by steam-activation method) from corn stover for the development of a biosensor (ABC/PLA-based platform) selective for NH_3. The analytical performances reported in Figure 5 [116] demonstrate the robustness of the method when used in smart food packaging control. Moreover, careful characterization in terms of polylactic acid (PLA, ranging from 15 to 50%), electrical conductivity and tensile strength were reported.

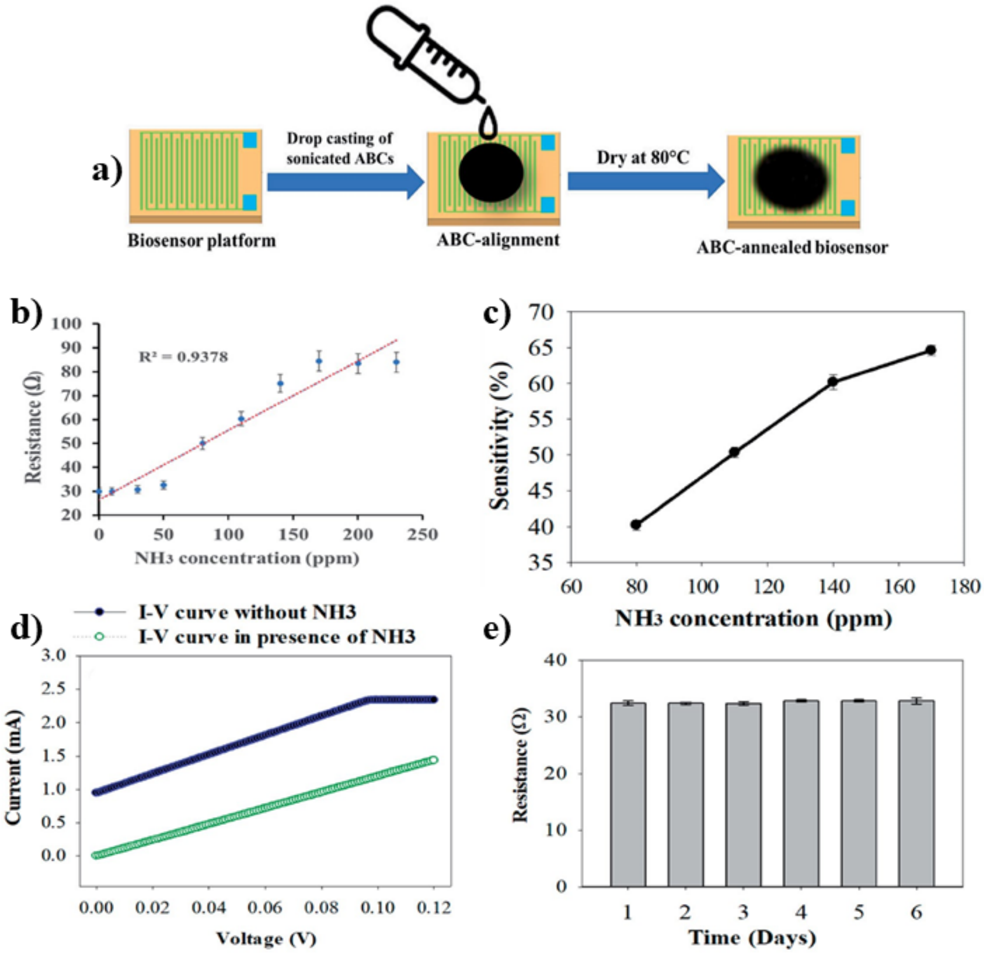

Figure 5. Schematic representation of ABC/PLA-based biosensor fabrication (**a**) and its analytical performances (from (**b**–**e**)). In particular, in (**b**) the resistance response to NH_3 concentrations, in (**c**) the sensitivity, in (**d**) the comparison of I-V data of 85% ABC/PLA-based biosensor in the presence/absence of NH_3 and (**d**) the stability of the biosensor using a fixed concentration of NH_3 (50 ppm) was reported.

Q. Zhou et al. (2021) reported on the fabrication of a sensor based on corncob biochar-modified GCE for the determination of dibutyl phthalate (DBP) [117]. In this tool, functional corncob biochar (F-CC_3) and MIP were combined, achieving high sensitivity (detection limit of 2.6 nM), reproducibility and successful application in the detection of DBP in rice wine [117]. However, there are also many examples of biochar-modified CPE-based biosensors. For instance, C. Kalinke and her co-workers proposed an electronic tongue based on biochar-modified CPE for the detection of phenolic compounds using stripping voltammetry (see Figure 6). In particular, this artificial neural network (2019)-based electronic tongue used stripping voltammetry as a discrimination technique for the analysis of a mixture of several phenolic compounds, such as catechol (CAT), 4-ethylcatechol (4-EC) and 4-ethylguaiacol (4-EG), showing good sensitivity (LOD in the micromolar range) and reproducibility [118].

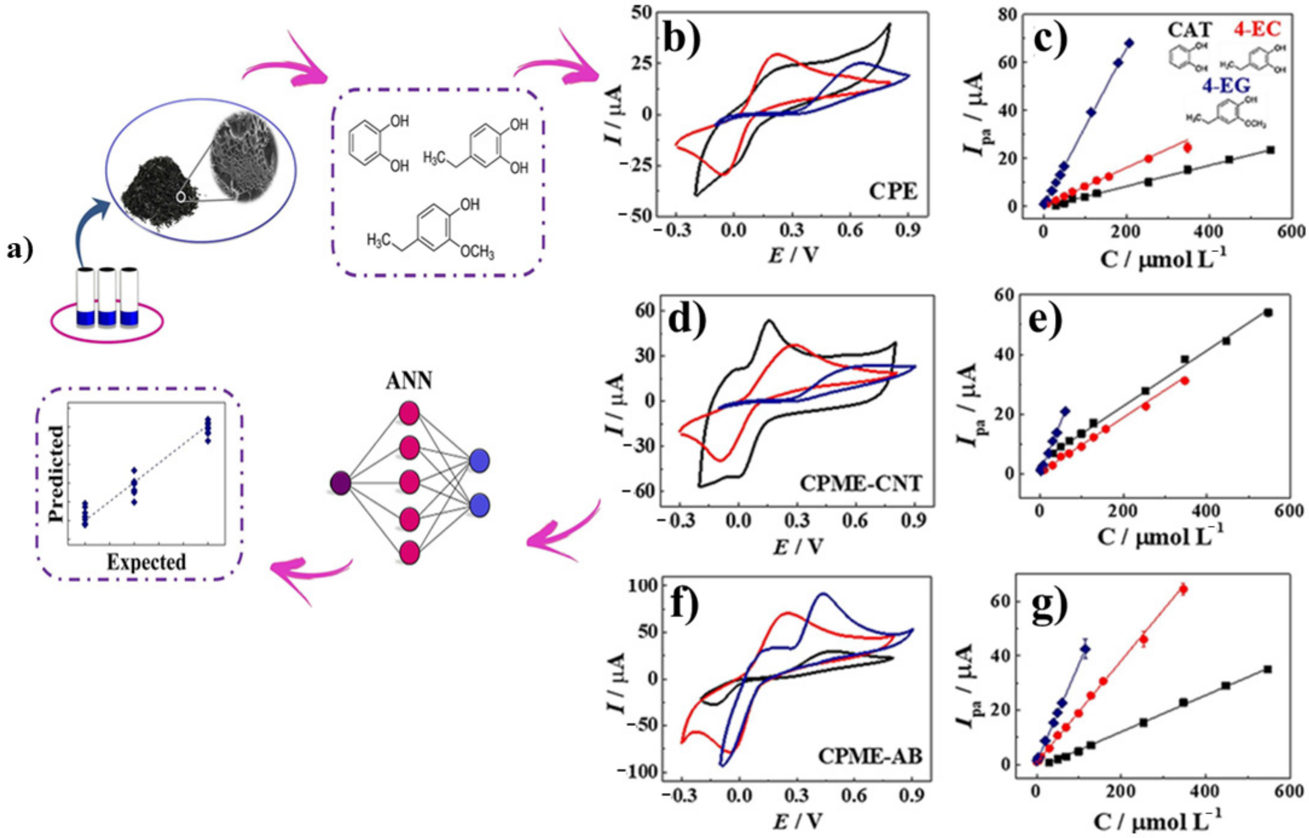

Figure 6. (**a**) Schematic representation of voltammetric electronic tongue-based biochar-modified CPE for phenolic compounds stripping detection. Cyclic voltammograms and analytical curves obtained for catechol (CAT), 4-ethylcatechol (4EC) and ethylguaiacol (4-EG) using CPE, CPME-CNT and CPME-AB (**b**–**g**, respectively).

Furthermore, other works based on biochar have been proposed by the same research group. For example, they developed a non-enzymatic sensor based on nickel-supported activated biochar modified-CPE (NiAB-CPME) for the determination of glucose [108]. The biosensor showed good performances in terms of repeatability (RSD% = 3.84%), sensitivity (LOD 0.137 μM) and linear range from 5.0 to 100.0 μM. Moreover, they applied this feasible green analytical procedure for the determination of glucose in human saliva and blood serum, also obtaining satisfactory results when a microfluidic methodology was used, thus proving to be a simple, robust and accurate method. In addition, in a second work, they presented a quick electrochemical immunoassay for the detection of pathogens based on biochar-modified CPE [119]. In this paper, the authors developed an immunosensor to detect Hantaviruses (single-stranded RNA viruses belonging to the Hantaviridae family) [119]. Matins and his co-workers exploited the highly functionalized surface of biochar able to bind covalently (by EDC/NHS conjugation) the specific antibody for Hantavirus in the construction of the immunosensor. The device showed good analytical performances with a LOD of 0.14 ngmL^{-1} and a linear range ranging from 5.0 to 1.0 μgmL^{-1}. Other biosensors dealing with biochar-modified CPE and GCE are reported in Table 5.

Table 5. Main source, preparation, application, limit of detection (LOD) and biosensors based on biochar.

Biosensor	Source	LOD (μM)	Analyte	Linear Range (M)	Real Samples	Reference
Biochar-GCE	Waste amla fruits	100	AA	$(0.01–3.57) \times 10^{-6}$	MI dose and soft drink	[120]
$ZnCl_2$ + KOH-Biochar-GCE	Kelp (algae)	0.004	Acp	$(0.01–20) \times 10^{-6}$	Human urine	[121]
Biochar-GCE	Mango leaves	0.16	p-NP	$(1–500) \times 10^{-6}$	Tap water and orange juice	[122]
Biochar-GCNS	BSF	0.67	Catechin	$(4–368) \times 10^{-6}$	Green tea	[123]
Pt-Re NPs-BC/GCE	Cassia fistula fruit	0.076	FRZ	$(0.1–300) \times 10^{-6}$	Human blood, serum and urine	[124]
Biochar-GCE	Cajeput tree bark	0.68	Vanillin	$(5–1150) \times 10^{-6}$	Chocolate and Biscuit	
Biochar-CPE	Castor oil cake	0.031	Caffeic Acid	$(1.0–3000) \times 10^{-6}$	White, Rose and Red wine	[125]
Nanop-Biochar-GCE	Pitch Pine	0.007	Pb^{2+}	$(1.5–850) \times 10^{-8}$ $(5.0–850) \times 10^{-8}$	Chinese cabbage and Navel orange	[126]
Biochar NPs-GCE	Bagasse	0.011	17-β-estradiol	$(0.05–20) \times 10^{-6}$	Groundwater	[127]
MIP-DBP-CTS/F-CC3/GCE	Corncob	0.003	Dibutyl Phthalate	$(0.5–1.8) \times 10^{-6}$	Rice and Wine	[128]
BCNP/Tyr/Nafion/GCE	Sugarcane	0.003	Bisphenol A	$(0.02–10) \times 10^{-6}$	Groundwater	[115]
CBPE-Tyr/SP CBPE	Beer	0.003	Phenolic Compounds	$(0.013–150) \times 10^{-6}$	Olive oil	[129]

Acronyms: Medical Injection (MI), p-Nitrophenol (pNP), Bouganvillea spectabilis flowers (BSF), Furazolidone (FRZ), Nano-powdered Biochar (Nanop-Biochar).

2.5.2. Biochar Application in Screen-Printed-Based Platforms

In the literature, several papers report the comparison of the analytical performances (using the same electroanalytical techniques) of SPEs with those of traditional (bulk) carbon or graphite electrochemical systems (GCE or CPE). The results underlined that, under the same experimental conditions, the combination of SPEs with portable electrochemical instruments represents the best possibility to have new reliable, reproducible, mass-producible and cost-effective electro-chemical tools [27,130,131]. Indeed, they can be easily modified with carbonaceous nanomaterials to improve their analytical performances [132,133]; in particular, this statement is confirmed by some examples of SPE modified with biochar, reported in the literature [13,17,27], used for its fascinating morphological and electrochemical features. In comparison with the bulk electrodes (the one reported in the previous paragraph, GCE and CPE), SPEs offer other advantages to coupling with cost-effective instrumentation, the capability of miniaturization and automation. However, it is well-known that these transducers, particularly when the working electrode (WE) is made of graphite-based materials, have serious issues due to their sluggish surface kinetics, which severely affect their performances [35]. Carbon materials and nanomaterials (CNMs) represent the most valid solution to overcome this problem, within which biochar is undoubtedly the most innovative and eco-friendly alternative. The modification of SPEs with CNMs and thus also with biochar provides several advantages. Among them, improved surface kinetics, enhanced electroactive surface area and amended adsorption and functionalization capability are, undoubtedly, the most important [132–134]. This represents a whole new field of research in full flow, but there are still few works dealing with sensors and biosensors based on SPE-modified with biochar. Table 6 reports the latest research works published about the development of biochar-based biosensors, and some examples of them are discussed in detail hereafter.

Table 6. Main source, preparation, application, limit of detection (LOD) and sensors based on biochar.

Sensor	Source	Analyte	LOD (μM)	Reference
nanoF-biochar-SPE	Eucalyptus scraps	o-diphenols m-phenols	0.6 3.8	[134]
PVB/biochar-SPE	Waste coffee ground	Humidity	20 RH%	[135]
Tub-biochar-SPE	Peachwood	Lead ions	0.02	[136]
Ty-biochar-SPE	BSG	Catecholamine	20	[34]
PVP-biochar-SPE	Conifer and rapeseed pellets	Humidity	5 RH%	[137]
Inverse-designed SPE	BSG	Potassium ferricyanide, AA, hexaammine ruthenium(III) chloride and NADH	3 3 9 12	[138]
Cnf-biochar-SPE	Eucalyptus scraps	Hydroxytyrosol (o-diphenol) and tyrosol (m-phenol)	≤0.5 ≤3.8	[139]
cMbOx-biochar-SPE	Chicken feather waste	HydroquinoneCatechol	0.063 0.059	[140]
Laccase-carboLign-SPE	Eucalyptus globulus	Catechol	2.01	[141]
Biochar-SPE	Kudzu vine	Clenbuterol	0.75	[142]
SPE/2D activated carbon	Desmostachya bipinnata	Roxarsone	1.5	[143]
Fdop-Ac-biochar-SPE	Corchorus genus (jute)	Nitrite	0.05	[144]
Biochar-SPE	Rice husk ash	Humidity	15% RH	[145]
SPE/hydrochar	Orange peels waste	Dopamine	0.2	[146]
nanoPd-Cu-Ac-biochar-SPE	Pistachio nutshells	Riboflavin	0.0008	[147]
nanoGF-Ac-biochar-nanoFeOx-SPE	Tamarind fruit shells	Rutin	0.027	[148]
Biochar-SPE	Bamboo	Humidity	10% RH	[149]

Acronyms: Nanofiber-biochar (nanoF-biochar), Brewer's spent grain (BSG), Tubular-biochar (Tub-biochar), Carbon nanofiber-biochar (Cnf-biochar), Carbon-based molybdenum oxide (cMbOx), carbonized lignin-based biochar (carbo-Lign), Fluorine-doped tin oxide/activated biochar (Fdop-Ac-biochar-SPE), palladium-copper nanoparticles/activated biochar SPE (nanoPd-Cu-Ac-biochar), graphitic-activated carbon/iron oxide nanocomposite (nanoGF-Ac-biochar-nanoFeOx).

An example of the electrochemical application of biochar as a serigraphic platform modifier (traditional SPE and inverse-designed IDSPE) was proposed by Cancelliere et al. (2019) [36]. The study, reported in Figure 7, showed the potentiality of biochar when used as a modifier of the WE with a remarkable improvement of the electrochemical performances in terms of electroanalytical parameters, such as peak-to-peak separation (ΔE), heterogeneous electron transfer rate constant (k^0) and current peak ratio (Ipa/Ipc). In this work, by comparing the analytical performances between biochar-modified and unmodified SPEs, an enormous gain in sensitivity and repeatability was assessed. This is ascribable to the actual benefits provided by this recycling material once cast on the WE surface, thereby demonstrating its effectiveness as an electrochemical enhancer.

The same research group also proposed two biochar-based biosensors. In one, they developed a tyrosinase-based amperometric biosensor (Ty/biochar-SPE) in which the enzyme was directly immobilized on biochar-modified SPEs. This biosensor showed very promising analytical performances when assessed for the detection of catecholamines, with a linear working range for epinephrine from 0.05 up to 0.5 mM and a LOD of 2×10^{-4} mM [34]. In the other, they used biochar both as an anchoring system and as an electrochemical enhancer, fabricating an ultrasensitive voltammetric label-free immunosensor for interleukin-6 (IL-6). The immunosensing platform was evaluated both in serum and in whole blood samples, showing excellent analytical assessment with LOD in the picomolar range (5.4 $pgmL^{-1}$), good reproducibility (RSD% < 11%) and recovery (always >85%). The peculiarity of this immunosensor resides in its time-saving ability coupled with powerful performances when compared with commercial spectrophotometric ELISA kits [35]. Another interesting work, reported in Figure 8, is that developed by the research group of Compagnone [134]. Precisely, they presented water-phase exfoliated biochar nanofibers from Eucalyptus scraps that they used both for the modification of the screen-printed electrodes (BH-SPE) and for the fabrication of a conductive film (BH-film). The BH-based sensors exhibited robust analytical performances with good repeatability (RSD $\leq$ 7%, n = 5), reproducibility (RSD $\leq$ 10%; n = 3), sensitivity for hydroxytyrosol (LOD $\leq$ 0.6 µM) and tyrosol (LOD $\leq$ 3.8µM) and recovery in the real sample matrix (91–111%, RSD $\leq$ 6%; n = 3) [134].

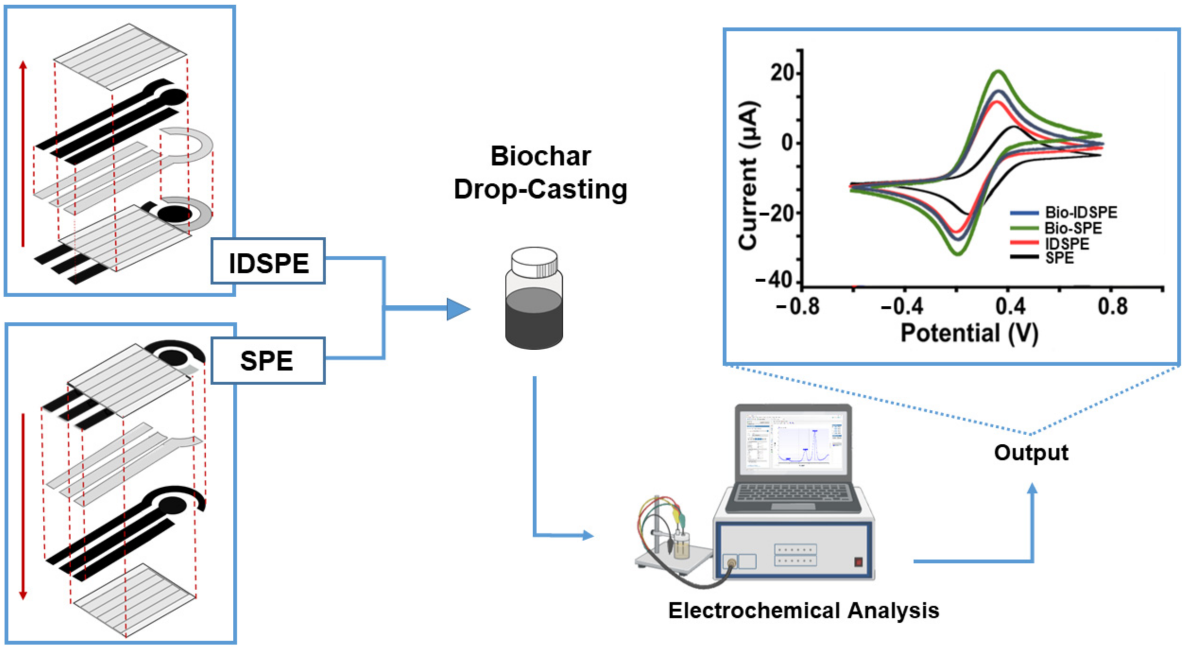

Figure 7. Two different configurations of serigraphic platforms and enhancing effect of the biochar.

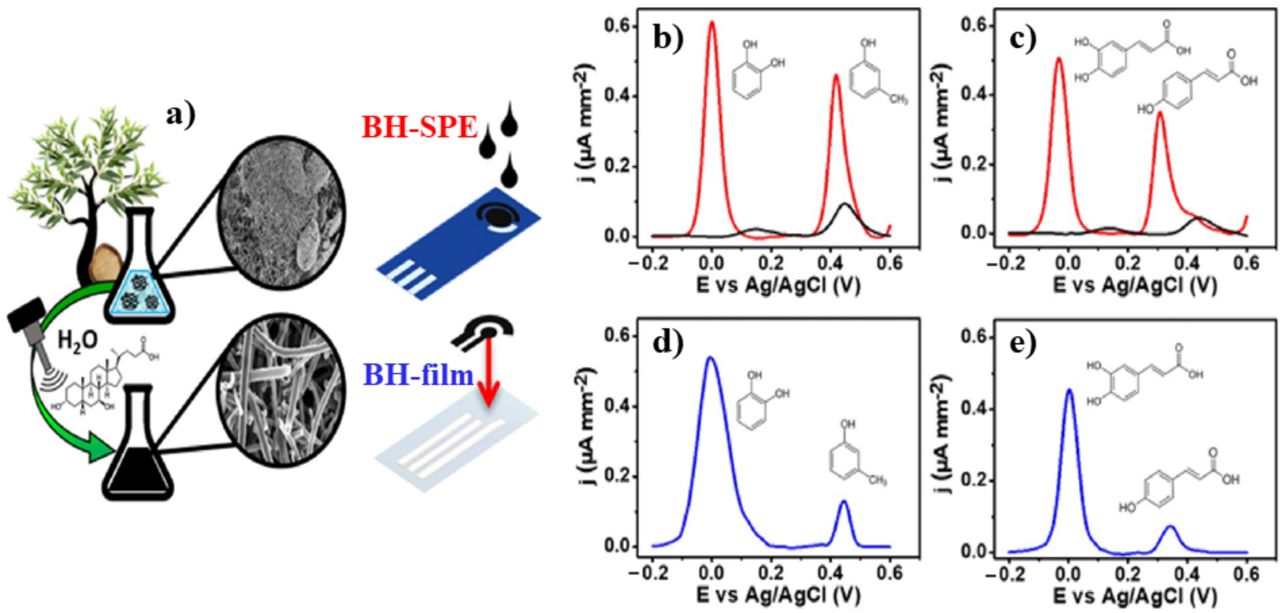

Figure 8. (**a**) Schematic representation of BH-SPE (red lines) and BH-film (blue lines) preparation and (**b**–**e**) their application in the detection of caffeic acid and dopamine (25 µM).

However, there are different works in which biochar is exploited for the development of powerful humidity sensors. Among them, one of the most promising is that of Jagdale et al. (2019) [135]. They proposed biochar from waste coffee as a material for the development of impedimetric humidity sensors (see Figure 9). The sensing platforms were produced (heated at 300 °C) using an ink-containing CGB with polyvinyl butyral (PVB), which acts as a temporary binder and ethylene glycol monobutyral ether, as an organic vehicle. The sensitivity tests (humidity), performed at room temperature under a flow of 1.7 Lmin^{-1} in a relative humidity range from 0 to 100%, showed an initial impedance of the film of 25.2 ± 0.15 MΩ, which changes to 12.3 MΩ under 98% humidity exposure, with a sensor response above 20% [135].

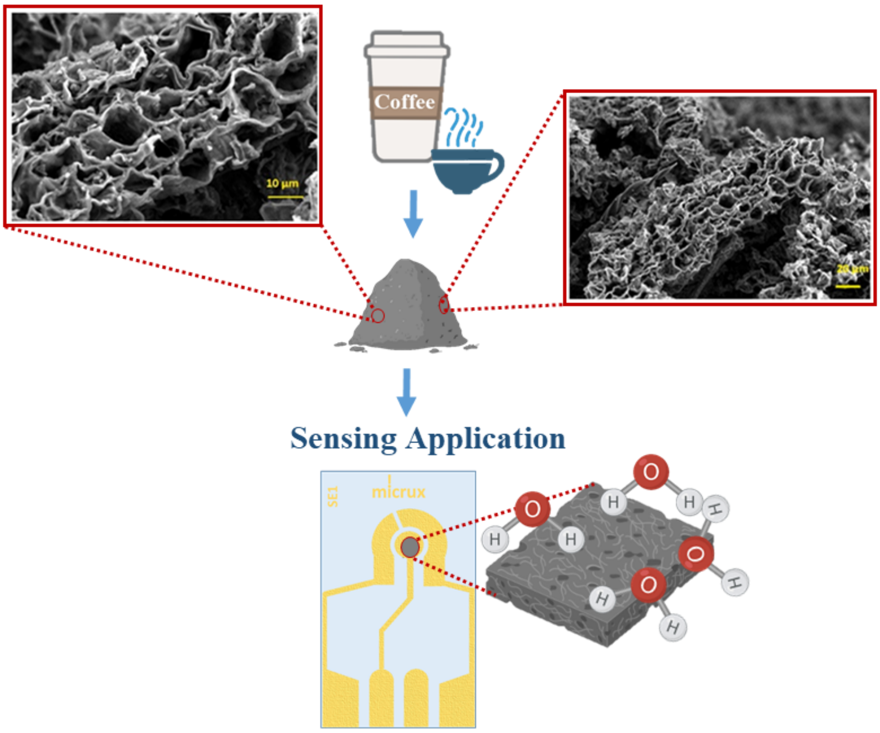

Figure 9. Schematic representation of humidity sensor based on biochar from waste coffee.

3. Conclusions

In this work, an overview of biochar's production techniques, its chemical and physical characteristics and the most recently analytical applications was reported. The numerous aspects of this sustainable waste-originated material were highlighted, ranging from its capability to sequester organic compounds in the soil to the reduction of reagents and toxic compounds in analytical procedures. It is in this area, and precisely in the development of electrochemical screen-printed-based sensors and biosensors, that we have focused on in this work. In the various examples reported, biochar has proven to be a versatile and economical substrate suitable for the fabrication of powerful electrochemical platforms employable in several areas, from clinical to food-safety control. In each case, biochar has provided very encouraging results, with analytical parameters comparable or superior to the other staple methods. Additionally, the adaptability of this green carbonaceous material was evaluated for the development of printed electrodes as a substitute for more expensive and difficult-to-produce CNMs. In this case, it has proven to be very advantageous, enabling the realization of sensitive, economic and rapid devices that are also suitable for field analysis. Nevertheless, although being immensely promising, biochar is a field of research in full expansion. Therefore, further investigations and rigorous optimisations are needed to make a green nanomaterial perfectly integrated for a whole new generation of biosensors.

Author Contributions: Conceptualization, R.C. and L.M.; methodology, R.C., K.C. and L.M.; software, R.C. and M.C.; validation, L.M.; data curation, R.C. and L.M.; writing original draft preparation, R.C., M.C., K.C. and L.M.; writing—review and editing, R.C., K.C. and L.M.; supervision, L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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