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Biochar-Based Screen-Printed Sensors: A Sustainable Solution for Electrochemical Applications

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ABSTRACT

Biochar, a sustainable and cost-effective material derived from biomass, has emerged as a versatile candidate for constructing advanced electrochemical sensors, particularly screen-printed sensors and biosensors. This review explores the utilization of biochar in the fabrication of screen-printed sensors, emphasizing its properties, such as a high surface area, chemical stability, and tunable functionalities, which are crucial for electroanalytical applications. Key factors influencing biochar properties, including feedstock composition, pyrolysis conditions, and activation methods, are thoroughly analyzed. The study highlights the integration of biochar into various screen-printed sensor platforms, demonstrating its effectiveness in enhancing sensitivity, selectivity, and durability across a range of analytes, from pharmaceuticals to environmental pollutants. Furthermore, the review discusses innovative applications, such as biochar-based flexible and wearable devices, biosensors, immunosensors, and resistive humidity sensors, underscoring their potential in real-world applications. Challenges, including variability in biochar properties and limited electrical conductivity, are also addressed, along with future perspectives for improving standardization and performance. By bridging waste valorization with cutting-edge sensor technologies, biochar-derived screen-printed electrodes represent a promising pathway toward sustainable and efficient electrochemical sensing platforms.

KEYWORDS
Biomass, biochar, screen-printed (bio) sensors, electroanalytical methods

1. INTRODUCTION

In line with the ongoing trend of miniaturization in science, electrochemistry has moved from using large instruments and materials to employing simple, portable, and point-of-care testing platforms. The screen-printed electrodes (SPEs) with low cost and ease and speed of mass production using thick film technology exemplify this shift. The main advantage associated with the miniaturization of the electrochemical sensors is the reduction of sample volume required, to as low as a few microliters, which in turn helps in reducing the overall size of the diagnostic system. The surface of SPEs can be easily modified to fit multiple purposes related to different analytes and to achieve a variety of

improvements. There are several effective strategies for modifying SPEs, including bulk modification (mixing all desired components into the sensor's ink before the printing process), layer-by-layer modification (printing the sensor components in sequential layers), and the drop coating method (depositing consecutive drops of a solution containing the desired modifiers onto the SPE surface and allowing the solvent to evaporate) (1–4). The choice of ink composition and the corresponding modification strategy are crucial aspects in the construction of SPEs, as they significantly determine the sensor's selectivity, sensitivity, and suitability for various applications. Among others, biochar (BC) traditionally utilized in agriculture and environmental remediation, has recently emerged as a promising candidate for constructing SPEs.

The term "biochar" is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a solid, multifunctional, porous carbon-rich product created by the pyrolysis of biomass at temperatures between 350 and 1000 °C, in the absence or limited presence of oxygen (5). In addition to pyrolysis, carbon-rich material can also be produced through alternative processes such as gasification or hydrothermal carbonizations (6,7). Among these processes, pyrolysis is the most commonly used, as the char obtained from gasification and hydrothermal carbonization generally does not meet the definition of BC (8,9). Feedstock for BC production includes a variety of organic materials such as agricultural residues, woody biomass, manure, and municipal waste, with the choice of feedstock significantly influencing the nutrient composition and properties of the final BC product (10,11).

BC offers several advantageous characteristics for a wide range of applications, including (*i*) a highly porous structure with a large surface area, providing additional active sites for electrochemical reactions (12), (*ii*) high carboncontent (60-95%), (*iii*) ion adsorption capacity, which seems to be very promising for applications including supercapacitors or electrochemical capacitors, where ion adsorption contributes to energy storage (13,14); (*iv*) chemical stability, ensuring its long-term performance as an electrode material (15); (*v*) environmental friendliness, (*vi*) low cost and (*vii*) tunable properties (16), where backdrop can be easily modified by the adjusting the production parameters, such as pyrolysis temperature, feedstock composition (origin, composition) (6,7), and activation methods (17,18). Beyond its traditional applications in remediation of

soil and water (19,20), contaminant degradation (21), BC's versatility extends to electrochemical technologies (9,22–26).

This review highlights the growing interest in BC as a dynamic material for constructing electrochemical devices, with a particular focus on SPEs. It covers recent advancements in BC-modified SPEs, including their applications in electrochemical sensors, enzyme-based biosensors, immunosensors, and humidity sensors. Future perspectives and challenges are presented, offering a comprehensive outlook on BC-based SPEs and their potentiality in electrochemical sensing.

2. CRITICAL FACTORS AFFECTING BIOCHAR QUALITY AND FUNCTIONALITY

The type of feedstock used plays a critical role in shaping the physical and chemical properties of BC. Common feedstocks include agricultural residues (such as straw), forestry residues (like wood chips and sawdust), organic waste materials (such as food waste and manure), energy crops (e.g., switchgrass or ornamental grasses), and even certain industrial by-products (23,27). The surface morphology, functional groups, hydrophobicity, stability, and other properties of BC are shaped by the varying proportions of cellulose, hemicellulose, lignin, and inorganic content in its feedstock. Biomass is primarily composed of these three components, each of which decomposes differently during pyrolysis, creating BC with distinct structures and characteristics (10,28–30). Feedstocks rich in cellulose and hemicellulose, such as straw and certain grasses, typically yield BC with a higher concentration of oxygen-containing functional groups, like hydroxyl (–OH), carboxyl (–COOH), ether (C–O–C), carbonyl (C=O), alkoxy (C–O), and aldehyde (–CHO), among which –OH and –COOH are the primary forms (31). These groups increase the BC’s hydrophilicity and promote interactions with specific analytes, enhancing its selectivity and sensitivity in electrochemical applications that depend on surface reactivity (32). In contrast, feedstocks with a high lignin content (e.g., wood, lignin-rich digestate) produce BC with a higher carbon content, greater aromaticity, and larger specific surface area (33). Such BCs exhibit enhanced stability and conductivity, properties that are beneficial in applications requiring durable and conductive materials (30). The higher carbonization level also contributes to better electron transfer capabilities, which can improve the performance of BC as a progressive electrode material in electrochemical sensors (28). In addition, feedstocks vary in their inorganic content,

with agricultural residues often containing more minerals like potassium, calcium, magnesium, and phosphorus than forestry residues (34). During pyrolysis, these minerals embed in the BC matrix and can create catalytic sites that enhance electrochemical activity (31). In the pyrolysis process, in addition to producing solid BC, liquid bio-oil and gaseous products (such as synthesis gas, which contains carbon dioxide, hydrogen, and nitrogen oxides) are also generated. The yield of BC depends on the type of biomass and the pyrolysis conditions, ranging between 10% and 35% (35).

Other important parameters that also significantly affect the yield and properties of BC are *pyrolysis temperature, pyrolysis time and heating rate*. Among them, *temperature* plays a critical role in optimizing both the yield and quality of pyrolysis products. For instance, the yield of BC from rice straw decreases from 51.36% to 32.75% as the pyrolysis temperature increases from 300 °C to 600 °C (36). At lower pyrolysis temperatures (300-450 °C), the feedstock undergoes less complete decomposition, resulting in a higher retention of solid material (37,38). As the temperature rises (450-650 °C), the yield of BC diminishes due to increased breakdown of the material into gaseous and liquid products. However, the BC produced at these intermediate temperatures exhibits enhanced quality, characterized by improved stability and a higher carbon content (39,40). At temperatures exceeding 650 °C, the BC yield becomes minimal as the organic matter undergoes extensive decomposition. However, the resulting BC is highly carbonized, less reactive due to the significant dehydration and deoxygenation at high temperatures, which reduce the presence of oxygenated functional groups (39,41). It is also more stable and typically exhibits an increased surface area and porosity (38). Furthermore, the pH of biochar is influenced by the pyrolysis temperature. Cong et al. (42) observed that as the pyrolysis temperature increased from 300 °C to 700 °C, the pH value of the BC rose from 7.05 to 9.29. The increase in pH may be attributed to the decomposition and volatility of nitrogen compounds and sulfides in the digestate at 700 °C.

The pyrolysis time affects the yield and carbonization of BC, with longer pyrolysis times leading to a higher degree of carbonization, which means that BC contains a higher proportion of carbon compared to other elements. This may result in greater stability and resistance to degradation. Lu et al. (43) manifested that within the temperature range of 500 to 900 °C, the specific surface area and pore

volume of BC initially increased with longer pyrolysis durations but begun to decline after exceeding 2 hours. It is important to note that the influence of pyrolysis time is often overshadowed by the dominant effect of temperature, making it challenging to isolate and directly assess the role of time in determining BC stability (44) .

The pyrolysis process can be divided into three types according to the *heating rate*: slow pyrolysis, fast pyrolysis and flash pyrolysis. Slow pyrolysis usually occurs at a heating rate of 5 to 7 °C/min and a long duration (several hours or days); it is characterized by high BC yields but low yields of bio-oil and syngas (45). In contrast, fast pyrolysis involves thermal decomposition at a heating rate of approximately 300 °C/min over a short period (< 5 seconds), primarily yielding bio-oil (up to 75%) (38,46). Flash pyrolysis, on the other hand, is a high-temperature process with an even faster heating rate (around 1000 °C/s) and an extremely short duration (30 milliseconds to 1.5 seconds), producing mainly bio-oil with yields of less than 70% (46).

2. THE ROLE OF BIOCHAR IN ELECTROCHEMICAL SENSING

2.1 Adsorbent

Due to its highly porous structure, BC has garnered significant attention as a promising adsorbent in various applications (47–49). The adsorption capacity of BC is primarily influenced by the functional groups on its surface, which modulate surface charge, polarity, and hydrophilicity. To enhance BC's adsorption performance, activation processes are employed, typically categorized as either chemical or physical (50). Physical activation is commonly employed due to its simplicity and lower cost compared to chemical activation, although it is generally less effective in enhancing pore structure. In physical activation, samples are exposed to high temperatures (usually 600–900 °C) in the presence of activating gases like steam or carbon dioxide, which increases surface area and porosity to some extent (50). Chemical activation involves using chemical agents such as acids (e.g., HCl, H_2SO_4, H_3PO_4, oxalic and citric acids), bases (NaOH, KOH), and oxidizing agents (e.g., $KMnO_4$, H_2O_2) (14,51–53). Acid treatment oxidizes the BC surface, adding acidic functional groups that enhance its affinity for example for heavy metals via ion exchange and complexation. Conversely, alkali treatment imparts a positive surface charge to BC, favoring the adsorption of negatively charged species. Although chemical treatments are more

intensive, they generate large volumes of acidic or alkaline waste and rely on costly chemicals (54). Electrochemical reactions have been reported as a cost-effective BC activation method that generates oxygen species, requires only low currents, and does not produce acidic/basic waste. The O-containing species generated at anode and/or cathode can modify the BC surface. Jong-Gook Kim et al. (55) found that electrochemical activation significantly increased the number of hydroxyl and carboxylic groups on the BC surface, which led to an increase in Pb adsorption from 27% (pristine BC) to 100% because the oxygenated-functional groups contributed to the adsorption of Pb. Pb capacity was 1.36, 2.64, 3.31, and 5.00 mg g^{-1}, corresponding to pristine, acidic, alkaline, and electrochemical activation, respectively (55).

In electrochemistry, BC is often utilized as a preconcentration material to enhance the sensitivity of electrochemical sensors. Its high surface area, porous structure, and the abundance of functional groups make it an ideal candidate for adsorbing target analytes prior to detection. This preconcentration step significantly boosts the local concentration of the analyte at the electrode surface, thereby amplifying the signal and improving the sensitivity and detection limits of the sensor. This approach is particularly useful in the detection of trace-level pollutants in water, food quality monitoring, and the determination of biologically or clinically significant analytes. However, its natural framework can become weakly conductive or non-conductive (56) during preparation, posing a challenge for electrochemical study. To overcome this feature and make BC beneficial for electrochemical detection, it must be combined with conducting materials or redox-active metals. For example, Gemeiner et al. (57) developed a new type of fully-printed electrochemical sensor using BC/ethylcellulose-modified carbon electrodes to detect a "phenol-like" analyte paracetamol. In this work, a layer of BC was printed over the conductive carbon working electrode. The performance of biochar SPE (B-SPE), unmodified carbon SPE (C-SPE), and biochar/ethylcellulose-modified SPE (BC-SPE) for preconcentration of the paracetamol was evaluated. The BC-SPE, combined with differential pulse adsorptive stripping voltammetry (DPAdSV), showed significant electrochemical performance (**Figure 1**). The sensitivity of BC-SPE was about 20 times higher than C-SPE and 280 times higher than B-SPE. Consequently, the limit of detections (LODs) were 3 μM for B-SPE, 0.4 μM for C-SPE, and 0.02 μM for BC-SPE. The authors highlight that

BC-SPE sensors are promising for detecting even trace amounts of analyte with longer accumulation times. The high sensitivity of BC-SPE for paracetamol is attributed to several factors. The highly porous structure of the BC provides a large surface area and promotes efficient mass transport of the analyte, improving electrocatalytic behavior towards paracetamol oxidation. Additionally, BC (produced from biomass containing 40% corn and 60% wood silage) has a high content of functional groups (O-H, C=O, COOH) (58) that interact with the hydroxyl groups in paracetamol through hydrogen bonds, resulting in substantial oxidation signals of paracetamol on the BC-SPE.

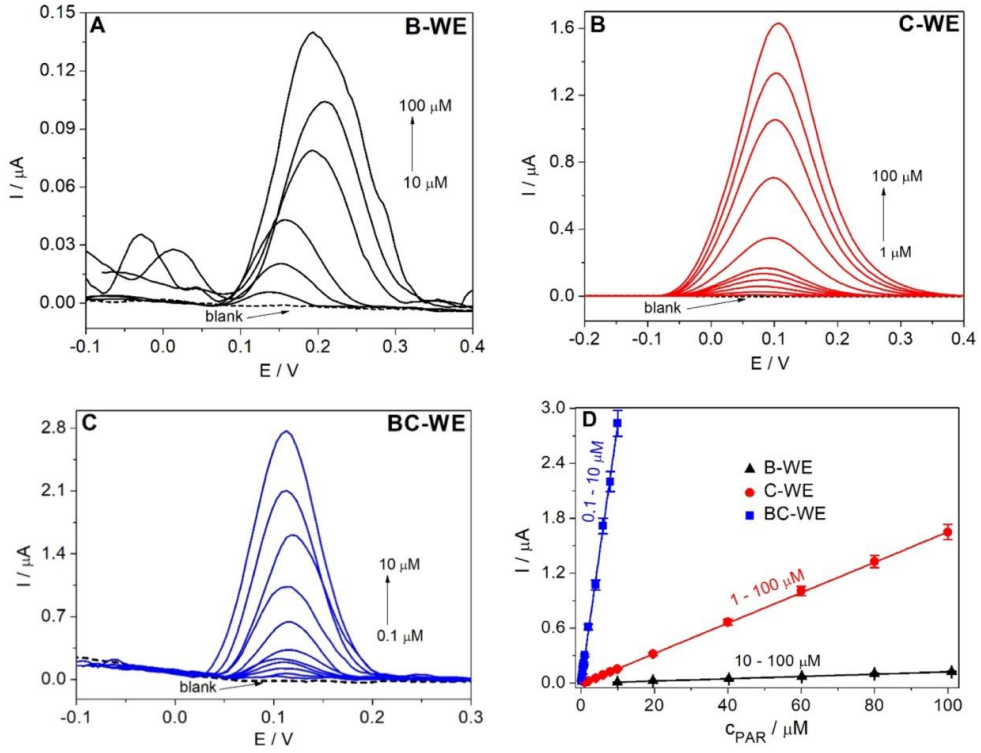

Figure 1. Baseline-corrected DPAdSV records for the various paracetamol concentrations in Britton–Robinson buffer pH 7 on the (A) B-WE (B) C-WE, (C) BC-WE (with accumulation potential of 0.0 V and accumulation time of 5 min). (D) The corresponding calibration curves (57)

2.2 Catalyst

BC could be served as an effective catalyst for contaminant degradation, primarily due to its surface functional groups and the incorporation of external transition metals (59–62). When combined with oxidizing agents such as hydrogen peroxide or persulfate, BC acts as an activator, generating hydroxyl radicals (•OH) or sulfate radicals ($SO_4^{\bullet 2-}$), respectively. These radicals have been shown to effectively degrade various organic

contaminants, including orange II (63), 2-chlorobiphenyl (64), sulfamethoxazole (65), and bisphenol A (66). Additionally, BC can act as a supporting material for photocatalysts, enhancing their photodegradation performance (66,67). Beyond its environmental applications, BC also shows promise as a catalyst in developing electrochemical sensors, expanding its utility in analytical and sensing technologies. Bukhari et al. (68) prepared a water-dispersed BC with a nanofiber structure from eucalyptus scraps using a Kraft process. These biochar nanofibers (BH-CNF) were utilized to construct sensors through two innovative strategies: surface modification of SPEs (BH-SPE) and fabrication of flexible sensors made entirely from biochar nanofibers (BH-films). The BH-CNF sensors exhibited exceptional conductivity and catalytic activity, particularly for detecting pairs of phenolic compounds. The selected couples were as follows: catechol/*m*-cresol as simple phenols, caffeic acid/*p*-coumaric acid as hydroxycinnamic acids, dopamine/*L*-tyrosine as phenols containing an amino group, and hydroxytyrosol/tyrosol as a representative of phenylethanoids. As can be seen from the presented DPV records (**Figure 2**), the BH-CNF brought a huge improvement in the electrochemical response, compared to bare SPE with the peak intensity increasing and cathodic shifts for the required oxidation overpotentials.

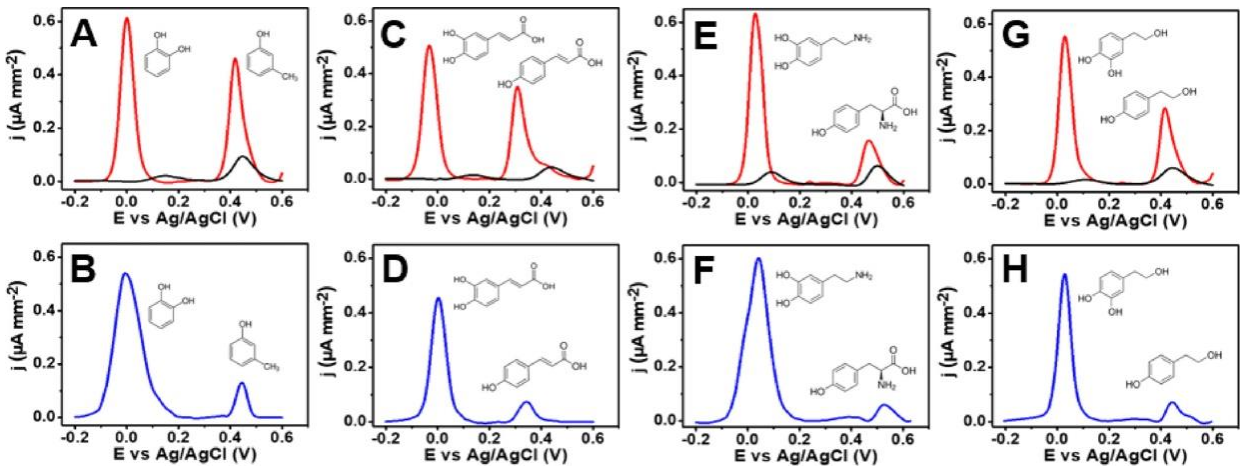

Figure 2. Differential pulse voltammograms of the simultaneous detection of couples of phenol-containing structures, performed with BH-SPE (red lines) and BH-film (blue lines), respectively, versus bare SPE (black lines). (A, B) 25 μM catechol and 50 μM *m*-cresol; (C,

D) 25 μM caffeic acid and 50 μM *p*-coumaric acid; (E, F) 25 μM dopamine and 50 μM *L*-tyrosine; (G, H) 25 μM hydroxytyrosol and 50 μM tyrosol (69).

2.3 Carrier

BC's porous structure significantly enhances its specific surface area, which is advantageous for adsorbing target analytes and carrying or loading various active molecules or materials. Summarizing the recent works about nanoparticles/BC composites, the key effect of BC is that its porous structure can effectively disperse metal hydroxide (70) and/or metal oxide (71). This setup ensures uniform dispersal, preventing agglomeration and maintaining high reactivity and functionality. Consequently, BC-nanoparticle composites exhibit enhanced properties, such as increased catalytic activity and improved conductivity, crucial for high-performance sensors. Moreover, BC's functional groups and surface area provide a suitable environment for *immobilizing enzymes*, enhancing their stability and activity. This can improve the analytical performance of biosensors by increasing the enzyme's ability to detect specific analytes, such as glucose or lactate (72–74).

3. THE USE OF BIOCHAR IN THE DEVELOPMENT OF SCREEN-PRINTED SENSORS

3.1 Biochar-based electrochemical sensors

Numerous studies have explored the utilization of BC as an electrode modifier for electrochemical detection across a range of analytes. This approach is underpinned by BC's sorption and interaction capabilities with both inorganic and organic compounds. Moreover, it can incorporate other species (metals, nanoparticles etc.) for indirect interaction with and detection of the analyte (23). BC-modified electrodes can be designed in various forms. Carbon-based electrode designs, such as carbon paste electrodes (CPE) and glassy carbon electrodes (GCE), have garnered significant attention in the literature for this purpose. This popularity can be attributed to the ease of modification and handling associated with these electrode types, ultimately resulting in improved analytical performance and sensitivity. CPE has proved to be a good platform for BC incorporation and the construction of sensors (75–82). BC-based paste electrode can be prepared by homogenizing BC with a conductive additive (e.g., graphite) and a binder (e.g., mineral oil) to achieve a consistent paste. The paste is then packed into an electrode

body and compacted with a plunger. A key advantage of this setup is its renewable surface: because BC as the modifier permeates the entire paste, simply reshaping or trimming the electrode surface restores its activity. This design ensures that the BC is evenly distributed, enhancing conductivity and stability over multiple uses, as the modification exists throughout the bulk of the paste rather than being limited to a superficial coating (83,84). Another approach is modification of GCE with BC using the drop-coating technique, where a dispersion of BC is applied directly onto the GCE surface (85,86). This method simplifies the process, as most studies have achieved stable modifications without the need for an immobilizing agent. The absence of a binding polymer suggests that BC can effectively adhere to the GCE surface on its own, making the modification both straightforward and stable (87–90). The drop-coating technique also allows for precise control over the amount of BC applied, facilitating reproducible sensor performance.

In this review, our focus will be paid on the third strategy involving the utilization of BC material, specifically in the preparation of SPEs (**Table 1**). SPEs have gained considerable popularity in voltammetric techniques owing to their straightforward design, facilitating the high-volume production of extremely inexpensive, highly reproducible and reliable single-use sensors. SPEs hold great promise for integration into small measuring devices, flow-through and batch cells, microfluidic systems, and are highly suitable for on-site monitoring and point-of-care testing (25).

One of the interesting example of using recycled waste material to boost the electrochemical performance of SPEs involved modifying the graphite working electrode with a BC-based nanomaterial derived from spent grains. This modification was achieved using the drop-casting technique. Three solvents – *N*,*N*-dimethylformamide (DMF)/H_2O (1:1, v/v), 1-methyl-2-pyrrolidinone, and ethanol – were tested for dispersing BC. Ethanol proved to be the most effective, providing homogeneous BC dispersion and yielding the most abundant electrochemical signal. To assess the electrochemical performance of the BC-modified SPE, various electroactive species were tested, including ferricyanide, benzoquinone, epinephrine, ascorbic acid, and uric acid. Comparing BC/SPEs with graphene/SPEs indicated that BC-modified sensors exhibited superior electrochemical performance, including improved resolution, reduced peak-to-peak separation, higher current

intensity, and lower charge transfer resistance (72). The same research group later developed an innovative design called inverse-designed SPEs (IDSPEs), where the reference (RE) and counter (CE) electrodes were swapped. Compared to traditional SPEs, IDSPEs achieved a four-fold increase in sensitivity and improved repeatability, with RSD of 3% lower among all tested electroactive species (potassium ferricyanide, ascorbic acid, hexaammineruthenium(III) chloride, and NADH). These enhancements resulted from a more stable reference potential and reduced overpotential due to the reversed electrode configuration. Moreover, surface modification of both electrodes (SPE and IDSPE) with BC obtained from recycled brewers waste (BC-SPEs and BC-IDSPEs), further improves their electrochemical performance, in terms, for example, of the heterogeneous electron transfer constant (0.0024 cm s^{-1} and 0.0018 cm s^{-1} for BC-SPE and BC-IDSPE, respectively) (91).

Biochar-based flexible devices are growing in popularity due to their design versatility, space efficiency, portability, and durability. These devices can be incorporated into applications like wearable technology, roll-up displays, and medical implants, enabling innovative designs and features. Their flexibility allows them to bend and fold, saving space in compact electronics, while their robust nature provides better resistance to damage than rigid alternatives (92). Rao et al. developed a portable sensing platform for hyperin detection, utilizing a lotus seedpod BC-decorated MoS_2 nanocomposite, prepared by co-hydrothermal synthesis. It was proved that BC improved microstructure, conductivity, electrode stability, and electrocatalytic properties of MoS_2. MoS_2-BC modified SPE in tandem with a compact U-disk potentiostat and a smartphone interface, performed detection of hyperin in the range from 100 nM to 3 μM, with a LOD of 50 nM (93). Similarly, Zhang et al. created a wireless sensing platform for detecting clenbuterol (CLB) in bovine serum, using a flexible carbon electrode (FCE) modified with kudzu vine BC (KVBC). FCE as flexible substrate electrode was prepared by the laser direct writing technique. Integrated with a smartphone and mini electrochemical workstation, the KVBC-FCE sensor demonstrated a linear response to CLB from 0.95 μM to 14.31 μM, with a LOD of 0.75 μM. The KVBC-modified FCE exhibited outstanding electrocatalytic activity toward CLB, achieving a broad linear concentration range from 0.95 μM to 14.31 μM and a low LOD of 0.75 μM (94).

Table 1. Examples of biochar-based screen-printed sensors applications

Analyte	Construction of sensor / biosensor	Biomass resource	Technique	Linear range, µM	LOD, µM	Analyzed samples	Ref.
Ascorbic acid	BC-SPE / BC-IDSPE	brewers waste	SWV	500–50000	1 / 0.2	–	(91)
$[Ru(NH_3)_6]Cl_3$	BC-SPE / BC-IDSPE				13 / 0,5		
NADH	BC-SPE / BC-IDSPE				6 / 3		
$K_3[Fe(CN)_6]$	BC-SPE / BC-IDSPE				5 / 1		
Catechol and hydroquinone	KSC@MoO_3/SPE	keratinous sludge	DPV	0.5–176.5 0.5–176.5	0.059 0.063	environmental water	(95)
Clenbuterol	flexible KVBC-Nf (IP)/FCE	kudzu vine plants	DPV	0.95–14.31	0.75	bovine serum	(94)
Dopamine	HC/SPE	orange peels	LSV	0–1000	0.18	–	(96)
Dopamine	Fabric-based flexible NMC	microalga *N. Oceanica*	DPV	0.02–2500	0.006	human serum	(97)
Hydroxytyrosol and tyrosol	BH-CNF/SPE	eucalyptus scraps	DPV	1–35 2–35	0.3 0.6	olive oil	(68)
	BH-CNF film			2.5–50 20–300	0.5 0.8		
Hyperin	MoS_2-BC/SPE	lotus seedpod	SWV	0.1–3	0.05	*Hypericum perforatum* samples	(93)
Lead	PTBC/SPE	wood (peach)	SWASV	2.4–579	0.097	tap water	(98)
Nitrite	AJCP-SP-FTO	jute (*Corchorus* genus) sticks	AMP	2.5–1300	0.437	tap water	(99)
Lead	PTBC/SPE	wood (peach)	SWASV	2.4–579	0.097	tap water	(98)
Paracetamol	BC/SPE	40 % wood and 60 % corn silage	DPAdSV	0.1–10	0.02	pharmaceutical formulations	(57)
Rutin	Fe_2O_3/GAC/SPE	tamarind fruit shell	DPV	0.1–130	0.027	tablets, nutraceutical formulation, river water	(71)

Abbreviations: AJCP-SP-FTO – a screen-printed fluorine-doped tin oxide electrode with activated jute carbon paste; AMP – amperometry; BH-CNF – water-dispersed biochar-nanofibers; BH-CNF/SPE – a screen-printed carbon electrode modified with water-dispersed biochar-nanofibers; BC-IDSPE – a inverse-designed screen-printed electrode modified with biochar; BC-SPE – a classically-designed screen-printed electrode modified with biochar; DPAdSV – differential pulse adsorptive stripping voltammetry; DPV – differential pulse voltammetry; Fe_2O_3/GAC/SPE – a screen-printed carbon electrode modified with porous graphitic-activated carbon coated iron oxide; HC/SPCE – a screen-printed carbon electrode modified with hydrochar; KSC@MoO_3/SPE – a screen-printed carbon electrode modified with a keratinous sludge biomass-derived carbon-based molybdenum oxide nanocomposite; KVBC-Nf (IP)/FCE – a flexible carbon electrode modified with a kudzu vine biochar treated with the nafion solution and isopropanol; LOD – limit of detection; LSV – linear sweep voltammetry; MoS_2-BC/SPE – a screen-printed carbon electrode modified with a biochar decorated molybdenum disulfide; NMC – nitrogen-rich macroporous carbon-based sensor; PTBC/SPE – a screen-printed carbon electrode modified with a hierarchical porous tubular biochar; SWASV – square wave anodic stripping voltammetry; SWV – square wave voltammetry.

3.2 Enzymatic biochar-based biosensors

Enzymatic biochar-based biosensors utilize enzymes as biorecognition elements, offering high selectivity in catalyzing specific reactions. Integrating enzymes with biochar enhances their catalytic activity, stability, and reusability in various applications. BC provides a stable, porous structure for enzyme immobilization, improving stability under harsh conditions (e.g., high temperatures, extreme pH) (100). Its large surface area and high adsorption capacity facilitate efficient enzyme immobilization and substrate binding, boosting catalytic efficiency. Additionally, BC allows for facile enzyme separation and recovery, enabling reuse across multiple cycles, supporting cost-effective and sustainable biotechnological processes (25,100). For instance, spent grain BC has been identified as an effective support for enzyme immobilization. Researchers utilized this material to develop an electrochemical biosensor for catecholamines, where tyrosinase (Ty) enzyme was directly immobilized onto modified BC/SPE (Ty/BC/SPE). The resulting biosensor demonstrated excellent analytical performance in detecting epinephrine, with quite constant in the first 7 days of storage In particular, Ty/BC/SPE maintained 98.2% of its initial current response with a decrease to 87.6% after two weeks, while the Ty/graphene-SPE showed an initial 98.5%, decreasing to 92.2% after two weeks (72).

BC's unique properties make it an excellent material for constructing *immunosensors*, where it serves as a substrate for immobilizing antibodies or antigens for selective detection of target analytes like proteins, viruses, or bacteria. An example is the development of an electrochemical immunosensor using BC derived from sugarcane bagasse to detect antibodies against SARS-CoV-2. In this approach, a SPE was modified with BC dispersion, allowing for the immobilization of the receptor-binding domain against the virus's S-protein. Even though the response profile of the immunosensor does not allow the analyte quantification, the device effectively distinguishes between negative and positive serum samples with a cut-off value of 82.3% at a 95% confidence level. Additionally, it showed high selectivity against yellow fever antibodies and maintained performance even after seven days of storage (73). Another impressive application of BC is the development of an activated BC-based immunosensor for detecting *E. coli* O157:H7, a major foodborne pathogen. In this study, BC was derived from corn stalks and activated using steam treatment. The immunosensor

was created by coating a gold interdigitated electrode with the activated BC, followed by functionalization with streptavidin as a linker, and the immobilization of biotin-labeled anti-*E. coli* polyclonal antibodies. The approach offers a rapid detection method for foodborne pathogens, with potential for integration into portable, multiplexed devices for use in the food industry (101). Cancelliere et al. utilized BC to enhance the electrochemical properties of SPEs by improving the sluggish electron transfer kinetics, and as a substrate for protein binding in the development of ultrasensitive voltammetric immunosensors for interleukin-6 (IL-6). Despite a lengthy overnight pre-coating step, the platform showed excellent reproducibility (RSD < 4%), wide linear ranges in human and blood serums, and LODs in the picomolar range. These results highlight the potential of BC-based immunosensors as effective diagnostic and therapeutic tools, offering a promising alternative to traditional IL-6 detection methods like ELISA and Western Blot (74).

3.3 Humidity sensors

BC-derived materials have been utilized as resistive humidity sensors, alongside synthetic nanostructured carbons like carbon nanotubes and graphene (102–105). These sensors typically consist of commercial α-alumina substrates with interdigitated platinum electrodes, onto which BC is screen-printed or drop-cast. The deposition often involves an organic polymer binder (e.g., polyvinyl butyral or polyvinylpyrrolidone) (102,103). The sensor's analytical signal is based on the change in impedance or resistance caused by the adsorption or desorption of gaseous species. To achieve high sensitivity, a very high specific surface area and porosity of the BC material are crucial.

Two types of pyrolyzed particles – carbonized bamboo and carbonized bamboo subjected to annealing – were evaluated for humidity sensor construction. The sensing materials were screen-printed onto commercial α-alumina substrates with platinum electrodes and fired at 300 °C for 1 hour. Sensors based on carbonized bamboo showed excellent response to humidity levels starting at 10% relative humidity, while annealed bamboo sensors began responding at 20% relative humidity. The difference was attributed to annealing, which reduced the specific surface area and decreased open porosity (105). More recently, waste brewed coffee powder pyrolyzed at 700 °C was used as an *n*-type semiconductor for measuring relative humidity in the range of 20-100%.

These sensors exhibited very fast response and recovery times (15-20 s at 50% humidity), with negligible cross-sensitivity to gases like carbon dioxide, ammonia, nitrogen dioxide, and ozone (103).

4. CHALLENGES AND FUTURE PROSPECTS

Despite the many advantages and promising applications of BC-based materials in electroanalytical devices, several challenges and limitations still need to be addressed (100). One significant limitation is the lack of reproducibility and consistency in the physicochemical properties of BC-based materials. This inconsistency arises from variations in feedstock and production conditions, including pyrolysis temperature and residence time (106). To address the issue of reproducibility and consistency in BC properties, standardized protocols and procedures for BC production including specific guidelines for feedstock selection, pyrolysis temperature, residence time, and other critical parameters should be established. Standardization can help establish a baseline for reproducibility and ensure that variations in BC properties are minimized.

An additional challenge faced by BC-based materials in electroanalytical devices is their comparatively low electrical conductivity when compared to conventional carbon materials such as graphene and carbon nanotubes. This reduced conductivity imposes limitations on their performance in electrochemical devices, especially in applications where high conductivity is essential for efficient electron transfer processes (57). Improving the electrical conductivity of BC materials can be achieved by incorporating conductive materials (e.g., carbon black, carbon nanotubes, or graphene itself), surface modification (coating the BC surface with conductive materials or functional groups) or doping techniques (e.g., nitrogen, phosphorus, and sulfur doping). Moreover, despite the good biocompatibility and chemical stability of BC materials, the long-term stability and performance of BC-based electroanalytical devices in real-world environments are yet to be comprehensively investigated (107). This involves a need to understand the impacts of fouling, biofilm formation, and other environmental factors that have the potential to compromise the sustained performance of these devices over time. To improve the long-term stability and performance of BC-based electroanalytical devices in real-world environments, a more detailed research is needed to understand the effects of fouling and other environmental factors on these devices.

Mitigation strategies, including the incorporation of antifouling coatings or the development of self-cleaning surfaces, should be actively explored.

The future prospects of BC-based electrochemical sensors are promising, offering numerous opportunities for advancements in environmental monitoring, healthcare, and other fields. As BC is known for its carbon sequestration properties in soil, BC-based electrochemical sensors could be designed to monitor and assess the effectiveness of carbon sequestration practices. This can contribute to sustainable land management and climate change mitigation efforts.

5. CONCLUSIONS

The integration of BC-based materials in SPEs represents a significant advancement in the field of electrochemical sensors. As the demand for sensitive, rapid, and selective analyte determination grows, the shift from conventional electrodes to SPEs is enabling the exploration of more cost-effective and efficient solutions. Screen-printing technology facilitates the mass production of reproducible and disposable electrodes, which are crucial for portable analytical devices. BC derived from renewable biomass resources has emerged as a promising material in this question due to its sustainability and economic benefits which also suitably fit into the concept of Green Analytical Chemistry. The future of BC-based SPEs lies in the synthesis of hybrid materials and their integration into flexible and wearable platforms. This evolving trajectory indicates a promising avenue for innovation, aiming to expand the functional scope of these electrochemical platforms. The journey of BC within electroanalytical applications, although challenging, holds immense potential. Overcoming the identified challenges, actively exploring emerging trends, and maintaining a commitment to sustainability are crucial for the successful integration of BC-based materials into the next generation of electroanalytical devices. This progress will pave the way for greener, more efficient technologies that align with the growing demand for sustainable solutions in analytical chemistry.

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7. Ethics approval and consent to participate

This study does not need any Ethics report.

8. Consent for publication

The Authors give consent for publication.

9. Availability of data and materials

All data and materials of the paper are available to the public.

10. Authors' contributions

Olha SARAKHMAN: Conceptualization, Writing – original draft preparation, Ján LABUDA: Literature search, Review and editing, Ľubomír ŠVORC: Literature search, Review and editing.

11. Disclosure statement

No potential conflict of interest was reported by the authors.

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