

Article

Evaluation of Activated Biochar Derived from *Sargassum spp.* as a Sustainable Substrate for the Development of Electrochemical DNA Biosensing [†]

Jorge A. Campoy-Ramírez ^{1,2} , Nikola Batina ¹ , Mauricio Castañón-Arreola ² ,
Eduardo O. Madrigal-Santillán ³ , José A. Morales-González ³ , Javier Jiménez-Salazar ⁴ ,
Pablo Damián-Matsumura ⁴ , José G. Téllez ⁵ , Xariss M. Sánchez-Chino ⁶ , Berenice Carbajal-López ⁷ ,
Abraham Cetina-Corona ⁸ , José A. Garcia-Melo ^{5,*}  and Luis Fernando Garcia-Melo ^{1,*} 

- ¹ Laboratorio de Nanotecnología e Ingeniería Molecular, Área Electroquímica, Departamento de Química, CBI, Universidad Autónoma Metropolitana-Iztapalapa (UAM-I), Av. San Rafael Atlixco 186, Iztapalapa, Mexico City 09340, Mexico; alfredo.campoy@alumnos.uacm.edu.mx (J.A.C.-R.); bani@xanum.uam.mx (N.B.)
 - ² Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México, San Lorenzo 290, Colonia Del Valle Sur, Alcaldía Benito Juárez, Mexico City 03100, Mexico; mauricio.castanon@uacm.edu.mx
 - ³ Laboratorio de Medicina de Conservación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Unidad Casco de Santo Tomás, Plan de San Luis y Díaz Mirón, Mexico City 11340, Mexico; eomsmx@yahoo.com.mx (E.O.M.-S.); jmorales101@yahoo.com.mx (J.A.M.-G.)
 - ⁴ Laboratorio de Endocrinología Molecular, Departamento de Biología de la Reproducción, CBS, Universidad Autónoma Metropolitana-Iztapalapa (UAM-I), Av. San Rafael Atlixco 186, Iztapalapa, Mexico City 09340, Mexico; jejs@xanum.uam.mx (J.J.-S.); pdamian@izt.uam.mx (P.D.-M.)
 - ⁵ Universidad Tecnológica de Tula-Tepetzi, UTTT. Av. Universidad Tecnológica No 100, El Carmen, Tula de Allende 42830, Mexico; josegabriel.tellez@utt.edu.mx
 - ⁶ SECIHTI, Departamento de Salud, El Colegio de la Frontera Sur-Villahermosa, Carretera Federal Villahermosa-Reforma Km 15.5, Ra. Guineo Segunda Sección, Villahermosa 86280, Mexico; xsanchez@ecosur.mx
 - ⁷ Instituto Nacional de Cancerología, Tlalpan, Mexico City 14080, Mexico; eb.carbajalopez@gmail.com
 - ⁸ Departamento de Madera, Celulosa y Papel, Universidad de Guadalajara, Zapopan 45110, Mexico; abraham.cetina@academicos.udg.mx
- * Correspondence: josealberto.garcia@utt.edu.mx (J.A.G.-M.); electronicfer@hotmail.com (L.F.G.-M.); Tel.: +52-(55)58044939 (L.F.G.-M.)
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Abstract

This study aims to develop an innovative electrochemical genosensor based on activated biochar (ABC) derived from the biomass of the seaweed *Sargassum spp.* The synthesis process begins with the pyrolysis of *Sargassum spp.* at 500 °C to obtain biochar (BC), which is chemically activated with nitric acid (HNO₃). The physicochemical properties of the resulting material, such as morphology and surface area, were characterized using techniques including scanning electron microscopy (SEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and the Brunauer–Emmett–Teller (BET) method for surface area. BET results showed an increase in surface area from 22.9367 ± 0.0879 m²/g (BC) to 159.2915 ± 2.2641 m²/g (ABC). For the development of the genosensor, a hydrolyzed collagen gel matrix enriched with ABC is created. This nanostructured, biocompatible mixture is used to immobilize a DNA probe on a graphite electrode, employing the large surface area of ABC and the formation of a functional HC-based coating. The system's viability was evaluated by cyclic voltammetry (CV), which showed changes in the maximum anodic peak current (I_{pa}) during fabrication: 27.78 ± 1.87 µA for the bare electrode,



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$35.25 \pm 1.24 \mu\text{A}$ for ABC 30%, and $39.25 \pm 1.84 \mu\text{A}$ for HC + ABC 30%. After ssDNA immobilization and hybridization to dsDNA, I_{pa} decreased to $28.81 \pm 1.565 \mu\text{A}$ and $23.10 \pm 1.25 \mu\text{A}$, respectively. Finally, hematoxylin (Hx) was used as an intercalating indicator from hybridization, reducing the maximum anodic peak current to $15.51 \pm 1.13 \mu\text{A}$, consistent with additional interfacial limitations associated with dsDNA formation. Overall, the developed system demonstrates a sustainable, promising platform for molecular diagnostics in electrochemical DNA biosensor development.

Keywords: genosensor; electrochemical; *Sargassum spp.*; collagen; activated biochar

1. Introduction

Over the past decade, the use of biosensors in healthcare has grown significantly, driving important advancements in analytical models for clinical diagnostics and overcoming the limitations of traditional methods [1,2]. This progress is reflected in the incorporation of nanomaterial-based platforms, which have diversified the applications of biosensors across various fields, including medical diagnostics and monitoring, the food industry, and the environmental sector [3,4]. Currently, biosensor platforms are distinguished by their high sensitivity, specificity, and speed in target detection. At the same time, the current trend focuses on the development of cost-effective, sustainable devices, driven by novel bionanomaterials and/or bionanostructures with improved physicochemical properties that enable lower detection limits [5]. In parallel, the use of polymeric biomaterials in the development of these devices provides a stable, biocompatible, and, in some cases, conductive matrix that, through structural and surface modifications, enables optimization of biosensor sensitivity and reproducibility to enhance performance in electrochemical detection and DNA immobilization [6–8]. In this context, electrode interface engineering with bionanomaterials and polymeric biomaterials is a key determinant of sensitivity, speed, and reproducibility in electrochemical biosensors [9,10]. Among the most widely used, accessible, and low-cost nanomaterials, carbon stands out for its versatility in designing biosensor surfaces, from its use as a plate to as a nanoparticle [11,12]. This material has exceptional electrical conductivity, high biocompatibility with molecules such as DNA, and is stable under physiological conditions, making it an ideal candidate for modifying electrodes in electrochemical biosensors [13,14].

When developing more sustainable biosensors, it is essential to use materials that are both analytically efficient and environmentally sustainable. In this context, carbon is considered an ideal material because of its abundance and versatility [15,16]. This study proposes using *Sargassum spp.*, an alga species that is proliferating uncontrollably on beaches in Quintana Roo, Mexico. This has significant ecological and economic consequences, particularly for reefs and corals. In addition to causing environmental damage, *Sargassum spp.* can also pose health risks to humans [17]. Contact with this alga may lead to allergies and skin irritation. Furthermore, when *Sargassum spp.* decomposes, it releases toxic gases such as carbon dioxide (CO_2), methane (CH_4), hydrogen sulfide (H_2S), and ammonia (NH_3). Excess biomass can lead to respiratory issues for both residents and tourists in coastal areas. In severe cases and with prolonged exposure, these gases may cause permanent damage to the lungs and nervous system. Managing this excess biomass entails high costs and does not provide a definitive solution for the waste generated [18]. Therefore, it is suggested that this biomass be valorized through controlled thermochemical processes to produce ABC. ABC has a high surface area, adjustable porosity, and excellent electroactivity, making it ideal for electrochemical biosensor applications. Due to ABC's biocompatibility, it can be

combined with biopolymers, optimizing the surface and enhancing the immobilization of the entire nanostructure, thereby facilitating the detection of the target of interest [19,20].

Interest in biopolymers such as HC has been growing due to their unique properties. Biopolymers are materials derived from renewable sources, providing a sustainable alternative to traditional synthetic polymers. Among the most used biopolymers are chitin, alginate, and HC [21]. These materials can form three-dimensional structures with properties that make them ideal for various technological applications. HC, a processed form of collagen derived from animal tissues, is especially desirable for its porosity, biocompatibility, and electroactivity in biosensors [22,23]. This biopolymer is widely used in regenerative medicine and the development of functional materials. Its porous structures can be easily modified to enhance performance in technological applications. The porosity of HC enables effective immobilization of nanoparticles or bioelements, thereby improving interactions with less-active surfaces. In addition to its structural advantages, HC's electroactivity makes it suitable for electrochemistry [20,23].

This work focuses on the development of an electrochemical genosensor that employs polymer biomaterials and ABC to detect a target DNA sequence from the adenomatous polyposis coli (APC) gene. APC is a tumor suppressor, and its inactivation is considered an early event in colorectal tumorigenesis. Notably, APC alterations are among the most prevalent genetic events in sporadic colorectal cancer and often involve protein-truncating mutations or loss of heterozygosity [24]. Furthermore, a substantial fraction of APC mutations accumulates in the mutation cluster region (MCR) in exon 15 (approximately codons 1286–1513), making this region a clinically relevant model for validating probe immobilization and electrochemical reading of DNA hybridization at our nanoengineered HC/ABC interface [25]. The process begins with the synthesis of ABC through the pyrolysis of *Sargassum spp.* biomass, followed by its chemical activation with nitric acid. The physicochemical properties of the resulting material, including morphology (SEM), elemental composition (EDS), surface area (BET), crystalline phases (XRD), and thermal stability (TGA), were thoroughly characterized. Next, a DNA probe is immobilized on a graphite electrode modified with a hydrolyzed collagen gel enriched with ABC, which serves as a porous matrix to increase the electroactive surface area and enhance immobilization efficiency. The development of the electrochemical DNA biosensor was evaluated using CV. The analytical signal was further amplified using Hx as an intercalating agent to enhance the electrochemical response upon hybridization with the target DNA. Overall, the developed electrochemical biosensor demonstrated optimized performance using biocompatible and sustainable materials for molecular diagnostic applications.

2. Materials and Methods

2.1. Reagents

The reagents used in this study were as follows: low-density agarose (LDA), hydrolyzed collagen (HC), potassium chloride (KCl) $\geq 99.0\%$, nitric acid (HNO_3) $\geq 70.0\%$, potassium ferricyanide $\text{K}_3[\text{Fe}(\text{CN})_6]^{3-}$ $\geq 99.0\%$, potassium ferrocyanide $\text{K}_4[\text{Fe}(\text{CN})_6]^{4-}$ $\geq 99.0\%$, phosphate-buffered saline (PBS) pH 7.4, ethylenediaminetetraacetic acid (EDTA) $\geq 99.0\%$, and Trizma[®] chloride (Tris-Cl) $\geq 99.0\%$, all of which were purchased from Sigma Chemicals (St. Louis, MO, USA). Alumina (Al_2O_3) powders with particle sizes of 0.3 and 0.05 μm , along with a polishing cloth (Buehler, Lake Bluff, IL, USA). Additionally, ultrapure sodium dodecyl sulfate (SDS) $\geq 99.0\%$ was obtained from MP Biomedicals, Inc. (Solon, OH, USA). Solutions were prepared using ultrapure Millipore Milli-Q water (resistivity 18.2 $\text{M}\Omega\cdot\text{cm}$) from Millipore Corporation (Milford, MA, USA).

DNA oligonucleotides corresponding to mutations associated with colorectal cancer were obtained from T4 Oligo (Irapuato, Gto, Mexico). Stock solutions of the oligonu-

cleotides were prepared in TE buffer (10 mM Tris-HCl/1 mM EDTA, pH 8.0) and stored at -20°C until use. Working solutions were prepared by diluting the stock solutions in 0.01 M PBS at pH 7.4. The specific oligonucleotide sequences used in the experiments are as follows:

Thiol probe sequence (ssDNA): 5'-SH-(CH₂)₆- TCC AAT CTT TTC TTT TTT TAT TTT -3'
Complementary sequence (dsDNA): 5'- AGG TTA GAA AAG AAA ATA AAA -3'.

2.2. Synthesis of Biochar from *Sargassum* spp.

The *Sargassum* spp. biomass was collected from the coast of Cancún, Quintana Roo, Mexico. This planktonic brown macroalga has a conical basal disk measuring 1.5 cm across and rhizoids at the ends. The alga used for biocarbon preparation was dried at room temperature for four days, and the entire biomass was pulverized. The resulting powder was heated to 500°C for one hour in an inert atmosphere. After this step, BC was obtained, which was washed to remove ash and then dried at 90°C for 24 h before storage [26].

2.3. Activation of Biochar from *Sargassum* spp.

For BC activation, 10 mL of concentrated HNO₃ was mixed with 190 mL of deionized water. Subsequently, 25 g of BC was immersed in the mixture for 24 h. After immersion, the BC was washed with distilled water and rinsed with deionized water. It was then dried at 90°C for 24 h before being stored for subsequent use and application in the development of an electrochemical DNA biosensor [26].

2.4. Physicochemical Characterization of Biochar and Activated Biochar

To obtain detailed information about their properties, BC and ABC were characterized using various physicochemical methods. The morphology was evaluated by scanning electron microscopy (SEM) using a JSM-7800F PRIME (JEOL, Tokyo, Japan). Additionally, elemental analysis of the materials was conducted using energy-dispersive X-ray spectroscopy (EDS) with an SDD X-max 80 EDS detector (Tokyo, Japan), operated without liquid nitrogen (LN₂). The specific surface area was determined by Brunauer–Emmett–Teller (BET) analysis. The Barrett–Joyner–Halenda (BJH) analysis and the t-plot method were used to determine the pore-size distribution and estimate the microporous volume, respectively. This analysis involved studying nitrogen adsorption–desorption isotherms at 77 K on a Micromeritics Tristar Surface Area and Porosity Analyzer ASAP 3000 (Communications Drive, GA, USA). Before textural analysis, the samples were pretreated at 120°C in a vacuum of 50 mTorr for 12 h. Phase analysis was carried out using a powder X-ray diffractometer (XRD) from Rigaku Smart Lab, under the following operating conditions: scanning range of $2\theta = 10^{\circ}$ to 80° , scanning speed of 30° per minute, voltage and current set at 40 kV and 50 mA, and employing Cu radiation ($\lambda \text{ K}\alpha = 1.54060 \text{ \AA}$). Finally, the absolute mass loss was measured using thermogravimetry (TGA) on a Simultaneous Thermal Analyzer from Instrument Specialists Incorporated apparatus. This analysis was performed in air at a ramp rate of $10^{\circ}\text{C}/\text{min}$ over a temperature range from 30°C to 1000°C .

2.5. Electrode Cleaning Procedure

Each electrode was polished using SiC #4000 paper (Struers, Rotherham, UK) impregnated with $0.3 \mu\text{m}$ alumina and deionized water for three minutes, employing a figure-eight motion without applying pressure and rotating the electrode every 20 movements to ensure homogeneous polishing. After cleaning, the electrode was polished with a cloth impregnated with $0.005 \mu\text{m}$ alumina and deionized water for an additional three minutes, applying the same figure-eight motion. Finally, the electrode underwent an ultrasonic treatment for 15 min. At each step, electrodes were rinsed with ultrapure water and dried

with nitrogen gas to ensure thorough cleaning. The electrodes were cleaned and evaluated by CV before each modification.

2.6. Electrochemical Analysis

All electrochemical measurements were carried out using CV at room temperature with a Bioanalytical Systems BAS-100 electrochemical workstation (West Lafayette, IN, USA), utilizing the traditional three-electrode system, which consisted of a graphite disk electrode (3 mm diameter) as the working electrode, a platinum electrode as the counter electrode, and Ag/AgCl as the reference electrode. All CV analyses were performed in a redox probe solution (0.01 M KCl containing 20 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$), with a potential scan range from -100 mV to $+600$ mV and a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$. Finally, all electrochemical measurements were performed by $n = 5$ for each modification process on the working electrode.

2.7. Assembly of the Biorecognition Interface

To construct the matrix, three different polymers with similar properties were first evaluated: HC, low-density agarose (LDA), and agar. Each was prepared at 0.5% (w/v) by dissolving 5 mg of each reagent in 1 mL of ultrapure water. 2.5 μL of each solution was deposited on the working electrodes. In the next step, optimal ABC concentrations were evaluated, 15% and 30% (w/v) ethanol suspensions of activated carbon were applied to the working electrodes for 20 min.

The matrix is generated with the results obtained. For this purpose, 2.5 μL of a 0.5% HC solution and 5 μL of the 30% ABC suspension were deposited on the working electrode, forming an HC/ABC film. Subsequently, 15 μL of single-stranded DNA (ssDNA) APC 1306 (100 μM) was immobilized on this coating and incubated for 1 h at room temperature. Unreacted sequences on the surface were removed by washing with 0.1% SDS in 0.01 M PBS (pH 8), followed by rinsing with ultrapure water. To detect hybridization, 15 μL of complementary double-stranded DNA (dsDNA) (100 μM) in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was added, and the mixture was incubated for 1 h at room temperature. Finally, unreacted sequences were removed by applying SDS/PBS solution washes. Each modification was verified independently by CV.

2.8. Surface Area of the Electrochemical Genosensor

Finally, the changes in the electroactive area were evaluated during each construction step of the electrochemical DNA biosensor. The Randles–Sevcik equation was employed for this analysis and carry on this equation $I_p = (2.69 \times 10^5)AD^{1/2}n^{3/2}\nu^{1/2}C$ where I_p is the current response of the graphite disk electrode (amps), D is the diffusion coefficient of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ($\text{cm}^2 \text{ s}^{-1}$), ν is the scan rate (V/s^{-1}), and C is the concentration of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (M). The changes in both the electrochemical and effective areas were analyzed following surface modification. The CV measurements were performed using a drop of 150 μL of 20 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.01 M KCl (pH 7.4) at scan rates of 10, 20, 30, 40, 50, 60, 70, 80, and 100 $\text{mV}\cdot\text{s}$ and a potential range from -100 mV to $+600$ mV.

3. Results and Discussion

3.1. Physicochemical Characterization of Biochar and Activated Biochar

The figure shows SEM micrographs of BC and ABC. Figure 1A and Figure 1B depict the morphology of BC at magnifications of $1500\times$ and $5000\times$, respectively. Both images reveal that the BC material has a heterogeneous, irregular morphology, with particles of various shapes and sizes. Additionally, the material shows polydispersity in the agglomerates. The particles' surfaces appear rough, with some slightly smoother areas, and exhibit a dense,

compact structure in certain regions, a characteristic of carbonized materials. The edges of the fragments are also visible.

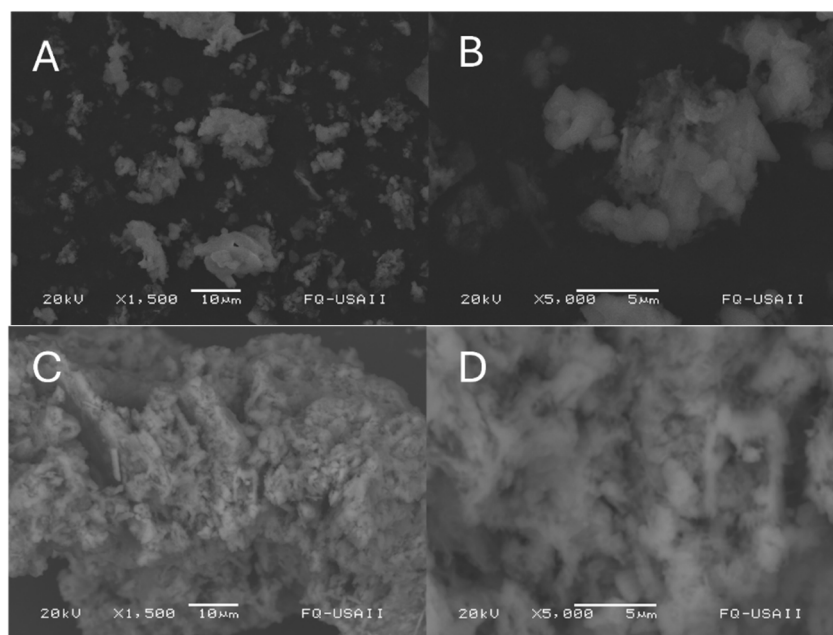


Figure 1. Surface morphology of biochar and activated carbon samples at magnifications of $\times 1500$ (A,C) and $\times 5000$ (B,D).

In contrast, Figure 1C,D display a significant transformation in surface morphology compared to BC. At a magnification of $1500\times$, the differences become clear. ABC does not show dense agglomerates; instead, it has a notably more porous structure.

The surface shows roughness and disorganization, indicating that the activating agent removed material. At $5000\times$ magnification, the structure appears even more porous and fragmented, with a surface that is not smooth but is filled with cavities, cracks, and a distinctive “honeycomb” or “sponge” texture. These features result from the formation of micropores and mesopores during activation, thereby increasing the material’s surface area and, consequently, enhancing its physicochemical properties. The results from the morphological analysis align with findings in the literature [22,27]. The processes of chemical and thermal activation create a network of pores of varying sizes throughout the carbon matrix, thereby increasing the surface area. This phenomenon is evident in the micrographs obtained in the present study. Additionally, Colomba et al. (2022) and Kulogljia et al. (2025) describe highly porous, heterogeneous surfaces that develop a three-dimensional network of interconnected pores during activation, a pattern observed in this study as well [22,28].

EDS analysis revealed significant changes in the elemental composition of biochar following chemical activation. In its untreated form (BC), as shown in Figure 2A, the biochar was primarily composed of oxygen, which accounted for 53.1% of its total mass, along with 23.1% calcium and 18% carbon, highlighting its organic nature. After activation, as illustrated in Figure 2B, the carbon content increased to 34.6%, while the oxygen content decreased to 45.3% and calcium to 11.7%. These changes were likely due to partial oxidation and gasification at elevated temperatures, leading to the release of carbon species, such as CO_2 and CO , as calcite. Both sargassum and calcite are rich in calcium and oxygen. The remaining carbon has the potential to enhance the structural morphology and adsorption capacity of the activated carbon, owing to the expected high degree of graphitization and thermal stability [29,30].

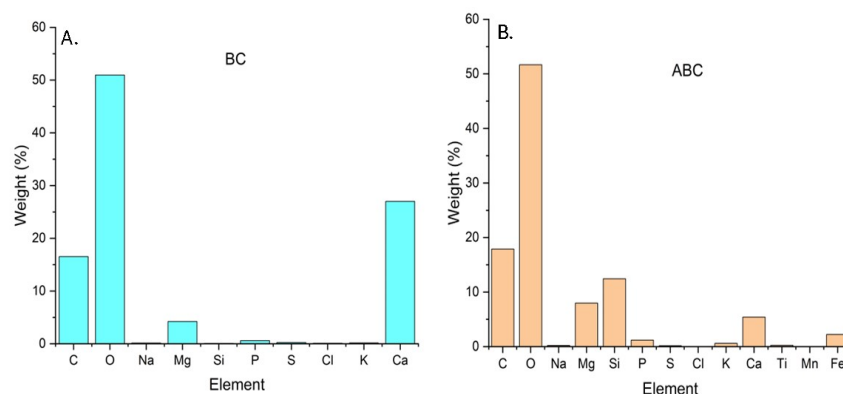


Figure 2. EDS spectra of (A) biochar before chemical activation (BC) and (B) biochar after chemical activation with HNO_3 (ABC).

To confirm the porosity and surface area of the materials, a BET analysis was performed. Figure 3 supports the morphological findings from SEM and shows the pore-size distribution derived from nitrogen adsorption–desorption data. The BC exhibited a surface area of $22.9367 \pm 0.0879 \text{ m}^2/\text{g}$, whereas the ABC showed a significantly larger surface area of $159.2915 \pm 2.2641 \text{ m}^2/\text{g}$. Comparing these findings with literature reports, such as [31], underscores the potential for process optimization to further enhance surface area and pore structure. Additionally, the activation process altered the pore diameter distribution.

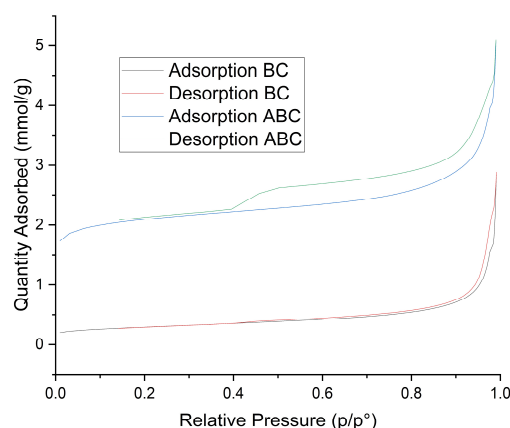


Figure 3. Nitrogen adsorption–desorption isotherms of biochar (BC) and activated biochar (ABC). The adsorbed amount (mmol g^{-1}) is plotted as a function of relative pressure (p/p°). The BC adsorption and desorption branches are shown in red and black, respectively, whereas the ABC adsorption and desorption branches are shown in blue and green, respectively.

In BC, the pore size distribution shows two main contributions: a peak centered around $\sim 3.5 \text{ nm}$ and a broader distribution centered around $\sim 60\text{--}70 \text{ nm}$, rather than a predominant population at $\sim 100 \text{ nm}$. After activation, the distribution shifts, with increased contributions from pores around $3.2\text{--}3.5 \text{ nm}$, reduced contributions in the $20\text{--}100 \text{ nm}$ region, and an additional contribution at larger pore diameters ($\sim 160 \text{ nm}$) in ABC (Supplementary Material, Figure S1). When comparing these results to those reported by [28], it is evident that the activation process increases the surface area, facilitating the formation of additional porosity through pyrolysis/activation. Moreover, as shown by [32], this process can be further optimized by increasing the synthesis temperature, resulting in a surface area up to 5.3 times greater and potentially enhancing the physicochemical properties of the ABC material.

Figure 4 presents the results of XRD analysis on both BC and ABC samples. The analysis reveals the presence of calcite (CaCO_3) and a type I cellulose structure. Calcite,

which forms as a result of CO₂ capture by *Sargassum*, is identified by characteristic peaks at 2 θ angles of 29.5°, 36°, 39°, 43°, 48°, and 57° [33]. The type I cellulose structure is confirmed by prominent peaks at 15°, 22°, and 34°. Additionally, signals were observed at 23° and 26°, which correspond to turbostratic crystalline carbon, a by-product of pyrolysis [34].

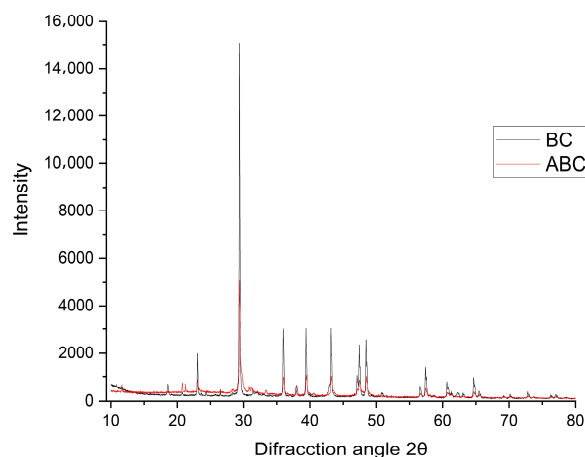


Figure 4. XRD pattern of (black line) BC and (red line) ABC.

When comparing the samples, the BC shows higher crystallinity than the ABC, highlighting structural differences that influence material properties. This conclusion is drawn from the relative intensity and the broader (greater FWHM) bands associated with turbostratic-type carbon in the ~23–26° (2 θ) region, linked to the broad (002) reflection, which appears less intense and more broadened in ABC. This variation primarily results from the chemical activation step, as oxidative treatment with HNO₃ introduces oxygen-containing functional groups and structural defects, increasing disorder and decreasing graphitic-like ordering (turbostratic carbon) observed by XRD [35–37]. As a result, a more amorphous/disordered carbon matrix forms, often accompanied by increased porosity and adsorptive capacity [38,39]. Both samples also exhibit high purity, with no diffraction peaks indicating the presence of contaminant crystalline phases.

Figure 5 presents the thermogravimetric analysis (TGA) results, showing that ABC exhibits a higher residual mass after the thermal process than BC. This higher residue indicates a larger non-volatile fraction remaining after heating under the applied conditions. The activation process effectively removes organic and inorganic materials that were not completely decomposed during pyrolysis, resulting in, according to XRD, ABC exhibiting a more disordered (more amorphous/turbostratic) carbon structure than BC, indicating reduced graphitic-like ordering after chemical activation. Complementary, TGA shows a higher residual mass for ABC at the end of the thermal program, suggesting a larger non-volatile fraction under the applied conditions (e.g., fixed carbon and/or inorganic content), which does not imply higher crystallinity. With a greater surface area and a more ordered structure [40]. Activation, particularly with agents such as nitric acid (HNO₃), introduces functional groups that enhance the material's thermal stability. This is evidenced by an increased degradation temperature and improved resistance, a phenomenon observed in both polymeric and carbonaceous materials [41–43]. For example, TGA studies of pequi and rice biochar have shown that BC loses 15–19% more mass than ABC. This confirms that the activation process removes volatile compounds and impurities, resulting in a purer and more stable material [44,45].

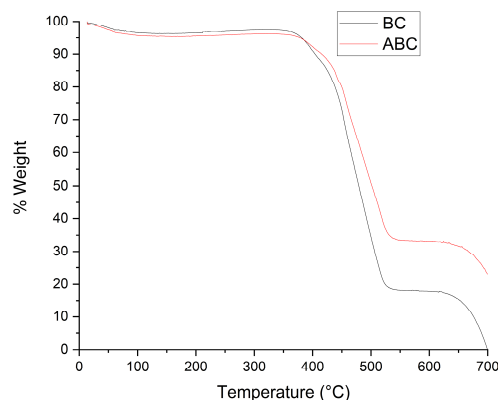


Figure 5. Comparison of thermogravimetric analysis of BC (black line) and ABC (red line).

3.2. Development and Electrochemical Characterization of the Genosensor

The CV data provided verify the electrochemical surface optimization achieved by the modifications. A comparative analysis of the voltammograms obtained from each sample confirmed that the three gels evaluated for modification and the employed ABC concentration significantly influence the electrode surface's electrochemical performance. Figure 6 illustrates the voltammograms of the redox system $[\text{Fe}(\text{CN})_6]^{3-/4-}$ for electrodes with different gel surface modifications: an unmodified graphite electrode (dotted red line), an electrode modified with HC (blue line), an electrode modified with LDA (green line), and an electrode modified with agar (purple line). The unmodified graphite electrode, referred to as "bare" (red dotted line), exhibited a maximum anodic peak current (I_{pa}) of $27.78 \pm 1.87 \mu\text{A}$. After coating with the biopolymers, the maximum anodic peak current was measured for HC ($24.6 \pm 1.74 \mu\text{A}$; blue line), LDA ($18.1 \pm 2.96 \mu\text{A}$; green line), and agar ($12.3 \pm 3.32 \mu\text{A}$; purple line).

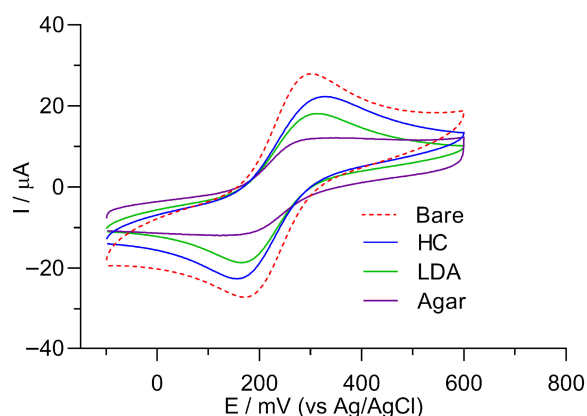


Figure 6. Cyclic voltammograms of the electrodes were measured in a 0.01 M KCl solution containing 20 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe. Graphite electrode (dotted red line), an electrode modified with HC (blue line), an electrode modified with low-density agarose (LDA) (green line), and an electrode modified with agar (purple line).

For the three analyzed biopolymers, the electrochemical response was lower than that of the unmodified electrode, indicating that all coatings partially limit redox accessibility to the surface. Among the gels evaluated, HC showed the best electrochemical response compared with agar and LDA. By controlling the deposition conditions, the thickness and permeability/porosity of the HC layer can be adjusted to maintain electrochemical access while allowing subsequent immobilization of the bioreceptor. This process enables the development of efficient electrochemical biosensors by balancing resistance to electron

transfer and bioreceptor accessibility to the target, thereby increasing device sensitivity and specificity [22,30].

Figure 7 shows the electrochemical response of the modified working electrode with ABC at 15% (green line) and 30% (orange line). The electrode modified with 30% ABC (orange line) showed a significant increase in current ($35.25 \pm 1.24 \mu\text{A}$), compared with the unmodified electrode (dotted red line, $29.32 \mu\text{A}$). Based on the results obtained from the gels and the ABC concentrations, a matrix was formed with HC and 30% ABC (blue line). The maximum peak current for this matrix significantly increased to $39.25 \pm 1.84 \mu\text{A}$, representing an 11.34% increase compared to 30% ABC. Posteriorly, the matrix was modified with a DNA probe (ssDNA, turquoise line) and its complementary strand (dsDNA, orange line). After the incorporation of these probes, a notable reduction in peak current was observed for both the DNA probe (light turquoise line, $28.81 \pm 1.565 \mu\text{A}$) and the complementary strand (purple line, $23.1 \pm 1.25 \mu\text{A}$). Finally, Hx (dark red line) was incubated on the working electrode previously modified with HC/ABC/ssDNA/dsDNA, confirming the hybridization reaction. In this step, the maximum anodic peak current decreased to $15.51 \pm 1.13 \mu\text{A}$, consistent with the formation of a more compact nucleic acid layer and interfacial limitations by Hx–dsDNA interactions [46].

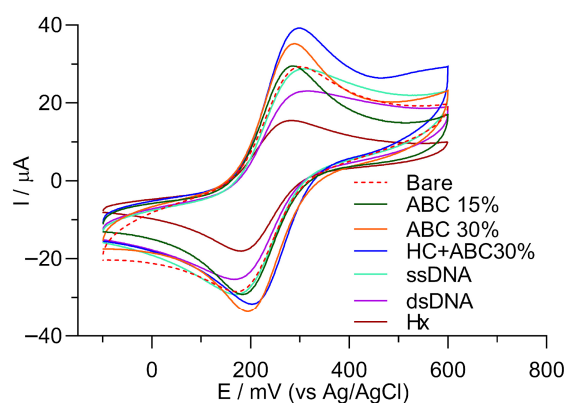


Figure 7. Cyclic voltammograms of the electrodes were measured in a 0.01 M KCl solution containing 20 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe. ABC 15% (green line), ABC 30% (orange line), HC + ABC 30% (blue line), DNA probe (ssDNA, turquoise line), complementary strand (dsDNA, orange line) and Hematoxylin (Hx, dark red line).

The results suggest that a higher ABC concentration (30%) increases the electrode surface area, thereby optimizing its electrochemical efficiency [47]. This result is consistent with previous studies reporting that higher amounts of ABC on the working electrode surface increase electrochemical [48]. This phenomenon could be explained by percolation theory: concentrations above this optimal level induce aggregate formation, thereby reducing the number of active sites [49]. Moreover, it was observed that an ABC concentration above 30% obstructs the porous structure, hindering the diffusion of analytes, a phenomenon widely documented in electrochemical impedance studies [50].

This decrease indicates an increase in the transfer resistance of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ions, suggesting that DNA hybridization has occurred with the electrode surface [51]. The reduction in current reflects the oligonucleotides' chemical and physical properties, which block the modified surface, thereby limiting electron transfer and affecting the electrochemical signal [52]. The HC + ABC (30%) matrix showed high surface area, controllable porosity, and good electrochemical response [53].

3.3. Surface Area of the Electrochemical Genosensor

The electron transfer kinetics of the designed graphite electrode were evaluated at different scan rates, from 10 to 100 $\text{mV}\cdot\text{s}^{-1}$, using the CV technique [51]. The CVs' maximum peaks increased with higher scan rates, as shown in Figure 8. The electroactive surface area of the bare graphite (A), HC (B), ABC 30% (C), HC + ABC 30% (D), ssDNA (E), dsDNA (F) and Hx (G) were calculated to be 0.00044 cm^2 , 0.00033 cm^2 , 0.00063 cm^2 , 0.00051 cm^2 , 0.00017 cm^2 , 0.00025 cm^2 and 0.00030 cm^2 , respectively. Electron exchange at the electrode surface occurs slowly at lower scan rates, leading to a narrower CV and a reduction in peak current [1]. In addition, each modification changes the working electrode's electroactive area, confirming the construction process [29].

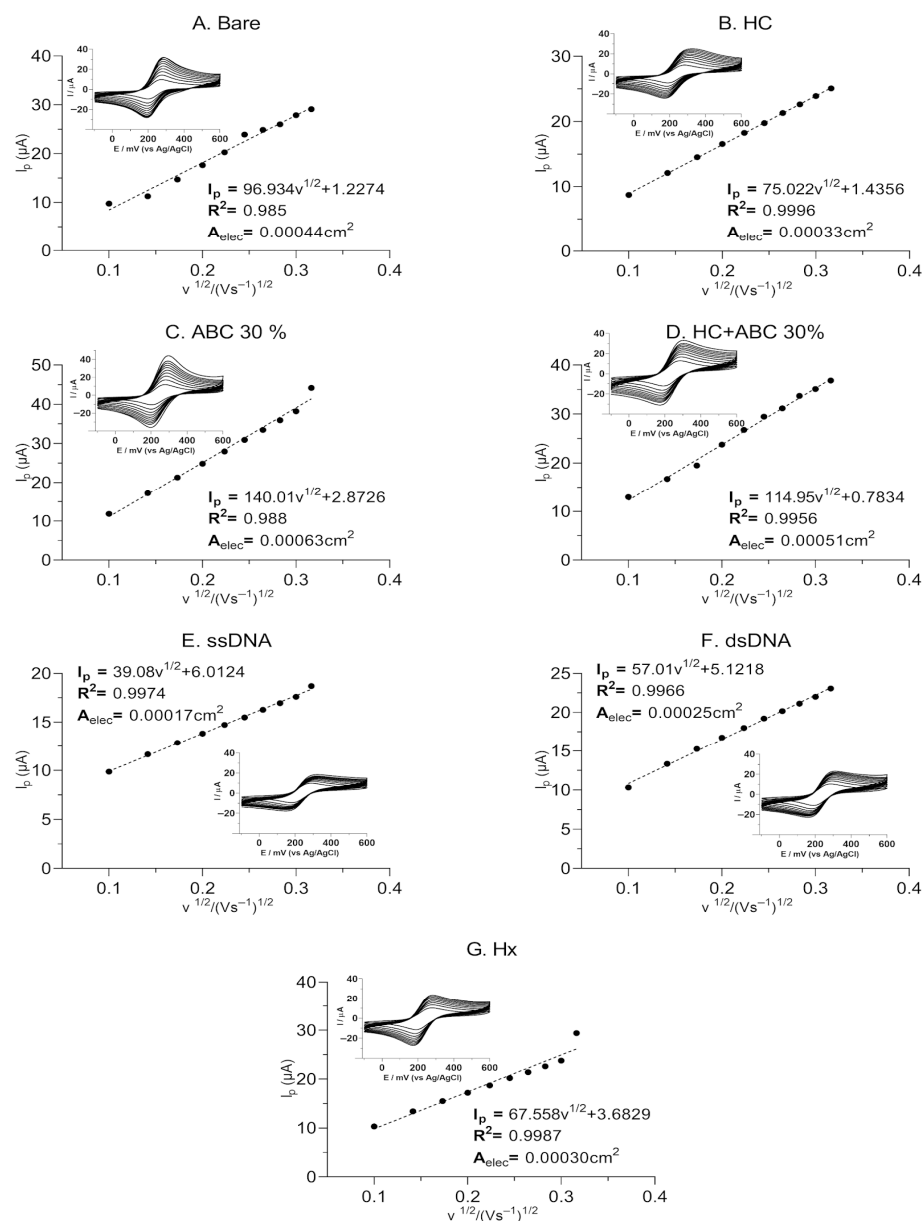


Figure 8. CV voltammograms of bare graphite (A), HC (B), ABC 30% (C), HC + ABC 30% (D), ssDNA (E), dsDNA (F) and Hx (G), on graphite electrode in a 0.01 M KCl solution containing 20 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe at different scan rates (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mVs^{-1}).

4. Conclusions

In summary, a study was conducted to evaluate the electrochemical response of a collagen matrix hydrolyzed with activated carbon obtained from *Sargassum spp.* biomass, which, when used as a substrate for DNA biosensor development, significantly improves the signal from conventional graphite electrodes. Structural and morphological characterizations obtained by pyrolysis and HNO₃ chemical activation showed that this process enabled the development of a nanostructure with a surface area of 159.3 m²/g, suitable for application as a substrate for the modification of the graphite electrode. Furthermore, in the electrochemical process using CV, it was observed that the biopolymer with the highest probe molecule ([Fe(CN)₆]^{3−/4−}) diffusion was HC. Together with ABC (30%), it formed a matrix with an optimal critical filling charge, increasing the electroactive area by 0.00063 cm² and raising the peak anodic current by 33.8% compared to the conventional, unmodified graphite electrode. The step-by-step construction of the electrochemical genosensor was evaluated by measuring the decrease in the peak current after SSDNA immobilization and subsequent dsDNA hybridization. Finally, Hx served as an intercalating agent and indicator molecule during hybridization, reflecting a decrease in electron flow due to the formation of a compact nucleic acid layer and the intercalation of Hx. Kinetic analyses demonstrated that electron diffusion is controlled, indicating high reproducibility and stability during construction. Therefore, this research provides a sustainable and efficient method for transforming and adding value to *Sargassum spp.* biomass through the synthesis of novel nanomaterials for biosensor development. Furthermore, the resulting HC/ABC matrix constitutes a promising, high-purity substrate suitable for advanced electrochemical biosensors with superior physicochemical properties.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/bios16020115/s1>, Figure S1. Pore size distribution from BET absorption analysis for (A) BC and (B) ABC.

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Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
$[\text{Fe}(\text{CN})_6]^{3-} / [\text{Fe}(\text{CN})_6]^{4-}$	ferricyanide/ferrocyanide redox couple
ABC	Activated biochar
Ag/AgCl	Silver/silver chloride
Al_2O_3	Alumina (aluminum oxide)
BC	Biochar
BET	Brunauer–Emmett–Teller (surface area method)
BJH	Barrett–Joyner–Halenda (pore size distribution method)
CH_4	Methane
CO_2	Carbon dioxide
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy-dispersive X-ray
H_2S	Hydrogen sulfide
HC	Hydrolyzed collagen
HNO_3	Nitric acid
Hx	Hematoxylin
$\text{K}_3[\text{Fe}(\text{CN})_6]$	Potassium ferricyanide
$\text{K}_4[\text{Fe}(\text{CN})_6]$	Potassium ferrocyanide
KCl	Potassium chloride
LDA	Low-density agarose
LN_2	Liquid nitrogen
NH_3	Ammonia
PBS	Phosphate-buffered saline
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
ssDNA	Single-stranded DNA
TE	Tris-EDTA buffer
TGA	Thermogravimetric analysis
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
XRD	X-ray diffraction

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