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Research Article

Electrospun PVA/CQD nanofiber–coated carbon anode for high–performance microbial fuel cells: A comparative study

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Abstract. This study details the development of a high-performance microbial fuel cell (MFC) utilizing a nanofiber-coated carbon anode, fabricated through the electrospinning of polyvinyl alcohol (PVA) integrated with carbon quantum dots (CQDs). A dual-chamber H-type MFC, with a working volume of 50 mL for both anode and cathode compartments, was operated in batch mode using sterilized sugarcane juice, adjusted to a pH of 7.0, as the organic substrate. Two electrogenic bacteria, Bacillus subtilis and Escherichia coli, were separately immobilized within the PVA/CQD nanofiber matrix to assess their electrochemical performance. Structural and chemical characterizations using SEM, FTIR, and UV–Vis spectroscopy confirmed the successful incorporation of CQDs and effective bacterial colonization within the nanofiber network. Electrochemical studies, such as CV and EIS, indicated low charge transfer resistance and improved electron kinetics especially when *B. subtilis* was present and an Rct of about 400 ohms. MFCs based on *B. subtilis* reached a maximum power density of 1754 mW/m² on day four of operation at a fixed external resistance of 100 0 and the electrode surface area of 9.45 cm², about 3.5 times greater than the power density obtained with *E. coli* (491 mW/m²). This has been due to the high performance of *B. subtilis* which can form a robust conductive biofilm, releases endogenous redox mediators, and has the ability to metabolize sugar rich substrates efficiently. These findings underscore the potential of PVA/CQD nanofiber-coated carbon anodes as an effective strategy for enhancing MFC performance and provide a promising foundation for future optimization and scale-up toward sustainable energy generation from organic waste at the laboratory level.

Keywords: Microbial Fuel Cell (MFC), Electrogenic microorganisms, Sugarcane juice, Carbon Quantum Dots, Electrospinning nanofiber.

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1. Introduction

Global dependence on fossil fuels (non–renewable), such as coal, oil, and gas, has led to the depletion of these resources due to overconsumption (Emodi *et al.*, 2023). This increase in demand is primarily driven by the accelerating growth of the human population (Grace *et al.*, 2020; Raihan *et al.*, 2023). Beyond the immediate concerns of long-term energy security, the persistent reliance on fossil fuels plays a significant role in the emission of greenhouse gases, thereby worsening climate change and contributing to environmental degradation (Grace *et al.*, 2020). Subsequently, it has made the quest of renewable energy technologies, which are efficient, cost-effective, and eco-friendly, a worldwide imperative which relates to the international adherence to the Sustainable Development Goals. (Trinh & Chung, 2023).

The microbial fuel cell (MFC) is one of the promising innovative technologies. Bioelectrochemical systems known as MFCs are systems that harness the biogenic nature of microorganisms that are electrogenic to break down organic compounds and produce electricity. This is because they have been introduced since the 1970s (Widharyanti *et al.*, 2021) and their use in household wastewater treatment in 1991 (Kurniawan *et al.*, 2022), MFCs have evolved into a dual solution for generating renewable energy while sustainably managing organic waste (Kurniawan *et al.*, 2022).

The working principle of MFC relates to the fact that microorganisms could oxidize organic substances and transfer the electrons produced to the anode electrode. The electrons then pass through an external circuit to the cathode producing an electric current. The conductive structures (nanowires or outer membrane proteins) may be involved in electron transfer directly, or indirectly with the assistance of redox mediators (menadione or methylene blue)(Kurniawan *et al.*, 2022; Widharyanti *et al.*, 2021). Organs are transformed into electricity in this process, and byproducts like water and carbon dioxide are produced due to the process of sustainable redox, which allows converting organic waste into energy and allows managing the environment sustainably (Christwardana *et al.*, 2024)

Microbial fuel cells (MFCs) have been widely recognized as a promising sustainable approach for integrating wastewater treatment with energy recovery (Hadiyanto *et al*, 2023). A range of configurations has been investigated, particularly two-chamber systems employing salt bridges, due to their straightforward design, cost-effectiveness, and suitability for

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laboratory-scale applications (Darmawan *et al.*, 2024; Mkilima & Baimukasheva, 2025). Despite these benefits, the performance of MFCs is still constrained, especially with regard to electron transfer efficiency and the resulting power output. The anode serves as a key component in the bioelectrochemical conversion process, where its characteristics—including electrical conductivity, chemical durability, biocompatibility, and surface area—play a decisive role in supporting the activity of electrogenic microorganisms (Yaqoob *et al.*, 2020). Within the anode chamber, the synergy between electroactive bacteria, organic substrates, and the electrode surface governs the effectiveness of electron production and transport (Yaqoob *et al.*, 2021). However, conventional anode materials remain limited in their ability to provide efficient electron pathways and adequate surface interaction for optimal microbial colonization.

Accordingly, the development of improved anode materials and optimized system configurations continues to be an essential area of research aimed at enhancing electron transfer processes and overall MFC performance. *B. subtilis* forms a stable conductive biofilm on the anode surface, whereas *E. coli* excels in growth rate and genetic manipulation ease, although its electrical performance is generally lower. Based on two studies, *B. subtilis* produces higher electrical power than *E. coli. E. coli* only produces a maximum power density of 204.5 μW/m² (Emodi *et al.*, 2023), whereas *B. subtilis* reaches up to 105 mW/m² (García-Mayagoitia *et al.*, 2019; Montoya-Vallejo *et al.*, 2023).

Sugarcane juice, an abundant agro–industrial byproduct in tropical regions, was used as a substrate in this study because of its high organic content (Christwardana *et al.*, 2021). This substrate supports efficient microbial oxidation, making it an ideal choice for waste–based sustainable energy solutions (Christwardana *et al.*, 2024). Various studies have confirmed the feasibility of using sugarcane juice or bagasse as a carbon source; for example, using Saccharomyces cerevisiae in single–chamber MFC configurations. Various studies have confirmed the feasibility of using sugarcane juice or bagasse as a carbon source, for example with Saccharomyces cerevisiae in a single–chamber MFC configuration (Christwardana *et al.*, 2024).

Electrodes in MFC serve for electrochemical reactions, namely substrate oxidation by microbes at the anode and oxygen reduction at the cathode, so the electrode material greatly influences the electron transfer efficiency and electrical output (Samudro *et al.*, 2021). Various materials, such as graphite, carbon cloth, carbon paper, carbon foam, carbon glass, stainless steel, and titanium mesh, have been used as electrodes, each with different biofilm formation and conductivity characteristics (Kosimaningrum *et al.*, 2021; Suriani *et al.*, 2020). Graphite is the primary choice due to its combination of high electrical conductivity, chemical stability (resistance to corrosion in the reactor environment), biocompatibility, and relatively low cost, enabling efficient electron transfer between microbes and the electrode (Logan & Rabaey, 2012).

To make the best use of anode performance in a MFC system, a polyvinyl alcohol (PVA)-based composite anode was created through the electrospinning procedure (Diep & Schiffman, 2023). PVA was selected due to its biocompatibility, hydrophilicity, and the ability to create porous nanofiber structures, which enable an optimal increase in microbial biofilms and an increase in the area of electron transfer (David *et al.*, 2021; Wijayanti *et al.*, 2022; Kumuthan *et al.*, 2021). Electrospinning technology creates nanofiber designs that support the easy diffusion of substrates and movement of electrons (Diep & Schiffman, 2023; Greiner & Wendorff, 2007).

PVA has low conductivity in nature, and the use of conductive dopants or additives is required in order to realize high levels of charge transfer (Hany & Mousa, 2017). Carbon quantum dots (CQDs) were used to enhance the conductivity of the anode by integrating them with PVA matrix (Zulfajri *et al.*, 2021). These particles are less than 10 nm in diameter, have aromatic structures that facilitate electron flow, high chemical stability, and excellent optoelectronic properties and enable the rapid transfer of electrons to electrode surfaces of bacteria (Rasal *et al.*, 2021). CQDs prepared using biomass waste, including rice husk, are also made based on renewable resources, and they possess good biocompatibility, preserving biofilm integrity (Angelov *et al.*, 2023; Suman *et al.*, 2024).

The proposed study presented a new methodology, and the strategy to be adopted in the research is the utilization of nanofiber-coated carbon anodes prepared using the electrospinning technique, with PVA/CQDs serving as the functional materials. While microbial fuel cells (MFCs) and the modification of carbon-based anodes have been widely investigated, the development of nanofiber-coated carbon anodes is still relatively underexplored. Moreover, the incorporation of functional nanomaterials into nanostructured anode systems has not yet received sufficient attention. This gap highlights the necessity for further research aimed at designing advanced anode materials capable of enhancing electron transfer efficiency and improving overall system performance. In response to this, the present study focuses on developing a nanofiber-coated carbon anode based on PVA/CQDs and assessing its effectiveness in enhancing electron transfer behavior and electrochemical performance in microbial fuel cells.

2.Materials and Methods

2.1 Materials

In this study different primary materials have been used in the development and testing of a MFC system, as shown in Figure 1. PVA, obtained at Sigma-Aldrich, served as the primary polymer backbone in making the nanofibers, whereas CQDs, which are the result of carbonizing rice husks with the help of a microwave heater served as a conductive additive. Graphite rods were used as the active anode. The bacterial culture was left to incubate at 28 °C in an orbital shaker until the cell density was attained and it was approximately 10^8 cells/mL ($OD_{600} \approx 1.0$). The organic substrate was fresh sugarcane juice and was filtered, pH was adjusted to 7.0 and its sterility was achieved by means of autoclave 121 °C and 151,987.5 – 202,650 Pa. According to the common compositional studies, fresh sugarcane juice contains about 10–20 wt% total sugars, and the majority of them is sucrose (Mulyadi *et al.*, 2022). H type MFC reactor was a two-chamber system that had a 50 mL anode and cathode chamber. The platinum carbon cathode electrode and a salt bridge consisting of 1.1 g of KCl (=0.738 M), 0.25 g of nutrient agar and 20 mL of distilled water were used as the cathode and separator respectively. This solution was heated up to a semi-solid solution. Redox mediator was used in the form of Methylene Blue provided by Merck (Montoya-Vallejo *et al.*, 2023).

2.2 Nanocomposite Anode Fabrication

PVA solution (10% w/v) was prepared by dissolving PVA in distilled water at 80 °C under magnetic stirring and then 15 min of sterilization in an autoclave. The sterilized solution was kept

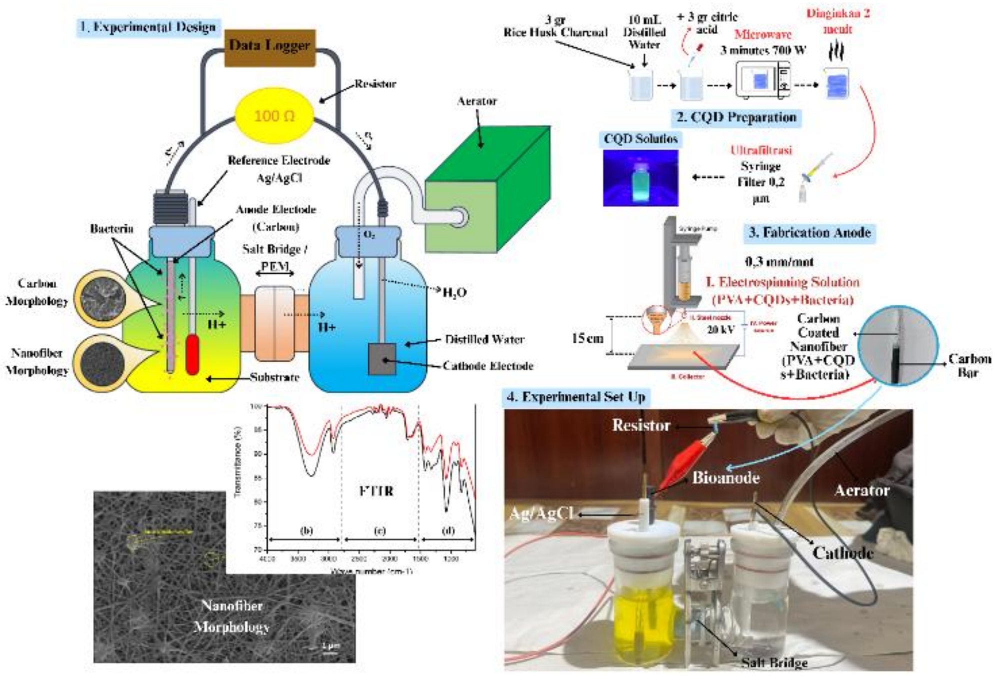

Fig. 1. Microbial Fuel Cell System Schematic (a) Experimental Design (b) CQD Preparation (c) Fabrication Anode (d) Experimental Set up

in a sterile container that had a cap to ensure stability (Khalaf *et al.*, 2023). The 3 g of rice husk charcoal was accurately measured to synthesize CQDs. The distilled water (10 mL) was put in a beaker and stirred with a magnetic stirrer. Afterward, 3 g of Citric Acid ($C_6H_8O_7$) was added and the mixture was mixed until it was dissolved. The husk charcoal of rice was then added to the mixture and mixed 10 min at 125 rpm. The solution was stirred after which it was put in a microwave and allowed to heat in a 3-min heating to expedite the synthesis. After the step of microwaving, 20 mL of distilled water was introduced to the beaker and the mixture was mixed vigorously followed by filtration using a 0.22 μm pore syringe filter to remove coarse particles or contaminants. The pure CQDs in the filtrate were added to a vial tube where they were kept until the electrolyte membrane fabrication stage (Suman *et al.*, 2024). Figure 1(b) demonstrates the process of preparation of the CQDs solution. Each type of bacterium was mixed with 7.5 mL of cooled PVA solution, 1mL of CQDs, and 1.5mL of bacterial culture ($OD_{600} \approx 1.0$) and used as an electrospinning polymer solution. A magnetic stirrer was used to stir this mixture gently over 5 min to have a homogeneous solution. The electrospinning process was conducted with parameters set at 18 kV voltage, 0.3 mL/h flow rate, and a 15 cm distance between the needle tip and the collector (Diep & Schiffman, 2023). Preparation of nanofiber making using the electrospinning method is illustrated in Figure 1(c). Nanofibers were collected on two substrates: aluminum foil for FTIR chemical structure analysis and graphite rods, which served as anode substrates in the MFC system, by manually rotating the rod to evenly distribute the nanofibers. The resulting electrospun nanofibers were dried at room temperature for 24 h (Diep & Schiffman, 2023).

2.3 MFC Assembly and Operation

The assembled dual–chamber MFC used a 50 mL H–type reactor. The anode chamber contained a graphite rod coated with PVA/CQDs/bacteria nanofibers and an Ag/AgCl reference electrode for electrochemical analysis (García-Mayagotia *et al.*, 2023). In the cathode chamber, a platinum carbon electrode is continuously aerated to supply oxygen as the electron acceptor (García-Mayagotia *et al.*, 2023; Wulan & Notodarmojo, 2020). A salt bridge composed of KCl solution and nutrient agar links the two chambers, facilitating ion transfer. The anode chamber is filled with a mixture of 10 mL of sterile LB medium inoculated with bacteria (~10^8 cells/mL), 39 mL of sterilized sugarcane juice, and 1 mL of methylene blue solution as an additional redox mediator. The experimental setup of MFC using H type reactor is shown in Figure 1(d). The MFC operated in batch mode with no substrate replacement during the experiment. Voltage output was recorded daily from 1^{st} day to 5^{th} day using a digital multimeter, while CV and EIS were conducted on 4^{th} day using a potentiostat.

2.4 Characterization

The anode chamber was equipped with a graphite rod that had been coated with PVA/CQDs/*B. Subtilis* nanofibers. To analyze the surface morphology and bacterial distribution on the anode, a field-emission scanning electron microscope (FE-SEM) was utilized at an accelerating voltage of 5 kV. Fourier-transform infrared (FTIR) spectroscopy was conducted on samples of PVA/CQDs/*B. subtilis* and PVA/CQDs/*E. coli* within the wavenumber range of 4000–400 cm^{-1}, using a PerkinElmer Frontier C90704 spectrometer with Spectrum IR software (v10.6.1). The UV–Vis absorption spectra of PVA/CQDs, PVA/CQDs/*B. subtilis*, PVA/CQDs/*E. Coli*, and a control sample were captured using an M21s Molar Series double-beam spectrophotometer to assess their optical properties. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) tests were carried out with a Corrtest E100 electrochemical workstation. EIS measurements were taken between 100 kHz to 0.1 Hz with an amplitude of perturbation in the AC form of 10 mV. Ionic conductivity (σ) was calculated with the help of the EIS data by means of the formula (1):

$$\sigma = \frac{L}{R \times A} \ (S/cm) \qquad (1)$$

The electrode area is A, the bulk resistance based on the Nyquist plot is shown as R and the sample thickness as L. Cyclic voltammetry (CV) was done at a scan rate of 50 mV s^{-1} and scanned between a potential of -0.2 V to +1.0 V relative to Ag/AgCl. The voltage output was measured on a daily basis throughout the operation of the microbial fuel cell (MFC) with high-precision digital multimeter. This was related to an extrinsic resistance of 100 Ω at an electrode surface area of 9.4 cm^2 that was used to determine power density.

To determine the electric current (I), current density (J) and power density ($P_{density}$), cell voltage (V_{cell}) data was measured three times per day and then average of these daily measurements was then used to determine the values using standard equations of electrochemical. These performance indicators allow to understand how well the electron transfer and the total output of the MFC system respond to the provided working conditions (Duarte–Urbina *et al.*, 2021):

$$I = \frac{Vcell}{Rext}\ (A) \tag{2}$$

$$J = \frac{I}{A_{electrode}} (A/m^2) \tag{3}$$

$$P_{density} = J\ x\ Vcell(\ (W/m^2) \tag{4}$$

3. Results and Discussion

3.1 Optical Properties Analysis (UV–Vis)

The existence of CQDs in the PVA nanofiber matrix and the impact of the bacterial colonization on the optical properties of the anode were checked with the help of UV-vis spectroscopy. According to the UV–Vis spectra in Figure 2, all three samples, including PVA/CQDs, PVA/CQDs/*E. coli*, and PVA/CQDs/*B. subtilis* showed high absorption in the ultraviolet area (approximately 250–300 nm). This intake is connected to the π–π* transition of the aromatic groups of the CQDs structure. In all samples, a reduction in absorption at 350–500 nm was observed, which indicates the transition of π–π* of the carbonyl group or other conjugated functional groups (Dominguez-Medina *et al.*, 2016).

The PVA/CQD curve (black) has the greatest absorption as compared to the two bacterial samples. The loss of absorbance at the PVA/CQD/*E. coli* (red) and the PVA/CQD/*B. subtilis* (blue) samples indicates how the CQDs interact with bacterial cell constituents, i.e. proteins or cell wall polysaccharides. This interaction may either screen the active surface of the CQDs or increase the light scattering effect. Nevertheless, the PVA/CQD/*B. subtilis* sample had a greater absorbance compared with *E. coli* at the range of 400–700 nm. This variation is probably because of superior bacterial colonization and development of a more secured biofilm over the anode surface.

This observation aligns with the study conducted by Kang *et al.*, where the authors found that the optical profile, such as a reduction in the absorbance of the surface aggregation or adsorption, depends on the interaction of carbon nanoparticles and bacterial biomolecules (Dominguez-Medina *et al.*, 2016). Accordingly, the positive outcomes of this UV-Vis indicate the effective incorporation of bacteria into the PVA/CQD matrix and indicate the possibility of improving the bioanode performance with the help of optical interactions and appropriate surface structures (Diep & Schiffman, 2023).

Besides establishing the optical interaction and effective incorporation of bacteria to PVA/CQD, the stability of PVA-based coating is important in its application as bio-interfacial layer in microbial fuel cells (MFCs). During the 5-day operation of MFC, PVA/CQD coating was sufficiently stable as shown by no abrupt variations in electrochemical activity as the coating still interacted with bacterial biomolecules at the functional surface. It is known that aqueous exposure to long-term exposure to electrolytes may cause polymer swelling, surface restructuring or gradual hydrolytic degradation, particularly in hydrophilic polymer matrices such as PVA. Nevertheless, in the experimental phase of the present study, no sudden performance deterioration and interfacial failure were observed, indicating that PVA/CQD composite has enough structural and interfacial integrity to remain operational in MFCs and be tested within a short time. Therefore, the present research represents a proof-of-concept evaluation of short-term operational stability rather than a comprehensive assessment of long-term durability.

3.2 Scanning Electron Microscopy Analysis

SEM characterization was done to note the surface morphology of the composite anode and bacteria distribution in it. SEM analysis of the anode made of PVA/CQDs nanofibers, inoculated with bacteria, as observed in Figure 3, indicates a network structure of interconnected nanofibers that forms a porous structure. The structure avails a high surface area which is highly significant in microbial attachment and colonization. These indicators in the photograph are that there are bacteria that are spread and confined in the nanofiber framework.

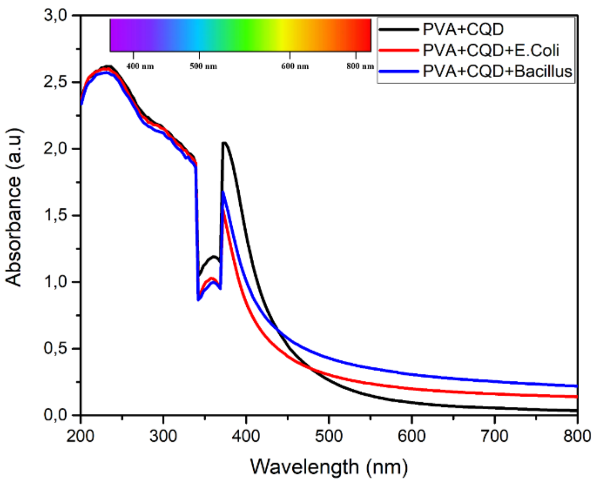

Fig. 2. UV–Vis Spectrum

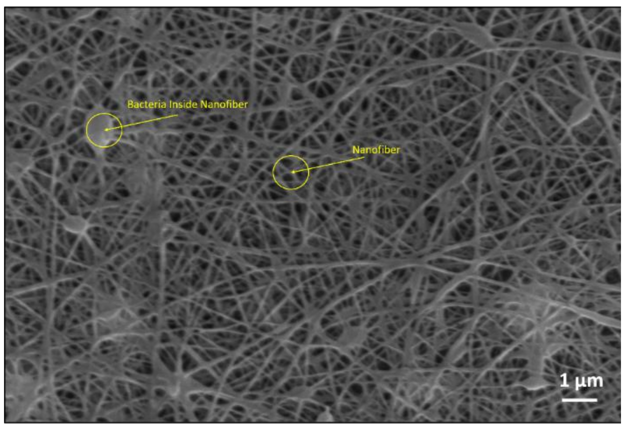

Fig. 3. SEM Image of Nanofiber Structure Containing Bacteria

These findings indicate that the electrospinning technique is effective in loading bacterial biomass into the anode structure. Inter-fiber pores offer the most ideal conditions to growth of biofilm, diffusion of substrates in the shape of sugarcane juice, and transfer of metabolite products (Zhou *et al.*, 2022). The general morphology observed suggests that the nanofiber composite anode is able to offer a three-dimensional scaffold. This framework aids in immobilization and even increase of bacteria. This distribution is a major factor in improving the performance and bio–electrochemical efficiency of the MFC system.

3.3 Functional Group Identification (FTIR)

Based on Figure 4, the FTIR results of the PVA/CQDs/*B. subtilis* and PVA/CQDs/*E. coli* samples indicate successful component integration, indicated by the appearance of several characteristic absorption bands. The FTIR spectrum displays a strong peak in the range of 3272–3273 cm^{-1} indicating O–H stretching, originating from hydroxyl groups in PVA and CQDs. The peak at 2921 cm^{-1} indicates aliphatic C–H stretching, while the band in the range of 1704–1709 cm^{-1} is associated with C=O (carbonyl) groups, likely originating from carboxylic acids or aldehydes resulting from the synthesis of CQDs using rice husks.

The presence of absorption in the range of 1419–1326 cm^{-1} indicates C–H bending vibrations as well as C–O groups from alcohols or esters, supporting the presence of CQDs with oxygen functional groups. The characteristic peaks at 1081 and 1079 cm^{-1} indicate C–O–C vibrations, while the bands at 915 and 832 cm^{-1} indicate the presence of aromatic C–H bonds or vibrations of heterocyclic rings.

These findings are in line with the report by García *et al.* (2023) (García–Mayagotia *et al.*, 2023), which reported that the FTIR spectrum of the PVA/CQDs composite presented these characteristic bands which means that there was successful incorporation of the carbon materials into the polymer mass. Therefore, the FTIR evidence of the present research contributes to the development of a composite anode system based on a blend of PVA, CQDs, and bacterial biomass and can be used to prove a high possibility of enabling biofilms and enhancing the efficiency of electron transfer in MFC systems.

3.4 Cyclic Voltammetry Analysis

Electrochemical analysis was done to evaluate the catalytic activity and charge transfer mechanism of anode-biofilm interface through cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). These methods provide a clear understanding of the efficiency of the electron transfer in the system of microbial fuel cell (MFC).

According to Figure 5, the results of the CV test indicate that there are significant differences in the electrochemical characteristics of *B. subtilis* and *E. coli* biofilms. The *B. subtilis* CV curve shows much better anodic and cathodic peak currents than the *E. coli*, which suggests that the oxidation activity of organic substrates is stronger. The CV curve of the *B. Subtilis* CV shows a steep rise in current towards the peak value in both the anodic and cathodic sweeps. On the other hand, the *E. Coli* curve demonstrates a slow and linear growth of the current without a redox peak (Santoro *et al.*, 2017).

The region beneath the *B. subtilis* CV curve is more extensive and more similar to a rectangle, which indicates a greater value of the double-layer capacitance and pseudocapacitance that increase the interaction of the electrode and the electrolyte

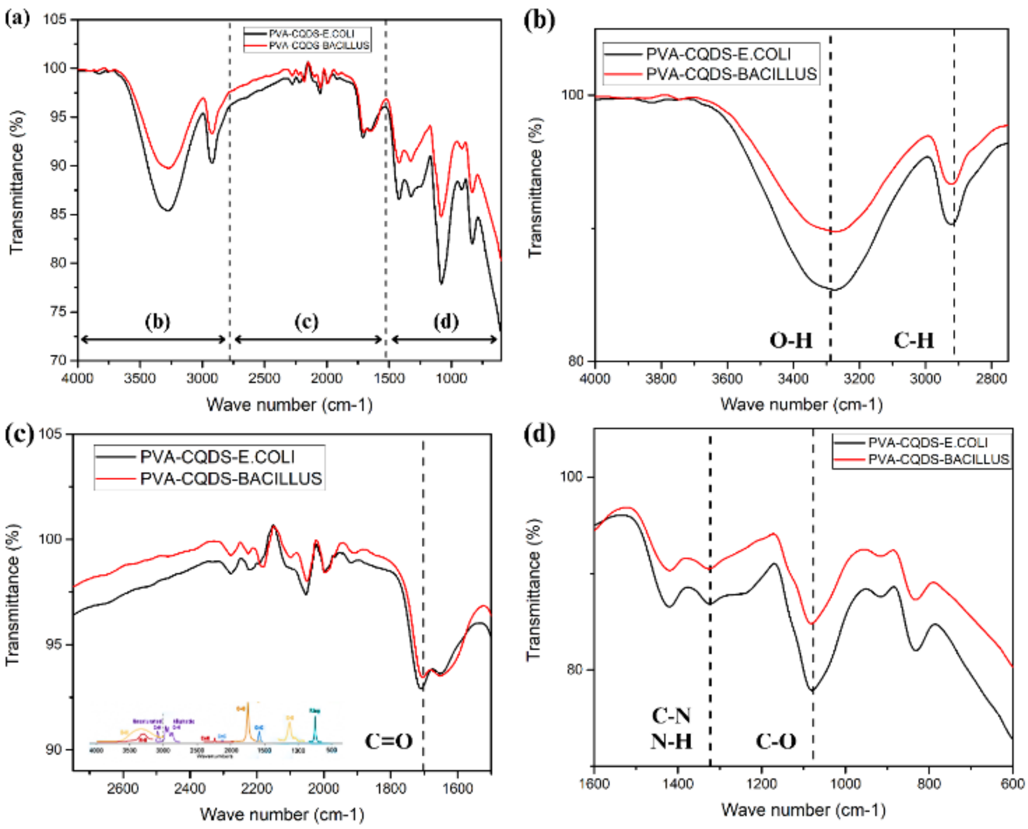

Fig. 4. FTIR Spectrum Bioanode PVA/CQDs with bacteria *E. coli* dan *B. subtilis*. (a) 4000–600 cm^{-1}(b) 3700–2800 cm^{-1}(c) 2500–1700 cm^{-1}(d) 1600–600 cm^{-1}

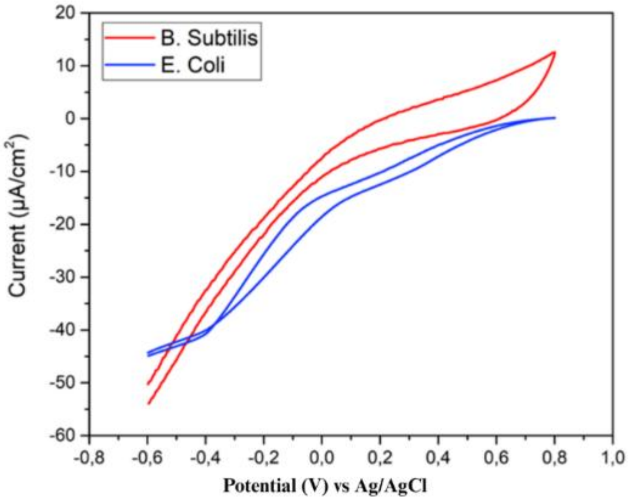

Fig. 5. Cyclic voltammetry (CV) curves of *B. subtilis* and *E. coli* based bioanodes

(Yavarinasab *et al*., 2025). Systems of *B. subtilis* make the most of the accumulation and effectiveness of the electrostatic charge and transfer of the electrons, and *E. coli* has a lower charge storage capacity because it cannot develop a favorable electrochemical architecture. *B. subtilis* only shows well-defined anodic and cathodic redox peaks with anodic potentials of between +0.10 and +0.15 V and cathodic current of between -0.60 to -0.65 V (vs. Ag/AgCl) indicating higher peak current densities.

The difference in behavior can be explained by the fact that redox compounds are naturally released by *B. subtilis*. The voltammetry findings reveal the liberation of endogenous mediators, e.g. riboflavin, which can have reversible reactions on the electrode surface. The *B. subtilis* CV curve has the peak of oxidation at +0.12 V and a peak of reduction at about -0.63 V (vs. Ag/AgCl), clearly stating that it is a redox active organism. Colasuonno *et al*. (2020) determined two present peaks indicating that two two-electron transfer processes were taking place. This fact also corresponds to the reversible character of riboflavin in oxidation and reduction processes (Colasuonno *et al*., 2020).

E. coli is able to produce small molecules only like indole which are not involved in redox activity. Consequently, the CV curve of *E. coli* resembles a wide capacitive current with no clear redox peaks. The CV data highlights the benefit of *B. subtilis* as an electrogenic bacterium. *B. Subtilis* is a good candidate to enhance MFC performance because of its ability to produce redox mediators and densely colonize the biofilm as well as produce voltammetric signals with strong electrochemical characteristics. Such results are consistent with the existing literature and add even more weight to *B. subtilis* as a microorganism with a significant potential in extracellular electron transfer (EET).

3.5 Electrochemical Impedance Spectroscopy Analysis

Sharp variations between *E. coli* and *B. subtilis* based MFC systems are seen in EIS. As can be seen, the Nyquist curves of both MFC systems have the shape of semicircles, which means that the influence of the charge transfer resistance (Rct) predominates over that of the total internal impedance. The fact that the semicircle diameter of the two systems is different gives significant data on the efficacy of electron transfer. The semicircle diameter in the MFC system based on the bacillus subtilis is comparatively small with Rct of approximately 400 ohms. The value shows the low charge transfer resistance and shows that the process of electron transfer of microorganism to anode is rapid and efficient. Compared to this, the MFC system based on Escherichia coli has a bigger diameter semicircle with an Rct of approximately 1400 ohm which means that its resistance to electron transfer is high and thus it is less efficient. This result is in line with existing literature on early MFCs, which writes that Rct is the most common component in the total system impedance (Ren *et al*., 2021). These variations in the features of EIS offer a significant ground that can be utilized in the analysis of the performance and development of MFCs. It is possible to reduce the values of high Rct by enhancing the conductivity of the electrodes, nanocarbon-based CQDs, and environmental parameters including pH, temperature, and substrate concentration (Chen *et al*., 2021). These are done to decrease the total impedance in order to enhance the overall efficiency of the MFC system (Ren *et al*., 2021).

Along with the resistance Rct, the general performance of the system depends also on the nature of the electrolytes. MFC system based on *B. subtilis* had a high electrolyte ionic conductivity as compared to *E. coli*. The ionic conductivity value can be determined with the help of equation (1). Variation in the conductivity between the MFC systems is due to the metabolic activity of the bacteria and the nature of the biofilm formed (Thulasinathan *et al*., 2021). *B. **s**ubtilis* exhibits active metabolism with sugar substrates like sugarcane juice and it forms a conductive biofilm uniformly on the anode face. This structure provides an effective ionic pathway between the microbial cells and the electrode. Conversely, *E. **c**oli* is a thin and uneven biofilm, and it is less bound with the electrode, increasing ionic resistance and decreasing ion mobility in solution. The system of *B. **s**ubtilis* has a solution resistance value of 900 ohms and ionic conductivity of 5.8789×10^{-5} S/cm. By comparison, the *E. **c**oli* system has an Rs value of 1800 ohms and a ionic conductivity of 2.9413×10^{-5} S/cm. The obtained calculation results indicate that the conductivity in the *B. Subtilis* system is nearly twice *E. Coli*, which means that the anode solution can conduct ions in a better way. This rise in conductivity is probably due to the active metabolism of *B. Subtilis* on the substrate of the sugarcane juice which generates abundant amounts of ions. Also, the ionic pathway between the microbial cells and the electrode is enhanced by the presence of a dense, conductive biofilm.

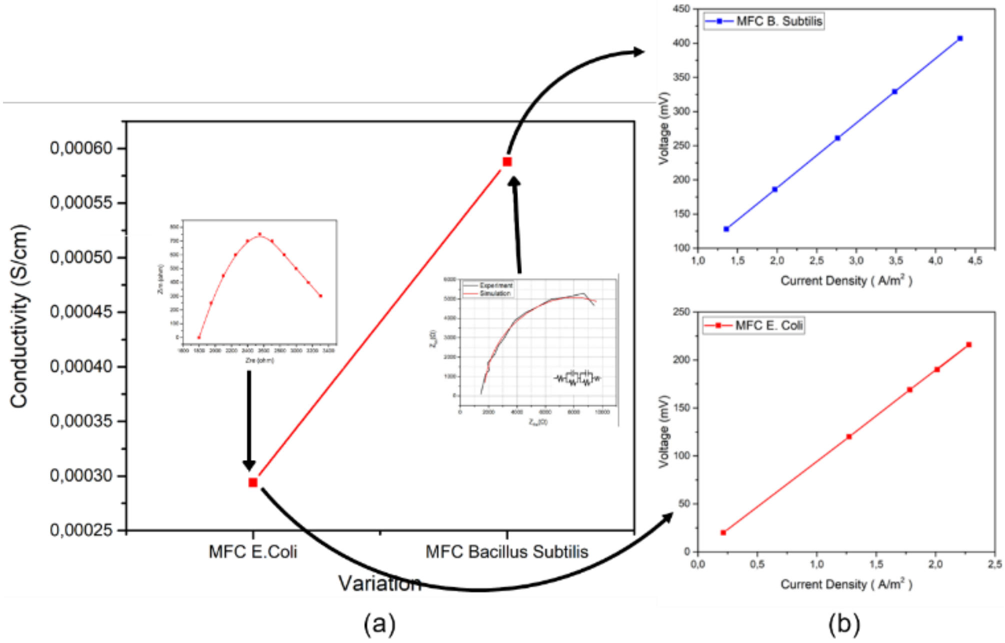

Fig. 6. (a) Ionic conductivity and (b) Polarization curve of MFCs

According to Figure 6, electrode and electrolyte dynamics can also be thoroughly visualized on the basis of the Nyquist curve. At medium to high frequencies, the semicircle form implies dominance of Rct, whereas at low frequencies, the slope of the curve tends towards a 45° angle, implying the contribution of the ion diffusion resistance (Warburg element). The *B. subtilis* system has a small semicircle diameter and a low slope which is indicative of low total impedance and high electrochemical efficiency. This state suggests that *B. subtilis* is not merely able to create a cohesive biofilm, but also produces redox mediators, e.g. riboflavin, which increase the speed of an electron transfer.

The equivalent circuit model of this system is composed of three key elements; the ohmic resistance of the solution (Rs), the charge transfer resistance (Rct) and the non-ideal capacitive element (CPE) at the interface between the biofilm and the electrode. Rs is the resistance to an electrical flow created by the electrolyte, electrodes, and connections in the circuit and which influences the total current flow. Rct is the resistance that is faced in a process of electron transfer between the electrode surface and the microorganisms or mediators that are important in defining the efficiency of an electrochemical reaction. Meanwhile, the CPE is the non-ideal capacitive response at the biofilm-electrode interface which is due to surface defects and the distribution of charge relaxation times, and has a considerable effect on the frequency response in impedance analysis. Rct with CPE produces a typical curve in the Nyquist curve, and Warburg contributes a slope at low frequencies. Low Rct systems with high conductivity will have a small arc with a small slope angle as is observed in the *B. subtilis* MFC.

The experimental findings generally indicated that the maximum power of the MFC with *B. subtilis* was ±1754 mW/m² on 4th day which was 3.5 times higher than the *E. Coli* system that was only able to produce a maximum of ±491 mW/m². This growth shows that the efficiency of the electron transfer, ionic conductivity and energy conversion in the *B. subtilis* system was higher. On the other hand, the Rct and ionic conductivity were very high, and the primary challenge in the *E. coli* system was these characteristics. Hence, *B. subtilis* exhibited better electrogenic properties as a result of conductive biofilm and high metabolic activity synergy that enhanced optimal MFC activity. The *B. subtilis*-based MFC recorded power output of approximately 3. 5 times higher than the output of the *E. coli* system, which can be attributed to the different extracellular electron transfer (EET) systems of both systems. Based on the electron transfer mechanism, *B. subtilis* mediated electron transfer (MET) pathway is used mainly, involving endogenous redox-active compounds such as flavins, to transfer electrons between the bacterial cells and the anode surface. These redox mediators enhance the mobility of electrons at biofilm-electrode interface and reduce the resistance to charge transfer as is demonstrated by the large redox peaks and enhances current response in the CV analysis. Conversely, *E. coli* is not electrogenic in nature and does not have an efficient EET pathway and its ability to transfer electrons is largely limited to weak direct contact or non-redox-active byproducts of metabolism and is mainly capacitive without discernible redox properties. Also, the biofilm of *B. subtilis* has a higher electrochemical activity because of its highly compact structure and its effective contact with conductive PVA/CQD matrix that contributes to adhesion of the biofilm and electron transfer. Conversely, the *E. coli* biofilm has a more of protective biological role than electroactive. These variations in EET systems explain why *B. subtilis* in the MFC system exhibited much better electrochemical performance.

3.6 MFC Performance Metrics

In this work, electrical characteristic of a MFC system under five days operating with the external resistance (*Rext*) of 100 ohms and with an area of electrode (*Aelectrode*) of 9.45 cm² (0.000945 m²) were studied. Figure 7 displays that the *B. subtilis* based MFC system exhibited a high voltage relative to 128 mV on the 1st day to a peak of 407.2 mV on the 4th day and then declined to 329.1 mV on the 5th day. This trend means that the electroactive biofilm on the anode was formed, thereby enhancing the efficiency of the electron transfer (Ren *et al.*, 2021). There was a peak power density of 1754.62 mW/m² on the 4th day, but it rose dramatically on the 1st day, then fell to

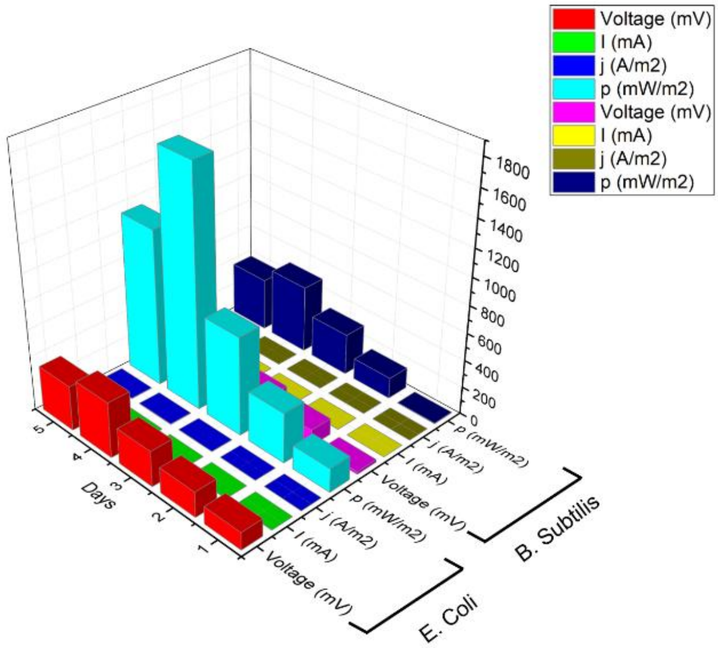

Fig. 7 MFC *B. subtilis* and *E. coli* performance

1146.34 mW/m². The MFC system also reached its maximum power density in the fourth day, but the electrical output was relatively steady until the expiry of the five days of operation. No unexpected voltage drops or failures of current occurred indicating the anode -electrolyte interface was electrochemically stable throughout this short term operation. The minimal difference in the performance seen after the peak power density could be attributed to the progressive depletion of easily biodegradable substrates and the growing mass transport resistance in the growing layer of biofilm. These results demonstrate that the system maintained the current generation during the experimental time, and it reflects the stability of short-term performance of the system that is relevant to practice in the field of MFC.

Such a reduction may probably be caused by the low levels of substrate, pH changes, or other microbial-related activities in the batch system. It can be assumed that the high performance of *B. subtilis* could be explained by the capacity of this bacterium to create a solid biofilm on the nanocomposite anode surface. The biofilm is known to directly transfer the electrons to the anode and the conductivity of the CQDs and the biocompatibility of the PVA are known to increase this process. Also, *B. subtilis* is reported to possess metabolic pathways that facilitate the generation of redox metabolites that enhance an increase in electrochemical activity (Nimje *et al*., 2009).

In MFC with *E. coli* case, the starting voltage on the 1st day was 0.0203 V with a power density of 4.4 mW/m² as shown in Figure 7. The voltage grew slowly until on the 4th day it peaked at 0.2155 V; the power density was 491 mW/m². On day 5, voltage went down to 0.1903 V and power density to 383.4 mW/m². Even though *E. coli* tended to exhibit a similar increasing trend as *B. subtilis*, its overall performance was lower, in terms of voltage as well as power density. The lower performance of *E. coli* may be due to its more limited electron transfer capability compared to *B. subtilis*. *E. coli* tends to rely on external mediators for electron transfer, and although the nanocomposite anode is designed to increase conductivity, the interaction between *E. coli* and the anode surface may be less optimal. In addition, the composition of sugarcane juice as a substrate may be less supportive of *E. coli* metabolism compared to other substrates such as organic waste.

B. subtilis consistently demonstrated superior performance compared to *E. coli* as demonstrated on Figure 8. The maximum power density achieved by *B. subtilis* reached 1754 mW/m², approximately 3.5 times higher than that of *E. coli*, which only reached 491 mW/m². The peak performance of both bacteria occurred on 4th day, indicating that optimal conditions for metabolic activity and electron transfer were reached at that point, before declining on 5th day. This superiority of *B. subtilis* is likely due to its ability to form a better biofilm, allowing for closer contact with the anode surface and increasing electron transfer efficiency. Furthermore, *B. subtilis* appears to be more adaptable to the sugar–rich sugarcane juice substrate, which supports metabolic processes and electricity production. Conversely, although *E. coli* is capable of generating electricity,

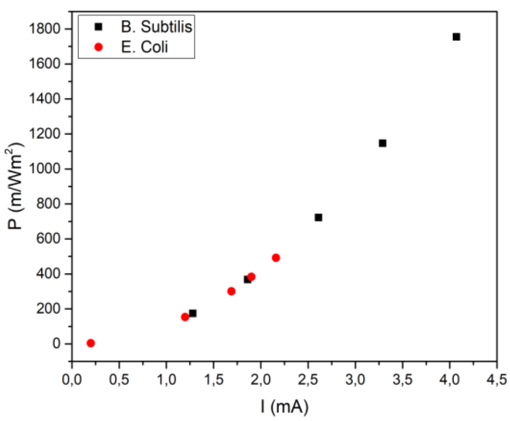

Fig. 8 Power and Current resulted from Microbial Fuel Cell

its lower performance is likely related to its reliance on indirect electron transfer mechanisms and its less than optimal adaptability to the nanocomposite anode and the type of substrate used. Compared with the literature, the maximum power density of 1.75 W/m² is within the competitive range. Studies on MFCs with *B. subtilis* for domestic wastewater treatment in the laboratory generally only produce 420–460 mW/m² (Obileke *et al*., 2021). Studies on MFCs with *E. Coli* for organic wastewater treatment in the laboratory generally only produce 11.7 mW/m² (Montoya–Vallejo *et al*., 2023) , much lower than the 491 mW/m² achieved in this study. Therefore, these MFC results reflect efficient performance at the laboratory scale with standard configurations and show great potential for improvement through further optimization.

4. Conclusion

This study reports on the design, fabrication, and performance evaluation of a novel MFC featuring a nanofiber–coated carbon anode prepared via electrospinning of PVA embedded with CQDs. SEM, FTIR, and UV–Vis analyses confirmed both the homogeneous incorporation of CQDs and the successful colonization of the nanofiber matrix by electrogenic bacteria. Electrochemical characterization comprising CV and EIS revealed that the CQD–enhanced nanofiber anode substantially lowered charge–transfer resistance and improved electron-transfer kinetics. When operated with sugarcane juice as the substrate, the *B. subtilis* based MFC achieved a maximum power density of 1,754 mW m^{-2} on the 4^{th} day of operation, representing a 3.5–fold increase relative to the *E. coli* system (491 mW m^{-2}). The markedly superior output of *B. subtilis* is attributed to its capacity for robust biofilm formation, secretion of endogenous redox mediators, and efficient metabolism of the complex organic substrate. These findings highlight the synergistic effect of nanofiber–coated CQD anodes and *B. subtilis* inoculation in advancing the efficiency and sustainability of MFC technology for organic-waste–derived power generation.

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