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Application of Low-Cost Plant-Derived Carbon Dots as a Sustainable Anode Catalyst in Microbial Fuel Cells for Improved Wastewater Treatment and Power Output

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Abstract: Microbial fuel cells (MFC) can generate electric energy from wastewater which can be enhanced further by anode catalysts. The recovery of electrons produced by oxidation of organics catalyzed by bacteria in the anode was enhanced when carbon dots(CDs) were added into the MFC. In this present study, a novel strategy for designing anode material and the fabrication of a high-efficient and environmentally friendly anode for energy generation from wastewater was reported. The CDs were synthesized by the pyrolysis of a peanut shell at the temperature of 250 °C for 2 h with a heating rate of 10 °C min^{-1}. Thus synthesized CDs were characterized by transmission electron microscopy (TEM), UV/Vis spectroscopy, and fluorescence spectroscopy. The TEM analysis showed morphology with an average size of 1.62 nm. The UV/Vis absorbance of the CDs shows a wide absorption band without a characteristic peak. The excitation spectrum of CDs recorded at the emission wavelength of 440 nm exhibits a peak around 320 nm. CDs were investigated as an anode material in a MFC utilizing acetate as the organic substrate. The average chemical oxygen demand (COD) removal in closed circuit operation mode was 89%. The maximum power density production (7.2 W/m^3) was observed in MFC containing 1 mg/cm^2 CD-impregnated anode (CDsIA). The CDsIA provides the ability to promote efficient biofilm formation. These results emphasize the application of CD-based electrodes in MFCs for the simultaneous treatment of wastewater and electricity generation while also providing additional benefits.

Keywords: carbon dots; anode catalyst; power density; microbial fuel cells

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1. Introduction

Microbe-catalyzed electrochemical systems, widely known as microbial fuel cells (MFCs) can metabolize organic compounds in wastewater and recover the resulting electrons for bioelectricity production [1]. In MFCs, anode chamber with bacteria and substrate (usually sewage sludge) under anaerobic environment promotes electron uptake by the anode due to sole available electron acceptor and redox potential of electrode employed [2]. MFCs offer a unified approach to wastewater-related environmental problems worldwide [3]. MFC technology is a promising technique for generating an electric current from different materials, such as natural organic matter and complicated organic waste, and can be advantageously integrated with wastewater treatment applications [4–6]. The capacity of certain electroactive bacteria to transport electrons extracellularly as part of their

metabolism close to a solid electrode is crucial to the operation of these novel systems. At the anode chamber, microorganisms oxidize the organic substrates, releasing electrons, protons, and carbon dioxide. The electrons produced by microbial metabolism are transferred to the anode surface by redox-active proteins or cytochromes and then carried toward the cathode via an electrical circuit [7,8] within the cathode chamber, and electron reduction occurs. An electron acceptor, such as oxygen or ferricyanide, is typically provided at the cathode. At the cathode, electrons combine with protons and oxygen to produce water. MFCs have certain distinctive properties that make this technology more advantageous than others. (1) The MFCs have a relatively superior ability to convert chemical energy into electric current. (2) MFCs can be operated at varying temperatures (from 20 to 40 °C), distinguishing them from other bioenergy approaches. Consequently, designing and developing efficient anode materials while maintaining outstanding biocompatibility and remarkable stability in the presence of microbial inoculum combinations and remarkably low electron transfer resistance has garnered an increasing amount of interest as potential solutions to the problem [9]. The performance of an MFC is determined in large part by the number of electrons transferred from the bacteria to the anode [10]. Scaling up of MFCs for use in practical applications in the real world has been hampered by the inefficiency of their electron transfer from the bacteria to the anode electrode [11]. It is possible for direct electron transfer to take place between the bacteria and the electrode through electron mediators that are either naturally produced or chemically introduced [12]. Therefore, the need to understand the mechanism of electron transfer from the tiny, insoluble molecules that serve as electron shuttles and facilitate electron transfer in MFCs is absolutely necessary [13].

Therefore, carbon-based materials such as lignocellulosic materials are important as they can boost the MFC's energy output. Hence, carbon felt, carbon paper, carbon brush, and graphite are widely employed as anode materials. CDs are composed of discrete, quasi-spherical nanoparticles and are a newly developed type of carbon nanomaterial. In 2004, they were identified as components of luminous nanoparticles during the purification of single-walled carbon nanotubes [14]. Prior to 2006, these carbon nanoparticles were referred to as "carbon quantum dots" by Sun et al., who described a method to produce CDs via simple surface passivation and chemical modification for boosting fluorescence emission [15].

Consequently, CDs have gained extensive and considerable attention owing to their unusual structure and fascinating properties in various disciplines [16–18]. As a novel carbon allotropes, CDs have numerous outstanding features, including reduced cytotoxicity, good biocompatibility, sustained chemical inertness, excellent light harvesting, and exceptional photoinduced electron transfer, making them promising material for numerous applications in biosensors, bioimaging, and optoelectronic devices. For instance, Thapa et al. have outlined the anode properties that impact electron harvesting and utilization [10]. The anode conducts the electrons generated by oxidized substrates and offers a surface for microbial growth. Because of their adaptability, conductivity, and biocompatibility, carbon dots (CDs) are one of the best choices of material as anode [19]. Carbon nanomaterials come in various forms and some have been employed as an anode in microbial fuel cells (MFCs) to boost efficiency [20]. However, their electron transfer efficiency is often rather low. The efficiency of an MFC relies on the microorganisms' ability to transfer electrons to the anode. Limitations in electron transfer have hampered efforts to commercialize MFCs in a significant manner [21]. Therefore, altering carbon-based anodes to improve microbial immobilization, lower the electron transfer barrier, and boost MFC energy production emerges as a clear scope of work.

Addressing these concerns, the present study demonstrates how CDs synthesized from a green technology, e.g., peanut shell, could be a promising new material for overcoming the abovementioned limitations and can help develop MFCs with higher efficiency. In this study, the graphite sheet anode of MFCs was modified using different concentrations of CDs (Figure 1). These anodes were used in MFCs to compare their performance to MFCs that used a raw graphite sheet as an anode. In order to develop a replacement anode

composition that might boost MFC performance, this study also investigated focused on whether switching the anode with impregnated CDs could increase MFC power density (PD) at a reasonable cost.

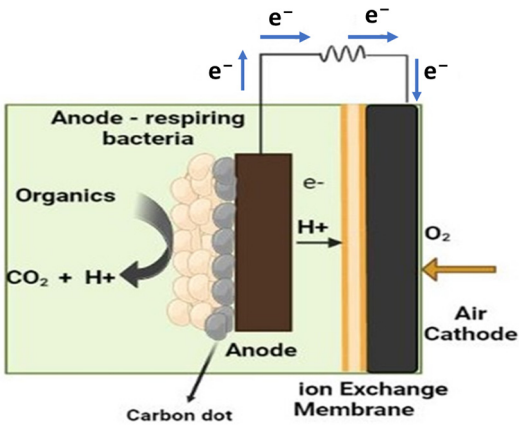

Figure 1. A schematic diagram of the MFCs using CDs as a catalyst on an anode.

2. Results

2.1. Characterization of Carbon Dots (CDs)

The morphology and structure of the CDs are characterized by TEM. Figure 2a shows the TEM images of the synthesized quantum dots, which show spherical morphology with a smaller diameter of CDs in the size range of 1.8–3 nm (shown in the yellow circle). Figure 2b shows the histograms of the particle size distribution of CDs obtained from the TEM micrograph, which shows the CDs are of a mean diameter of 2.47 ± 0.3 nm size with a rather broad range from about 1.8 nm to 3 nm. Figure 2c shows the DLS technique-derived size distribution profile of carbon nanodots. The hydrodynamic diameter of CDs is measured to be about 2.7 nm, as shown in Figure 2c.

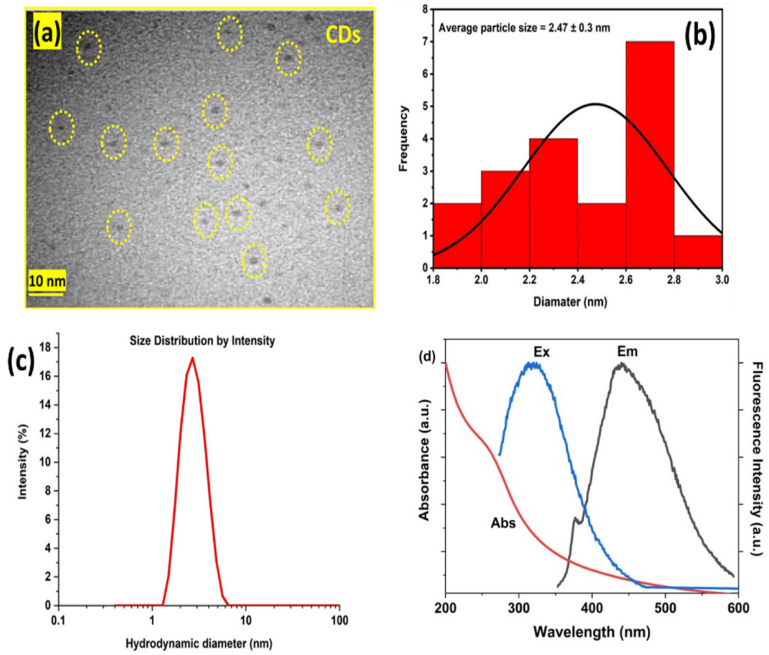

Figure 2. (**a**) TEM image of the CDs; (**b**) particle size distribution histogram of carbon nanodots obtained from TEM micrograph; (**c**) dynamic light-scattering spectrum of carbon nanodots; (**d**) fluorescence spectra and UV/Vis absorption spectrum of the obtained CDs. The emission spectrum was recorded at the excitation wavelength of 320 nm; the excitation spectrum was recorded at the emission wavelength of 440 nm.

Further, the optical properties of as-prepared CDs are investigated using UV/Vis spectroscopy and fluorescence spectroscopy techniques. When characterizing CDs in UV absorption spectra (Figure 2d, red curve), CDs show a broad absorption spectrum, possibly because of the relatively broad size distribution of the CDs. The absorption of CDs in UV absorption spectra corresponds to the π-π* transition of the aromatic C=C bond, suggesting the formation of CQDs with more aromatic sp^2 domains in the CDs sample. When the CDs are excited at 320 nm, a strong fluorescence light around 440 nm is observed, confirming the successful synthesis of the CDs. The emission may be ascribed to the presence of abundant surface defects in the synthesized CQDs.

The fluorescence spectra of the CDs under the excitation of different wavelengths of light (340–370 nm) are shown in Figure 3. The CDs fluorescence emission depends on the excitation wavelength. This excitation-dependent phenomenon is due to the difference in particle size and the distribution of diverse emissive trap sites of the CDs.

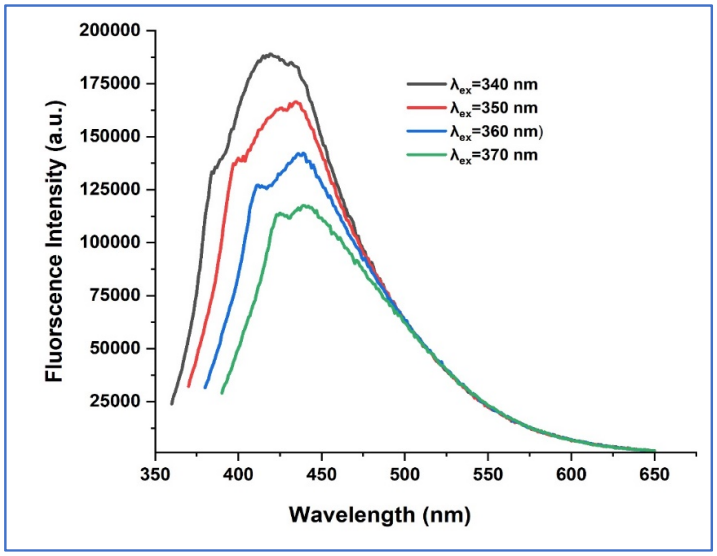

Figure 3. Fluorescent emission spectra of CDs at different excitation wavelengths in the 340–370 nm range.

The Raman spectrum of CDs (Figure 4) displays two bands at 1377 and 1634 cm^{-1}, representing the D and G bands of CDs, respectively which indicates that CDs were composed of a graphitic sp^2 carbon atom and sp^3 carbon defects.

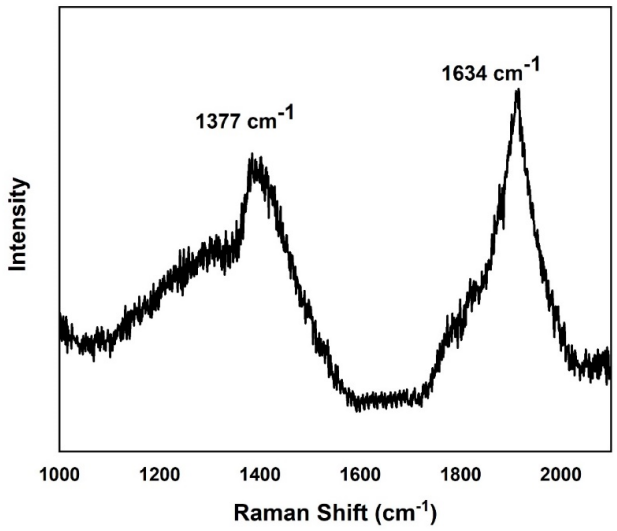

Figure 4. Raman spectrum of CDs.

The surface state of the CDs is confirmed by XPS measurements (Figure 5), which showed three typical peaks at 532, 400, and 286 eV in the XPS spectrum of the CDs, representing the O1s, N1s, and C1s, respectively.

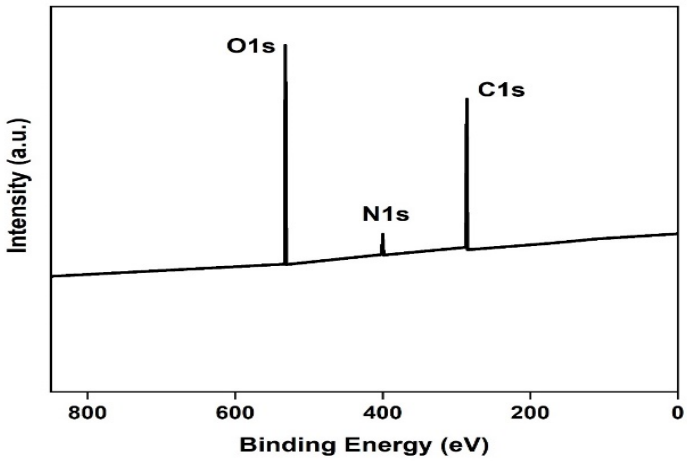

Figure 5. XPS spectrum of the CDs.

2.2. Power Generation

The polarization curve is used to determine the maximum volumetric power density (PD_{max}) in MFC (Figure 6a). At the same time, the viability of anodic biofilm and constant voltage production also need to be maintained. Thus, the batch period was ~36 h with a pH value of 7.0 ± 0.1. The effect of carbon dots with a graphite anode on the power output in MFC is studied using different concentrations including 0, 0.25, 0.5, 0.75, and 1.0 mg/cm^2 carbon dots impregnated on graphite anode. A significant variation in the anodic half-cell potential is observed with different concentrations of CDs in the MFC (Figure 6b). The anode impregnated with 1.0 mg/cm^2 CDs containing graphite produced a maximum volumetric power density (PD_{max}) of 7.2 W/m^3. On a contrary, with the introduction of 0, 0.25, 0.5, and 0.75 mg/cm^2 of carbon dots in graphite anode, the PD_{max} has significantly increased to 2.9, 3.6, 5.7 and 6.9, W/m^3, respectively. Increasing the carbon dots' concentration from 0 to 0.25 mg/cm^2 leads to a nearly two-fold enhancement in the magnitude of PD_{max}. However, increasing it further from 0.75 to 1.0 mg/cm^2, the improvement in PD_{max} is only 4.3%. The maximum Coulombic efficiency (CE) and COD removal efficiency are observed to be increasing, whereas the internal resistance decreases with the increasing CD concentration (Figure 7a). It can be stated that the supplementation of CD content in the anode plays a significant role in power output in the MFC.

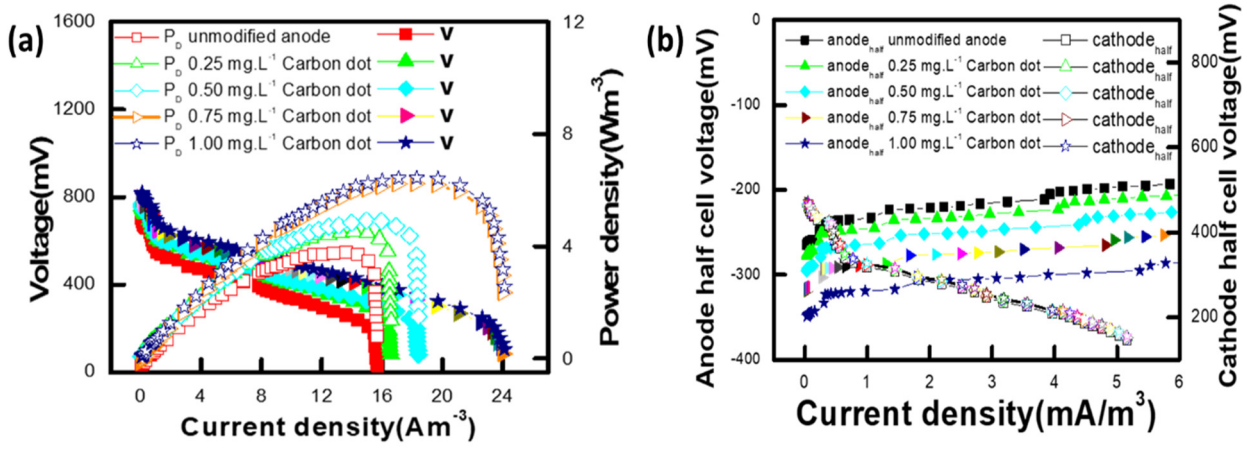

Figure 6. (**a**) Polarization plots for MFCs (power density and D.C. voltage as a function of current density) with different concentrations of (1 mg/cm^2 to 0.25 mg/cm^2) CD loaded anode (full-cell and

half-cell). The power density and voltage data points are presented as solid and open symbols. The power density and voltage data points are presented as solid and open symbols. (**b**) Anode and cathode polarization curves of sMFC with different concentrations of carbon dot supplemented to the anode surface. Open and solid symbols represent cathode and anode half-cell voltages, respectively.

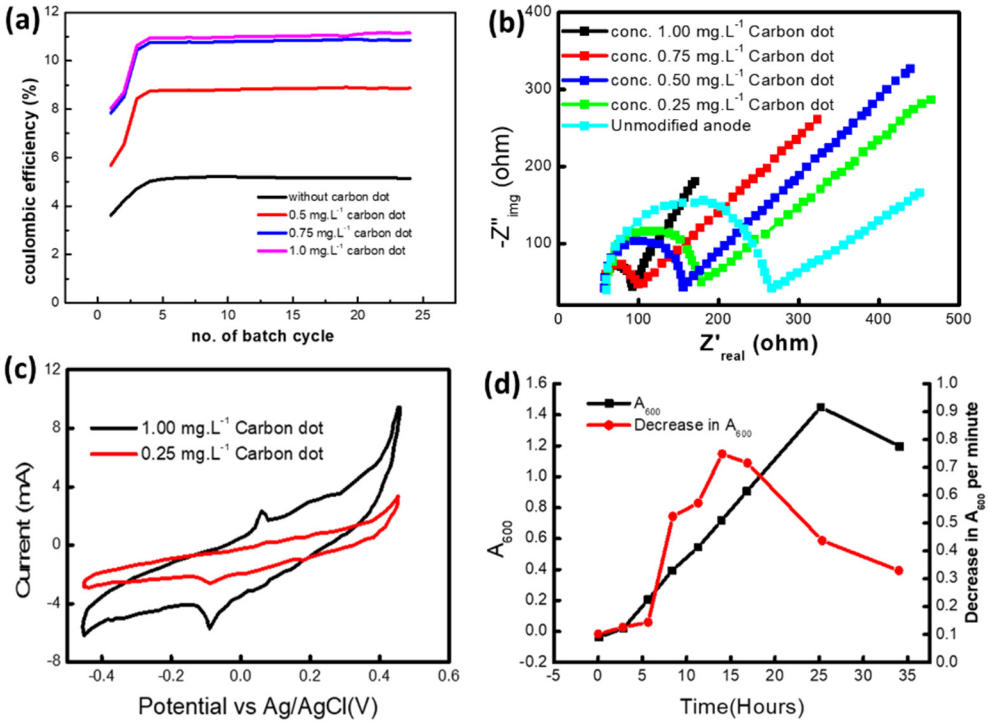

Figure 7. Showing Coulombic efficiency (**a**), impedance spectroscopy (**b**), cyclic voltammetry (**c**), and DREAM assay (**d**) of MFC reactors. CE of the reactor increased with the increase in CD concentrations in the medium. EIS, CV, and DREAM also showed similar observations, where an increased concentration showed higher activity and better performance.

2.3. COD Removal and Coulombic Efficiency

Wastewater treatment is one of the key large-scale applications of MFCs. Thus, the MFC batch operation is monitored for COD elimination (Figure 7). Over 25 cycles (38 days) of service, MFCs with anode chamber having different concentrations of CD displayed up to 89% COD withdrawal with 1.0 mg/cm^2 carbon dots in MFC. The CE gives the number of electrons recovered as current versus that initially present in the biodegradable matter of the anolyte in MFC under close circuit mode at a particular external resistance (R_{ex}), Figure 7a. The maximum CE of 10.9% is achieved with 1 mg/cm^2 of CD anode this is followed by 10.78% for CD loading of 0.75 mg/cm^2. The next highest CE is 8.8%, obtained for CD loading of 0.5 mg/cm^2. These CE values are found to be higher thaan the bare anode which showed 5.2%.

2.4. Electrochemical Impedance Studies

The anode's impedance plot presents a significant variation in the semicircle region (Figure 7b). The R_{ct} values of the MFCs exhibit the following order: MFC having anode—without carbon dots (272 Ω) > 0.25 mg/cm^2 CDs (171 Ω) > 0.5 mg/cm^2 CDs (152 Ω) > 0.75 mg/cm^2 CDs (113 Ω) > 1.0 mg/cm^2 CDs (106 Ω). The minimum R_{ct} value observed in the anode in the presence of 1.0 mg/cm^2 carbon dots in MFC indicates the maximum electron transport due to high substrate oxidation. This process, in turn, increases the anodic voltage losses and improves the current generation. The EIS results also support the results of the half-cell polarization study. The results indicate that the major reason for

high internal resistance in MFCs without carbon dots is the inefficient extracellular electron transfer from EAB biofilm at the anode surface.

2.5. Cyclic Voltammetry Investigation of Anode

The bioanode (Working Electrode), Pt wire as counter and Ag/AgCl electrode (Reference Electrode) was placed into the anolyte containing acetate medium. The scanned rate was at 2 mV s^{-1} using a potential window of −0.5 V to 0.5 V (Figure 7c). A distinct oxidation (0.1 V) peak were observed with 1 mg/L CD, while 0.25 mg/L CD did not showed noticible distinct oxidation peak. This might be due to the influence of CD which facilitate electron tranfer during acetate oxidation of EABs on the anode surface, where active electron shuttling is observed with an increase in CD concentrations.

2.6. DREAM Assay Evaluates the Electron Transfer Activity of Microbial Cultures

The DREAM assay is a general test developed to quantify microbial electron transfer activity quickly, cheaply, and easily. It has been transformed into a screening technique for detecting antibiotic resistance and evaluating MFC inocula for bioelectrical activity (Figure 7d). MB is a colourigenic redox dye used for a long time as a bacterial activity indicator. MB is employed as the terminal electron acceptor in the DREAM test, and electrons produced during microbial nutrient oxidation convert it to a colorless leuco form. The DREAM assay coefficient, a measure for calculating methylene blue reduction, shows how active the microbes are in transferring electrons within a particular sample. Cultures show a better capacity to convert MB to its colorless counterpart when CDs is included on the bioanode and there was active interaction with anolyte. These bioanode comprised electroactive bacterial (EABs) populations. Such populations oxidize substrate which act as an electron donor (Figure 7d). However, EABs grown without CDs on anode possess a 27% lower DREAM coefficient. This fact suggests that such EABs have a lower capacity to decrease the MB dye.

3. Discussion

A DREAM assay was carried out to determine the electron transfer activity of microorganisms [22]. Even though the CDs possessed an inherent reduction capability, they could not independently decolorize the methylene blue dye. The assay results revealed that the mixed culture of microorganisms utilized in this investigation contained electron transfer activity, which was then improved upon by the inclusion of CDs. This improvement was brought about because of the role that CDs played in acting as electron shuttles between the bacteria and the dye. During the DREAM test, a competitive electron acceptor with a high redox potential, such as oxygen, has been found to previously limit electron transfer to the dye. This was reported by a similar study [23]. The small size of the conducting CDs and the existence of functional groups on their surface that may either draw or donate electrons made them ideal choices for electron shuttles. The role of functional groups in surface passivation enabled their usage as a stable dispersion in an MFC anode chamber.

CDs of the nanoscale size provided a large surface area for electron transport in the anode chamber. CDs have been utilized as reducing agents for the synthesis of gold nanoparticles and nanocomposites in the past [6]. It has been shown that CDs that are abundant in hydroxyl groups have a reducing property, but CDs that are abundant in carboxyl groups are known to facilitate electron transport by creating strong hydrogen bonds with bacterial cytochromes [23]. The movement of electrons from the microorganisms to the anode is the fundamental process that drives MFCs. In MFCs, the role of mediator or electron shuttle has been played by molecules of a small size that undergo reduction and oxidation with relative ease. Since the revelation of the direct transmission of electrons by bacteria to the anode of an MFC, research on electron mediators in MFCs has not inspired much attention from the scientific community [24]. Only those types of microorganisms that do not rely on foreign electron shuttles or mediators have attracted the attention of the MFC research community [25]. In addition, artificial mediators have not been favoured

because of the instability and toxicity associated with them. The demonstration of CDs as electron shuttles in this study is not intended to serve as a prerequisite for electron transfer; rather, it boosts performance efficiency. In addition, the CDs utilized in this investigation are biocompatible and did not inhibit the expansion of the microbes in any way [26]. CDs' dispersion in the anode chamber and continuous stirring of the anolyte improved electron transfer by enhancing anolyte conductivity and facilitated electron collection from the anode chamber. CDs' higher surface area reduced activation and Ohmic overpotentials. The electron harvest from a mixed microbial culture in an MFC increased in the presence of CDs. The CDs increase the conductivity and stimulate the cells to secrete many electron shuttles, which helps improve the effectiveness of the bacterial extracellular electron transfer (EET).

Although significant progress has been made in the study of carbon dots (CDs), there are still major obstacles that prevent the commercial use of CDs, including the complexity of large-scale synthesis, purification, low quantum yield, lack of clarity in structure-property correlation, electronic structures, and photophysics. CDs are potential nanomaterials for use in various settings owing to their unusual features, such as their biocompatibility, great photostability, outstanding light-harvesting, up-conversion, efficient electron transport, and shrinking bandgap. Based on resource recovery, microbial fuel cell technology has recently received much attention in the scientific community. These MFCs were promoted as a sustainable, non-conventional answer to the current energy issue. Even though this system has been the subject of extensive study ever since its inception, it has failed to meet its economic potential because of several practical and conceptual obstacles. There are often three obstacles to overcome when bringing new technology to market: price, efficiency, and user acceptance. These statistics suggest that MFCs' high price, poor power production, limitations/losses, and lack of stability pose the greatest challenges to their commercialization and widespread use. Owing to irreversible losses, the actual voltage output of an MFC is lower than the thermodynamically anticipated value (i.e., overpotentials). Activation losses, mass transfer losses, and ohmic losses have been identified as the primary limiting factors (losses) in MFC performance. The expense of key components, including current collector, catalysts, separator material, and electrode material, contributes significantly to the overall price of MFCs. The main technical obstacles to the mass production of MFCs include concerns over the stability and robustness. Neither the fuel cell costs nor the concerns of its durability, accuracy, and robustness are significantly reduced by investments in an MFC. Therefore, compared with other bioenergy technologies, fuel cells' high costs and technical concerns (such as robustness) provide the greatest barrier to their widespread adoption.

These bioelectrochemical systems can be used in the real world by reducing electrochemical losses, improving performance, and lowering costs. This research, using synthesized CDs produced from ground nutshells as electron shuttles, is a promising first step in this area. The study's findings could lead to a deeper understanding of how carbon nanostructures influence microbial electron transport. Table 1 shows CDs' enhanced properties compared with other carbon materials.

Table 1. Previous studies and literature where other carbon materials have been used.

Electrode Material	Size	Bacterial Source	MFC Type	Power Output (mW/m^2)/(W/m^2)	References
Carbon brush	4 cm by 3 cm dia	Pre-accumulated bacteria from active MFC	Cube air cathode	(2400)/(2.4 W/m^2)	[27]
Graphite plate	155 cm^2	*Shewanella oneidenis* ($MR-1$)	Two-chamber air cathode	(1410)/(1.41 W/m^2)	[28]
Activated carbon cloth	1.5 cm^2	*D desulfuricans strain*	Single-chamber air cathode	(0.51)/(0.00051 W/m^2)	[29]

Table 1. *Cont.*

Electrode Material	Size	Bacterial Source	MFC Type	Power Output (mW/m^2)/(W/m^2)	References
Carbon mesh	7 cm^2	Pre-accumulated bacteria from active MFC	The single-chamber cube air cathode	(893)/(0.893 W/m^2)	[30]
Carbon dot	-	-	Single-chamber air cathode	5.2 W/m^2	This study

4. Materials and Methods

4.1. Preparation of Carbon Dots (CDs)

The overall procedure for the carbonization method of synthesis of CDs and the MFCs used in the study are shown in Figure 8. The CDs were synthesized by the pyrolysis approach using the peanut shell as the carbon source. In a typical procedure, pieces of peanut shell were placed into a ceramic crucible and carbonized under vacuum at the temperature of 300 °C for 2 h with a heating rate of 10 °C min^{-1}. After cooling to room temperature, the dark black products were mechanically ground into fine powders. Then, 0.1 g of obtained sample was dispersed by ultrasonication in 10 mL of distilled water to achieve a homogeneous solution. Finally, the CDs were collected by removing larger particles through a vacuum with a filtration membrane (0.22 µm of pore size). The obtained CD solution was kept at 4 °C for further characterization and use.

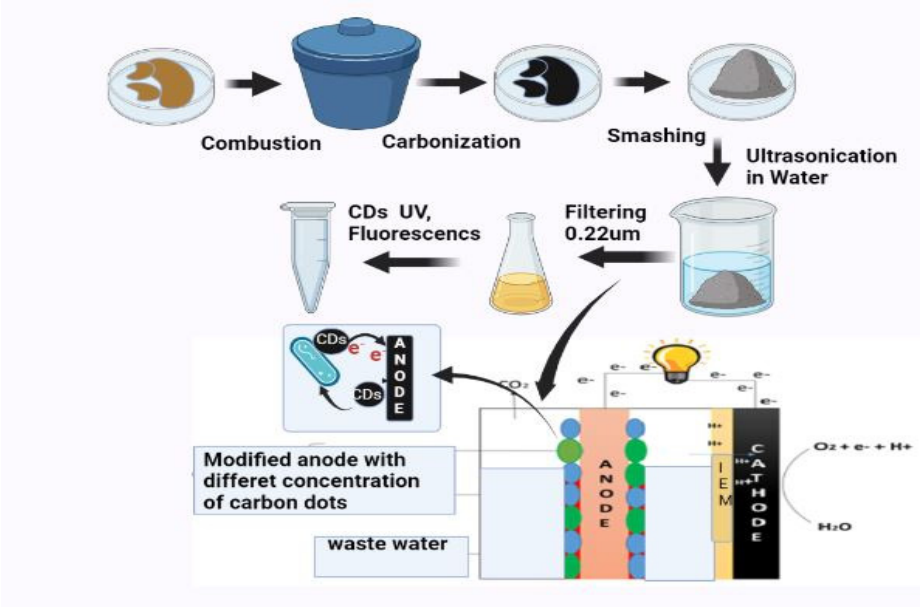

Figure 8. Schematic representataion of CDs synthesis and MFCs used in the study.

4.2. Characterization of Carbon Dots (CDs)

UV/Vis absorption spectrum was acquired using Shimadzu UV–1800 spectrophotometer (Shimadzu Analytical (India) Pvt. Ltd., New Delhi, India); photoluminescence spectrum was acquired using Varian Cary Eclipse Spectrophotometer (Agilent Technologies, Delhi, India). The sample's transmission electron microscopy (TEM) micrograph was obtained using an FEI Tecnai G2 S-Twin transmission electron microscope with a field emission gun operating at 200 kV (Denton, TX, USA). Dynamic light scattering (DLS) spectrum was taken using Nano ZS90, Malvern instrument (Malvern Panalytical Ltd., Malvern, UK). The X-ray photoelectron spectroscopy (XPS) was obtained with an ESCALAB250 XPS using Al Kα radiation (1486.6 eV). Raman spectrum was recorded using a laser confocal micro-Raman spectroscopy (InVia-Reflex). Sample transmission electron microscopy (TEM) micrography.

4.3. Fabrication of CDs@ Graphite Anode

The CDs were dissolved in 50 cc of deionized water using a titanium horn edge sonicator (Piezo-U-Sonic, Delhi, India). The composite mixture was sonicated for 3 h, transferred to 0.5 mL of 5% polyvinyl alcohol (PVA), then sonicated for an additional 30 min. Graphite sheet clumps were washed in 1 M HCl to remove any dirt or dust. The total contact area was around 9 cm^2 (3.0 cm by 3.0 cm). First, the pieces were sonicated in a tank of distilled water for 30 min to loosen any lingering particles; then, they were rinsed multiple times in a mixture of 35% ethanol and distilled water; finally, they were subjected to thermal treatment in a muffle furnace for more than 30 min at 400 °C. The CD-modified anodes were prepared by spraying different concentrations of sonicated CDs of the mixture mentioned above on a graphite sheet and kept for a whole night at 60 °C. The same method was used to create a raw graphite sheet anode without adding a CD to the solution.

4.4. MFC Construction

Nine identical MFCs were taken for the experiments. The MFC consisted of an anode compartment and a membrane cathode assembly (MCA) on opposite sides. The anode comprised cuboidal chambers of transparent polyacrylic material of outer dimensions $7 \times 8 \times 3.5$ cm^3 with 110 mL capacity. The anode chamber had two ports at the top, one for the electrode terminal and the other for the reference electrode (Ag/AgCl, saturated KCl; +197 mV, Equiptronics, Mumbai, India) and sampling. The anode consisted of a carbon cloth with a working surface area of 12 cm^2 with a stainless-steel wire welded to form the terminal. The membrane cathode assembly (MCA) was prepared by coating the membrane with a catalyst-loaded cathode. To prepare the cathode, conductive ink containing cathode catalyst (0.15 mg/cm^2 lab prepared manganese cobaltite nanorods ($MnCo_2O_4$-NRs) dust and carbon black Vulcan XC−72 (0.35 mg/cm^2; Cabot Corp.; Navi Mumbai, India)) were dissolved in 20 mL 1:1 acetone–isopropyl alcohol solution with 0.3 mg/cm^2 PVA (1% *w*/*v*) aqueous solution as a binder [31]. Ultra-sonication of the PVA-MnO_2-NRs-loaded carbon black aqueous-acetone solution was performed in 30 min and used as ink to spread on the cathode. Ink-containing cathode catalyst was sprayed on the preheated membrane and kept in the oven at 60 °C. The MCA was manufactured by bonding the carbon ink-coated anion exchange membranes (AEMs) directly onto a flexible stainless-steel mesh as a current collector. The AEM comprised polyvinyl alcohol (PVA) and poly diallyl dimethylammonium chloride (PDDA) [32].

Further, the SS mesh (8 cm^2) was attached to all of the membranes using an Ag conducting paint (Siltech corp., Bengaluru, India) on the air-facing side [1]. The SS mesh used in the present study was of SS–304 type with 50×50 openings per square inch. The wire used in the SS mesh had a diameter of 0.17 mm. It was connected with a concealed copper wire as a cathode terminal. Thus, the concealed copper wires connect the external resistance to close the circuit. The inter-electrode distance was kept constant at about 2.5 cm in all experiments. The anodes were placed equidistant from the MCA. The additional ports were sealed with clamped tubes to ensure an anaerobic environment. The MFCs were washed with 70% alcohol and placed in the UV chamber for 30 min before the experiment.

4.5. Anodic Mixed Consortia and Anolyte

Anaerobic mixed microbial consortia obtained from pH-neutral bottom sludge from a local septic tank were used as the parent inoculum. Dewatered anaerobic sludge (pH—7.4, VSS—25.54 g/L) was acquired from a septic tank's bottom and subjected to heat pretreatment procedures. Initially, the inoculum sludge was sieved through a 1 mm sieve. During the heat-shock pretreatment procedure, the anaerobic inoculum was subjected to heat treatment at 105 °C for 15 min. It was applied as the anodic inoculum.

Anolyte: Acetate medium was prepared using the composition prescribed by [33] and adjusted with a COD of 3000 mg/L. The resulting enriched culture was inoculated along with the feed. These MFCs were operated at about 37 °C under batch mode with repetitive cycles (each cycle = 36 h). In order to perform multiparameter optimization after selecting

suitable pre-treated inoculum, the initial anolyte pH was adjusted by weak acid or base solution to the desired values.

4.6. Performance Evaluation of MFCs

The COD values of the anolyte were measured using a COD measurement instrument set (TopLab Pvt. Ltd., Mumbai, India). The potential was measured using a data acquisition system (National Instruments, Bengaluru, India). Prior to the polarization study, the MFCs were kept in open circuit mode to let them reach the maximum voltage (Figure 1). Polarization curves were obtained by varying the external resistance of the closed circuit using a variable resistance box (range 90 kΩ–20 Ω) in discrete steps and measuring the corresponding voltage drop. The average time required for a stable reading was about 5 to 10 min. The current density, volumetric power density, and coulombic efficiency were calculated following earlier work [31,34]. A three-electrode configuration consisting of bioanode, Pt wire, and Ag/AgCl as the working, counter, and reference electrode, respectively, was used for electrochemical measurements. CV was recorded in the potential window of −0.5 to 0.5 V at a scan rate of 2 mV/s. Electrochemical impedance spectroscopy (EIS) of bioanode was performed with the same electrode configuration. Thus, the EIS was performed over a frequency range of 100 kHz to 1 Hz with a sinusoidal perturbation of 5 mV [32,35].

4.7. DREAM Assay for Microbial Extracellular Electron Transfer Activity in the Presence of CDs

At the log phase, each subculture (with and without CDs on the anode) was subjected to the DREAM (dye reduction-based electron-transfer activity monitoring) assay. A cuvette was filled with 2 mL of log phase culture. The culture was exposed to 0.2 L of methylene blue (MB), having a final concentration of 50 mg mL^{-1}, and the cuvette was closed with a lid. Next, the UV/Vis absorbance at 660 nm was measured for 3 min at 10 s intervals to determine the microbial electron transfer activity. Microbial electron transfer activity responsible for MB dye reduction was determined using the DREAM coefficient. As mentioned above, this is done by measuring the difference between the beginning and final absorbance readings at 660 nm during the three minutes.

5. Conclusions

In this study, the effects of anode material in MFCs were evaluated. The biocompatible zero-dimensional CDs supported by graphite sheets were manufactured as a novel MFC anode for high electricity generation. In addition, the electrode exhibited a lower barrier for transmitting charges. The maximum power density was 7.2 Wm^{-3}. Upscaling testing of efficient anodes is an area that needs significant attention in the future. An excellent membrane-based electrode assembly is crucial for real-world use, and developing an anode/membrane combination is crucial in this direction. However, current anode efficiency is inadequate for widespread commercial application. Research is needed to determine the best way to address the existing problems while also exploring the use of waste materials in the production of anode electrodes.

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