Comparative Electricity Generation by Two Locally Produced Corncob Pyrochar Electrodes and Graphite using Microbial Fuel Cell Technology

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Abstract

There are growing interests in microbial fuel cells (MFCs) for anaerobic bioenergy generation. MFC uses electrodes and organic wastewater as substrate for electrogenic bacteria to catabolize and generate power. Researchers in this discipline continue to be most interested in finding suitably affordable electrode materials. The focus of this study was on comparative bioelectricity generation from process water of hydrothermal carbonization (HTC) (pH = 5.99) and treated–biogas digestate (pH = 7.97) using locally developed corncob pyrochar electrodes and graphite in dual-chambered MFC. The electrodes used in this study were graphite rod (non-porous and very low surface area), potassium hydroxide (KOH)–activated corncob pyrochar (KAC) of Brunauer-Emmett-Teller (BET) surface area, 1626 m^2 / g and steam- activated corncob pyrochar (SAC) with 485.8 m^2 / g. Each electrode was separately tested in the MFC, some charged with HTC process water, and others with treated biogas digestate. An overnight culture of actively dividing cells of *Shewanella oneidensis* MR-1 (Electro-active bacterium) at logarithmic phase of growth was seeded into each of the anode chamber as inoculum. The anode chambers were sealed off to achieve anaerobiosis and the cathode chambers sparged continuously with air. The MFCs were operated for 30 d and results obtained were recorded. The highest power outputs achieved were 323.8 µW and 316.8 µW from HTC process water with SAC and biogas digestate with KAC electrodes respectively at an external load of 47 Ω. The initial Chemical Oxygen Demand (48780 mg / L), Dissolved Organic Carbon (4000 mg / L), and Total bounded Nitrogen (5600 mg L^{-1}) of the biogas digestate decreased significantly to 36405, 3610 and 4300 mg / L respectively in the MFC with KAC electrodes. A Coulombic efficiency of 75 % was recorded from the MFC operated with treated biogas digestate and KAC electrode in a significantly shorter residence time, making it more efficient than its counterpart with SAC electrode, which had a lower Coulombic efficiency of 64 %.

Keywords: *Microbial Fuel Cell, Electrodes, Wastewater, Electrogenic Bacteria, Bioelectricity*

1. Introduction

The demand for energy is generally increasing worldwide, meanwhile fossil fuels supply 80% of it. By 2050, it is predicted that global energy need is likely to have increased by a factor of two compared to current levels [1]. Furthermore, increased use of fossil fuels not only contributes to the depletion of limited natural resources, but also has negative environmental consequences, as seen by rising pollution levels and climate change around the world. As a result, major efforts are being made to produce bioenergy using alternative approaches based on renewable resources, which will result in considerable reductions in carbon emissions to the environment [2]. The development of environmentally friendly ways for waste management and remediation along with addressing the issues of climate change, is crucial for sustainable development [3].

To address these aspects, Microbial Fuel Cell (MFC) is an evolving bio-electrochemical concepts and emerging technologies employed. It principally produces electricity using a biodegradable organic substrate by anaerobic oxidation and gets the benefit of the energy produced by microorganisms and simultaneously providing the habitat to sustain their growth and metabolic activities [4] while the wastewater is being treated. In an anoxic environment, bacteria in the MFC's anode chamber breakdown and oxidize the organic substrate to electrons. These electrons first get delivered to the anode electrode before it flows via a conductive wire loaded with a resistor to the cathode. The protons generated, on the other hand, flow through a selectively permeable cation-exchange membrane to reach the cathode. This cation-selective membrane also limits oxygen crossover into the anode chamber. Simultaneously, in the cathodic chamber, chemical and/or microbiological reduction of electrons occurs, thus, completing the circuit and forming water [2].

The types of microorganisms involved and their metabolism, the MFC design (double or single chamber), the substrate type and its concentration, the type of electrode material, the anode type and operating conditions, the cathode type and pH buffer, as well as the proton exchange membrane are all factors that influence MFC performance [5, 6]. Wastewaters from various sources can be used as substrates in MFC ranging from wastewaters of domestic origin, biogas digestate (BD), to industrial wastewater like the brewery wastewater [7, 8, 9] as well as the process waters from carbonization processes [10]. Hence, MFC shows the potentials of treating wastewater by decreasing the Chemical Oxygen Demand (COD) with concomitant electricity generation [11, 12].

Anaerobic digestion (AD) of agro-industrial residues [13] and biodegradable urban wastes for energy production is becoming more popular every year [14]. Digestate is a heterogeneous solid–liquid by-product of the AD process that is produced in huge quantities [15]. It is ideal for organic soil addition due to its organic residual content [16]. It is however necessary to manage the digestate in a more sustainable manner by utilizing it as a substrate for further bio-transformation [17] such as MFC technology, and thermal transformation [18] such as the hydrothermal carbonization (HTC) [19, 20] in a framework of circular economy.

HTC is simply a wet thermochemical process that transforms source materials into carbonaceous biofuels at 180–250 °C and 10–50 bar in the presence of water [21]. Because of the water initially present, HTC treatment also generates some process water and both dissolved organic and inorganic salts are contained this process waters [22]. Process waters, just like the solid products, are substantially influenced by feedstock type and the set conditions of the HTC process [23]. They can either be reused into the HTC process or processed in other ways, like in microbial fuel cells.

Electrodes are considered important in the production of exo-electrogenic biofilms as well as the electrochemical reactions that improve MFC efficiency and performance. Large surface area, high electrical conductivity, cost-effectiveness, biostability, and biocompatibility are all characteristics of an excellent electrode [24, 25]. A thermal process known as slow pyrolysis (350-600° C, 1-30°C / min heating rate in the absence of oxygen) of biomass is used to produce pyrochar which upon physical or chemical activation [26] can be a potential electrode material with equal or better electrochemical properties than the fossil-based commercially available electrodes, most of which are expensive [27].

Shewanella oneidensis MR-1 used in this study is known to be facultative anaerobic bacterium since it can survive under both aerobic and anaerobic environments [28]. This offers *Shewanella oneidensis* an advantage over other highly electroactive bacteria such as the *Geobacter* species, which is an obligate anaerobe. *S. oneidensis MR-1* has been used for bioremediation in anaerobic contaminated environments, particularly with heavy metals such as lead, uranium, and iron [29]. It is also an excellent candidate for MFC, since it can breakdown organic matter and transforms the chemical energy contained therein into electrical energy. *S. oneidensis*-MR-1 transfer redox equivalents to the electron acceptor (anode electrode) through the cytochrome C proteins MtrC and OmcA located in its outer membrane [30]. Furthermore, *S. oneidensis* secretes electron shuttles compounds known as flavins that aid extracellular electron transfer (EET) [31].

The novelty of our research is the successful application of cheap and locally produced biobased electrodes of desirable properties and better performance than graphite electrode, in microbial fuel cells, this makes our research unique. This is simply because, most of these electrodes used in microbial fuel cell technology are cost prohibitive and a search for alternative becomes necessary.

2. Materials and Methodology

2.1 Electrode Materials and Electrode Production

Two sets of both the anode and cathode electrodes for both MFC experiments were prepared using a stainless mesh

of 50 x 30 mm dimension. A non-reactive binder, Polytetrafluoroethylene (PTFE) known as Teflon was used to bind the grounded activated pyrochar on the electrode. A grounded activated corncob pyrochar of 1.25 g from the work of Musa *et al*. [32], was mixed with seven milliliters (7 mL) of Teflon to make a semisolid mixture before it was evenly spread on both sides of the stainless-steel mesh. Millipore water was used to immerse the pyrochar-coated mesh for 5-10 mins after which it was allowed to dry at room temperature as also described by Musa *et al*. [33]. The electrodes for steam-activated pyrochar (SAC) and KOH-activated pyrochar (KAC) were separately prepared following the same procedure. On the other hand, a cylindrical graphite rod (0.00173 m^2) was used as control in both MFC experiments. An electrically conductive wire was then connected to each electrode for transport of electrons to the external circuit [34].

2.2 Substrates

Two different substrates namely HTC process water (HTC-PW) and biogas digestate (BD) were selected and used in this study. The HTC-PW used in this study was produced from Brewer's spent grains in a two-step-processing (150-160 °C for 1 h & 210-220 °C for 5 h) in a pressurized reactor, at an industrial scale plant located at Relzow, Germany. Approximately 60 L of the process water were homogenized and refrigerated at a temperature of 4 °C until required. Its chemical parameters before use are displayed on Table 1.

The BD used in the second MFC experiment was obtained from the secondary fermenter of biogas plant at the research station "Unterer Lindenhof" of the University of Hohenheim in Eningen unter Achalm, Germany. The BD resulted from mesophilic anaerobic digestion carried out using a seasonally varying composite waste including 48.8% silages (maize, ryegrass, triticale), grains (wheat, barley, oat, winter triticale) and roots (sugar beet) and 51.2% animal manure (swine, cattle, chicken, sheep). Approximately 5 L of the original digestate was collected in two clean plastic containers of 2.5 L capacity and refrigerated at 4 °C before use. The BD was pre-treated with 2 g L^{-1} of ammonium heptamolybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) and allowed to stand for 24 h.

2.3 Physicochemical and Analytical Methods

Physicochemical characteristics of the process water from HTC and the treated biogas digestate used in the two MFC experiments were determined using established analytical methods, including pH, total nitrogen bound (TNb), chemical oxygen demand (COD), dissolved organic carbon (DOC) and electrical conductivity (EC) [35].

2.4 Bacterial Inoculum Preparation

The electroactive bacterial strain used in this study was *Shewanella oneidensis* MR-1 [36]. Glycerol stock culture was inoculated onto freshly prepared analytical grade Tryptic Soy Agar (Merck Life Science GmbH) and incubated overnight at 32 °C. The actively growing colonies which appeared were scrapped off using a sterilized wire loop to prepare a bacterial suspension which optical density (OD) was determined photometrically (DR6000, HACH LANGE GmbH, Germany) before seeding into the anode reactor. *S. oneidensis* MR-1 naturally contains electrochemically active redox proteins on its outer layer such as the cytochromes or nanowires (pili) that aid in electrons shuttle from the bacterial cells onto the anode electrode [37, 38].

2.5 MFC Design and Configuration

The design, construction, and the experimental setup of the MFC were performed based on the following steps: two separate MFC experiments were carried out using graphite as control electrode in each case. The first experimental set up involves the steam-activated corncob pyrochar (SAC) electrode and process water of HTC as substrate. The second set of experiment involved the use of KOH-activated corncob pyrochar (KAC) electrode and biogas digestate as substrate.

First experimental set up

Two sets of dual-chambered MFC (experimental and control) were setup each having separate anode and cathode made up of a rectangular plastic of 1000 mL capacity. The anode and cathode chambers for each set were connected to each other through a valve and were partitioned on the valve by a special cation exchange membrane; Proton Exchange Membrane (PEM) called the Nafion® N-117 membrane of 0.180mm thickness (ThermoFisher GmbH) as also reported by Du *et al*. [39]. The protons generated passed internally through the PEM to the cathode [40]. The anode chambers of both sets were filled with 800 mL of HTC process water each (pH 6). A 4.75 mL of the freshly prepared culture of *Shewanella oneidensis* MR-1 (OD = 2.3 at 450nm) was then seeded into each anodic chamber as the inoculum. A potassium phosphate buffer of pH 7.0 (KH_2PO_4 6.309g + K_2HPO_4 9.344g per litre) was used to fill the cathodic chambers which were continuously aerated with air from an electric air pump [40, 41, 42]. The SAC electrode served as both anode and cathode in the experimental MFC set up, just as graphite (cylindrical area of 1.7281 x 10^{-3} m^2) in the control MFC set up. The anodic chambers were tightly sealed to maintain an anaerobic condition after connecting the electrodes with external circuit via conductive wires to the high-resolution PicoLog data logger. The data logger was connected to a notebook for continuous recording of the voltage produced on the Picolog6 software (Pico Technology Ltd., Cambridgeshire, United Kingdom).

Second experimental set up

The setup of the second experiment was done in the same way as the first experiment, except in the following ways: the substrate used in the anodic chamber was biogas digestate (BD), which prior to use was treated with 2 g L^{-1} of ammonium heptamolybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) and allowed to stand for 24 h to inhibit methanogenesis. This was to avoid competition for nutrient between the electrogenic bacteria added and methanogens

originally present in the biogas digestate. The anodic chamber was filled up with 800 mL of the treated BD pH 7.97. A 12 mL of overnight cultured *Shewanella oneidensis* MR-1 at exponential growth phase (Optical Density of 0.7 at 450nm) was seeded as inoculum into the MFC's anodic chamber. All other processes were the same as in the first experiment.

2.6 MFC operation and Voltage Generation

Electricity was generated by the catalytic microbial activities and the voltage produced was continuously recorded in the Picalog6 Software. The MFC was left opened for several days without applying any external resistance to record the Open Circuit Voltage (OCV).

2.7 Polarization Studies

After 48 h of "pseudo-stabilization" and seven days of OCV, polarization studies were carried out on both the experimental and control MFCs to determine the power produced during the first experiment. These measurements were recorded daily by separately applying external resistance of 4700 Ω, 2200 Ω, 1000 Ω, 470 Ω, 220 Ω, 100 Ω and 47 Ω and allowed to stay for five (5) min to achieve "pseudo-stable" state before taking the readings. The polarization readings were recorded for 21 d [43]. In the second experimental set up with KAC electrode, the MFC system was initially left open for OCV for nearly two weeks. The polarization studies were then carried out using the same resistance loads as in the first experiment. The polarization readings were first recorded for 8 d before 200 mL of the effluent was replaced with equal volume of the fresh substrate. This was then left open for 7 d of Open Circuit Voltage before polarization studies continued for another 8 d (Figure 10).

Ohm's law, [I = V / R; I *current* (Amps), V *voltage* (Volts) and R *external resistance* (Ohms)], was applied in determining the current *I*. The power law equation [P = I x V; V *voltage* (Volts)], was used to determine the power in Watt. The function of electrode surface area (m^2) was used to calculate power density (PD) in mW / m^2 and current density (CD) in mA / m^2.

2.8 Semi-continuous MFC Operation

During the MFC operation, 200 mL of the mid-operation effluent each from the first and second experiments were replaced with fresh HTC process water and treated biogas digestate respectively. This procedure adds more nutrients for the bacteria to establish more biofilms and to generate electricity. The pH, COD, and EC of all the effluents were measured.

2.9 Coulombic Efficiency (ε^c) of the MFC Systems

The ε^c for each MFC was obtained using Equation 1 [42].

$$\varepsilon^c = \frac{MI}{Fbq\,\Delta COD} \cdot 100\% \quad (1)$$

While F represents the Faraday's constant (96.485 C / mol), M is the oxygen's molar mass, q the volumetric influent rate is represented by q, b is the number of exchanged electrons per mole of oxygen (b = 4), and ΔCOD (g / L) is the difference in the fresh and last COD of the effluent.

2.10 Scanning Electron Microscopy (SEM)

Surface morphologies of all the electrodes used in this study were examined microscopically, before and after the MFC operation, under the Scanning Electron Microscope (SEM) (JSM-IT100). Pore sizes of the pyrochar before and after activation and the development of biofilms (crucial in transferring electrons to the anode) after the MFC operation were observed under the electron microscope and the SEM photomicrographs were recorded.

3. Results and Discussions

3.1 Substrates' Composition

For a better understanding of the role of substrate components in electricity generation, the consumption of the saccharides and the changes in carbon percentages of their degradation products like the aldehydes, ketones, organic acids, alcohols, and phenols were monitored initial, mid, and final operation of the MFC. HPLC analysis was used to calculate the Carbon (C) percentages illustrated in Figure 1.

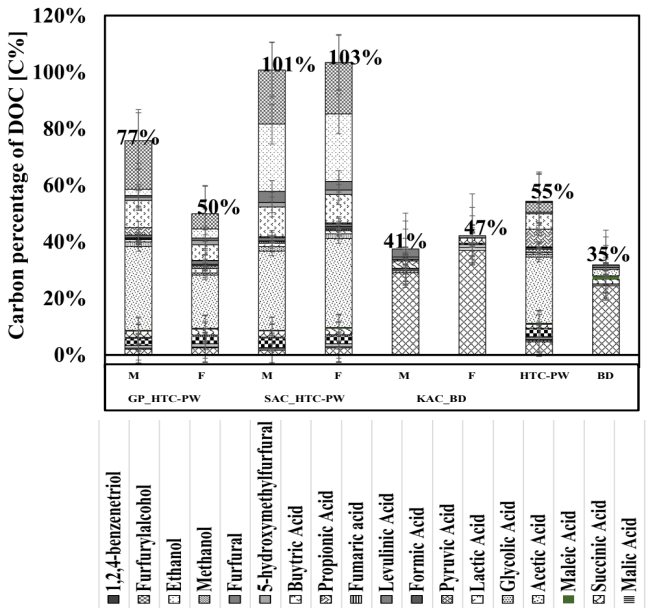

Fig. 1. Carbon percentages of detected organic components in the substrates used.

Key: Initial materials (IM); Biogas Digestate (BD) and its effluent after Mid-/Final-Operation (KAC-M/-F); Process water from Hydrothermal Carbonization of Brewer's Spent Grains (HTC-PW) and its Mid-/Final-operation effluent used with graphite electrode (GP-M/-F); Mid-/Final-operation effluent used with electrodes produced from steam-activated pyrochar (SAC-M/-F). "0%" means below the detection limit of 50-1000 ppm for sugars and 50-500 ppm for organic acids, aldehydes, ketones, and others.

Figure 1 depicts the HTC-PW composition as determined by HPLC analysis and displayed as C percentages related to DOC. More details on the reaction pathways of HTC which resulted in the displayed components in HTC-PW are described by Wüst *et al* [44].

During MFC operation, oligosaccharides were further decomposed to derivatives and saccharides such as sucrose, glucose, and fructose. Di- and monosaccharides were firstly consumed, mostly within 4-6 h, coupled with a simultaneous production of acetate, lactate, butyrate, propionate, fumarate, and formate then followed by succinate, maleate, malate, pyruvate, glycolate, levulinate production which are later consumed and metabolized by the electroactive bacteria with corresponding voltage increase during electricity generation. The changes are depicted on Table 1 and Figure 1.

Kiely *et al* [45]. reported that formate and lactate were mostly consumed again within about 25 hours by *Shewanella putrefaciens*, while *S. oneidensis* kept both C percentages to low final levels. According to Lamberg and Bren [46], lactate acted as self-produced mediator for electron transfer of *S. oneidensis* as well as *S. putrefaciens* [47]. Whereas, acetate as well as butyrate provided the basis for electricity generation, in comparison to acetate and lactate, formate consumption resulted in higher voltages but lower power densities [45]. Although, the C percentage of acetate dropped in the case of GP, the electricity production was quite low. This means that the bacteria were re-directing this component for their growth, but the electron transfer was weak because of poor biofilms production on the electrode due to a lower surface area. Whereas propionate obviously contributed to electricity generation by using a GP rod electrode, propionate kept on a comparable C percentage level by using S rod electrode after M and F operation. *Desulfobulbus propionicus* has also demonstrated significant electricity production using propionate [48]. Fumarate is an electron acceptor that can be incorporated into cells, and it has been shown that *Geobacter sulfurreducens* is not capable of utilizing fumarate as source of carbon or energy since the succinate produced from formate reduction is excreted into the medium, instead of being oxidized in the tri-carboxylic acid (TCA) cycle [49]. Fumarate is reduced using a membrane bound fumarate reductase complex, FrdCAB, that also functions as a succinate dehydrogenase for *Geobacter metallireducens* [50]. Since fumarate is internalized, it has a comparatively simple electron transport chain (ETC), and its role in respiration has been extensively studied with *Geobacter sulfurreducens* [51, 52].

In the second experiment where biogas digestate was used as a substrate, acetic acid concentration was naturally lower than that in HTC process water because of its prior utilization in the biogas plants and most of the nutrients were already consumed during the biogas production. The continuous reduction of acetic acid concentration from the initial concentration of 2.474 g L^{-1} to the mid-operation and then to the final effluent compared to the reduction in glucose indicated that part of the acetic acid might have been used in electricity generation as also observed by the study of Linke *et al.* [53]. In the work of Deval *et al*. [54], the role of acetic acid was studied by observing a spike in voltage generation on the addition of acetic acid into the substrate. This suggested the importance of acetic acid since it is used as the simplest source of carbon for electricity generation by the electrogenic bacteria.

Additionally, pure culture of *Shewanella oneidensis* was used as the inoculum in both cases, thus serving as the only bacterium in the MFC with HTC process water. However, there might have been a synergistic or augmentative effect between the *Shewanella oneidensis* and the naturally derived microbial consortia present in the biogas digestate. Though other factors need to be in considered such as the nature of the electrode used, a better performance from the MFC with biogas digestate therefore indicated the advantage of having a wide variety of microbial species carrying out different roles during electricity generation in the system. However, the amount of nutrients present in the biogas digestate is usually limited since it is from the final stage of biogas production and most of the nutrients most have been depleted prior to use in the MFC system: Therefore, the lower organic matter content observed was attributed to the source of the biogas digestate which was the secondary fermenter of biogas production. In this case, the methanogens, which were still present must have already utilized most of the acetic acid present and will continue to convert the residual acetic acid into methane. This account for the low concentration of acetic acid observed.

3.2 Physicochemical Properties of the Substrates

The physicochemical properties of the studied substrates including HTC process water and biogas digestate treated with ammonium heptamolybdate tetrahydrate are shown in Table 2. The digestate was pre-treated with ammonium haptamolybdate tetrahydrate to reduce or suppress the methanogenic activity. This compound is known to inhibit the growth of the methanogens, and there will be limited or no competition between the *Shewanella oneidensis* added and the methanogens originally present. This helps to avoid redirecting the residual carbon source to undesirable methane production. Interestingly, other bacteria which were also present may augment the role of the *S. oneidensis* in electricity generation or even work synergistically as previously explained.

The parameters include pH, EC, COD, DOC and TN_b. The pH increased in both the substrates after MFC compared to the original samples. The bacterial metabolic consumption of organic acids and transport of protons (H^+) to the cathode chamber increased the pH. According to Linke *et al* [53], the association between MFC performance and substrate pH values revealed that electroactive bacteria are

more tolerant of high pH. Lower pH causes poor performance of MFC and partial substrate decomposition. The EC values decreased in the mid-operation effluent and the final effluent compared to the original EC of the HTC process water and biogas digestate. The electrical conductivity of the wastewater is due to the presence of dissolved ions; therefore, as the microbes started utilizing them, the reduction of ions in the wastewater results in a lower EC, which ultimately suggests the proper bacterial metabolism in the MFC. The EC values increased slightly in the final effluents after MFC compared to the mid-operation effluent and this might be due to the addition of fresh substrate in the middle of the operation. In the first experiment, the initial DOC of 13,440 mg / L of the HTC process water reduced to 9,940 mg / L in the mid-operation effluent, and then to 9,170 mg / L in the graphite electrode reactor, demonstrating the utilization of carbon by the microbes. In the case of the SAC electrode chamber, the DOC decreased in themed-operation effluent and increased slightly in the final substrate after MFC operation compared to the replaced effluent value which was due to the addition of substrate. The MFC operation was stopped before the bacteria utilized all the available nutrients, hence the DOC was still higher in the final substrate. COD decreased from 41,815 in the original HTC process water to 29,980 mg L^{-1} (28.3 %) in the graphite electrode reactor before it slightly increased to 30,400 mg L^{-1} (1.4 %) in the final substrate. The COD removal rate in the SAC electrode reactor was highest of all where up to 51 % of COD was removed. The reason for the highest COD removal rate but a lower performance of the MFC with SAC electrode than that with the KAC electrode, could be that microbes were able to remove COD efficiently. However, most of the organic content was used for their growth rather than electron production. The second reason could be that the produced biofilm was thick and that it was hindering the proper transfer of the electron to the surface of the anode electrode. Therefore, a thin and homogenous biofilm is ideal for a better conductance of electrons. TN_b analysis showed a similar pattern as DOC in both the reactors.

In the second MFC experiment, which was operated with pre-treated biogas digestate and KAC electrode, the pr-treatment was carried out to suppress the growth of the methane-producing bacteria (methanogens) which were originally present in the biogas digestate. This helps to avoid competition for nutrients between the electroactive bacteria (*Shewanella oneidensis*) and the undesirable methane-producing bacteria. In this way, the organic matter in the digestate is not redirected to methane production but instead it is mainly utilized by the *Shewanella oneidensis* for growth and electricity generation. Table 1 is showing the physicochemical properties of the digestate.

The DOC value deceased by 14.5 % from 4,000 to 3,420 mg L^{-1} in the effluent and then increased slightly due to the addition of fresh substrate. A similar pattern was observed with TN_b with a slight decrease in the mid-operation effluent compared to the original sample followed by an increase in the final substrate after MFC (Table 2). The COD value decreased by 25 % from 48,780 to 36,405 mg L^{-1} in the final substrate after the MFC operation. The increase in COD value after the MFC operation was mainly due to the addition of substrate and the development of the biofilm because microbes release chemical compounds while developing biofilms which contribute to the increase in COD value.

The resulting rise and fall of the power against time with external loads (see Figure 5) showed the microbial growth pattern where the microbial growth starts with the lag phase accustoming the environment followed by the log phase where the microbes multiply logarithmically and lastly a negative phase when the available nutrients are being exhausted. The highest power achieved was after fresh substrate was added and started decreasing when the MFC operation is towards completion suggesting the exhaustion of the available nutrients as observed following ICP-OES analysis of the effluents (see Table 3). A good example is the depletion of Ca^{+} from an original concentration of 203 µg/ml in the HTC process water to final concentrations of 171 µg/ml in effluent of MFC with SAC electrode and 15.6 µg/ml in effluent of MFC with graphite rod electrode.

Table 1. Composition of the HTC process water and biogas digestate before MFC, mid-operation effluent during the MFC and final effluent from the MFC operation for each reactor obtained by HPLC in g L^{-1}

Samples	Acetic acid	Glyceraldehyde	Formaldehyde	Levulinic acid	HMF	Furfuryl Alcohol	Ethanol
HTC-PW original	8.9	0.10	0.11	0.19	0.21	2.47	4.78
GP-M_HTC-PW	6.29	0.12	0.24	0.06	0.18	2.19	1.09
GP-F_HTC-PW	4.04	0.05	0.19	0.05	0.15	1.73	0.28
SAC-M_HTC-PW	6.28	0.08	0.17	0.05	0.16	1.24	3.64
SAC-F_HTC-PW	6.30	0.07	0.19	0.06	0.15	1.42	3.89
Samples	**Acetic acid**	**Sucrose**	**Glucose**	**Levulinic Acid**	**HMF**	**Furfural**	
TDB	0.17	0.29	0.10	0.0	0.02	0.03	
KAC-M_TBD	0.02	0.35	0.17	0.0	0.0	0.0	
KAC-F_TBD	0.0	0.43	0.15	0.0	0.0	0.0	

Key: HTC-PW = Hydrothermal carbonization process water; TBD = Treated biogas digestate; GP_M = Mid-operation effluent from MFC with graphite rod electrode; GP_F = Final effluent from MFC with graphite rod electrode; SAC_M = Mid-operation effluent from MFC with steam-activated electrode; SAC_F = Final effluent from MFC with steam-activated electrode; KAC_M = Mid-operation effluent from MFC with KOH-activated electrode; KAC_F = Final effluent from MFC with KOH-activated electrode; HMF = Hydroxy methyl furfural

Table 2. Physicochemical properties of HTC process water and treated biogas digestate

Samples	pH	EC (mS / cm)	DOC (mg / L)	COD (mg / L)	TN_b (mg / L)
HTC-PW original	5.99	19.7	13440	41815	4410
GP-M_HTC-PW	8.6	17.1	9940	29980	3880
GP-F_HTC-PW	8.8	17.8	9170	30400	2730
SAC-M_HTC-PW	8.2	15.2	9510	27760	3830
SAC-F_HTC-PW	8.1	15.8	9604	20470	3910
TDB	7.97	26.7	4000	48780	5600
KAC-M_TBD	9.6	22.1	3420	30370	4040
KAC-F_TBD	9.5	22.4	3610	36405	4300

Key: HTC-PW = Hydrothermal carbonization process water; TBD = Treated biogas digestate; GP_M = Mid-operation effluent from MFC with graphite rod electrode; GP_F = Final effluent from MFC with graphite rod electrode; SAC_M = Mid-operation effluent from MFC with steam-activated electrode; SAC_F = Final effluent from MFC with steam-activated electrode; KAC_M = Mid-operation effluent from MFC with KOH-activated electrode; KAC_F = Final effluent from MFC with KOH-activated electrode; HMF = Hydroxy methyl furfural; **COD** = Chemical Oxygen Demand; **EC =** Electrical conductivity; **DOC** = Dissolved Organic Carbon; **TN_b** = Total Nitrogen bound

Table 3. ICP-OES values of the nutrients present in the original substrate before MFC and effluents after MFC operation

Sample name	B_ICP	Ca_ICP	K_ICP	Mo_ICP	Na_ICP	P_ICP	S_ICP	Cu_ICP
	µg / ml	µg / ml	µg / ml	µg / ml	µg / ml	µg / ml	µg / ml	µg / ml
SAC-M_HTC-PW	4	144	1330	ND	162	<20	346	ND
SAC-F_HTC-PW	4	171	2070	ND	168	<20	379	ND
GP-M_HTC-PW	4	143	1480	ND	163	<20	352	ND
GP-F_HTC-PW	4	15.6	2550	ND	177	<20	419	<10
KAC-M_TBD	2	131	6260	492	188	198	50	<20(4)
KAC-F_TBD	2	157	6140	390	182	172	38	<20(10)
TBD	2	75.6	2860	437	93,8	140	26	<20
HTC-PW original	4	203	292	<4	178	51	396	ND

Key: **HTC-PW** = Hydrothermal carbonization process water; TBD = Treated biogas digestate; **GP_M** = Mid-operation effluent from MFC with graphite rod electrode; **GP_F** = Final effluent from MFC with graphite rod electrode; **SAC_M** = Mid-operation effluent from MFC with steam-activated electrode; **SAC_F** = Final effluent from MFC with steam-activated electrode; **KAC_M** = Mid-operation effluent from MFC with KOH-activated electrode; **KAC_F** = Final effluent from MFC with KOH-activated electrode; ND Not detected

3.3 Open Circuit Voltage (OCV) with SAC, KAC and Graphite Electrodes

MFC was operated in the first experiment with a SAC electrode in one MFC setup (with anode and cathode) and graphite rod electrode in the other setup using HTC process water as a substrate. Figure 2 shows the OCV with SAC electrode for the whole MFC operation time excluding the daily hours of polarization readings. The OCV was measured by taking a record of the generated voltage daily at inestimable resistance when no current was flowing, hence no power was generated. The highest OCV observed was 459.63 mV, which was recorded after adding the fresh HTC substrate during the MFC operation. There was a drop in the voltage during the 9th day, which was likely due to nutrient exhaustion. The voltage increased after the addition of the fresh substrate until it reached its maximum OCV and started dropping again after a few days. In MFC operation, OCV states the capability of the produced biofilms to store charges [54]. The highest OCV obtained with MFC containing graphite rod electrode was 114.83 mV with the same substrate, as mentioned before. The OCV was much lower compared to the SAC electrode MFC (Figure 3).

The OCV curve obtained to check the open circuit potential from the second MFC system wth KAC electrode is shown in Figure 4. It was operated using the biogas digestate treated with Ammonium-Heptamolybdate-Tetrahydrate and KOH (15:3) activated corncob pyrochar electrode under similar conditions and total time frame of 1 month before. The OCV produced during the initial days was quite stable after an initial increase due to the initial stabilization time and bacterial acclimation to the new environment. Furthermore, the added bacterial density was lower compared to the added in the first experiment. Therefore, the microbial metabolization on the available nutrients for the growth multiplication and production of stable electrogenic biofilms until the OCV started rising rapidly after the 18th day, probably due to the addition of fresh substrate. The maximum OCV obtained from this MFC was 494.24 mV. The OCV was still increasing before the MFC system was stopped. The reason is that the short-intended duration of the operation. The OCV obtained in this experiment is relatable to the OCV obtained with the reactor containing SAC electrode and HTC water. Although the former one showed maximum OCV on the 15th day and showed fluctuation, while this one showed a steady increase after the 13th day.

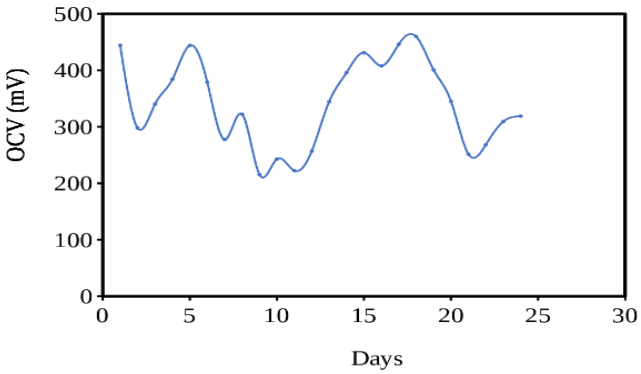

Fig. 2. Open Circuit Voltage (OCV) in MFC with SAC electrode and HTC process water during the whole MFC operation (excluding polarization readings hours)

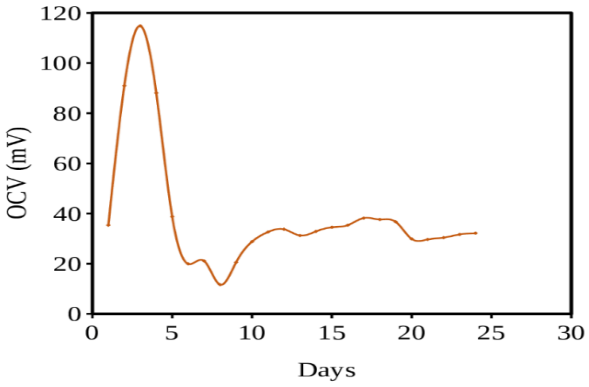

Fig. 3. Open Circuit Voltage (OCV) from MFC with HTC process water and Graphite rod electrode during the whole MFC operation (excluding polarization readings hours)

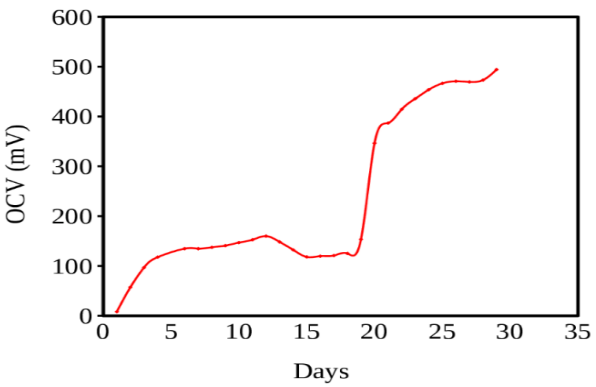

Fig. 4. Open Circuit Voltage (OCV) for MFC with KAC electrode and treated biogas digestate (excluding polarization readings)

3.4 Polarization studies of MFC with SAC, KAC and Graphite Electrodes

Polarization studies were performed against the external loads of 47 Ω, 100 Ω, 220 Ω, 470 Ω, 1000 Ω, 2200 Ω, and 4700 Ω, every day. The maximum power output achieved was 323.83 µW at 47 Ω followed by 272.15 µW at 100 Ω as shown in Figure 5. When the external resistance equals the internal resistance in an MFC, maximum power generation is achieved. Due to the increasing biomass of the electrogenic bacteria, it is only possible when the external resistance is almost the same as the internal resistance [55]. The power output of the present study with SAC electrode and HTC process water substrate revealed that the internal resistance of the system was between 47 Ω and 100 Ω. The lowest power obtained was at the highest resistance of 4700 Ω, at this point, the current was not passing efficiently through the system due to a higher external resistance and increased Ohmic losses. As a result, a term known as the *cell design point* is the resistance at which the highest current, voltage and power are obtained. Therefore, an MFC operation above this point performs efficiently, while below this point causes instabilities due to higher current and lower voltage [12, 43].

The MFC with graphite electrode yielded a maximum power output of 2.37 µW at the highest resistance of 4700 Ω (Figure 6). This shows that the system had a high internal resistance since maximum power output is achieved when the external load equals the internal resistance. The highest current produced was 0.185 mA at a voltage of 8.68 mV.

The resulting curve of the power against external loads with respect to time for MFC with KAC electrode is shown in figure 7. The power produced during the initial days of the polarization readings was quite lower, probably because the internal resistance was higher as observed in the curve of internal resistance (see SI file). The highest power obtained was 316.78 µW at 47 Ω on the 13th day, while the MFC reactor equipped with SAC pyrochar also produced the highest power at 47 Ω on the 15th day. The lowest power obtained was at 4700 Ω, which corresponded to the previous findings with a SAC electrode reactor. When the MFC operation was completed, the voltage produced was still high and higher power output was being generated, suggesting the availability of enough nutrients in the substrate for bacterial utilization. The relatively higher power output of 316.8 µW obtained with the KAC electrode was probably because of the highest surface area of the KAC electrode which denotes higher binding sites for microbial colonization and transfer of electrons. Similarly, the KAC pyrochar added in the anode chamber with KAC electrode might have served as a nutrient sequester for bacteria and a mediator for transfer of electrons from the bacterial cell to the electrode and enhanced generation of power. Additionally, the concentration of acetate was higher in the HTC process water compared to the biogas digestate making the former an excellent carbon source for electrogenic bacteria for their growth and electricity production than the latter.

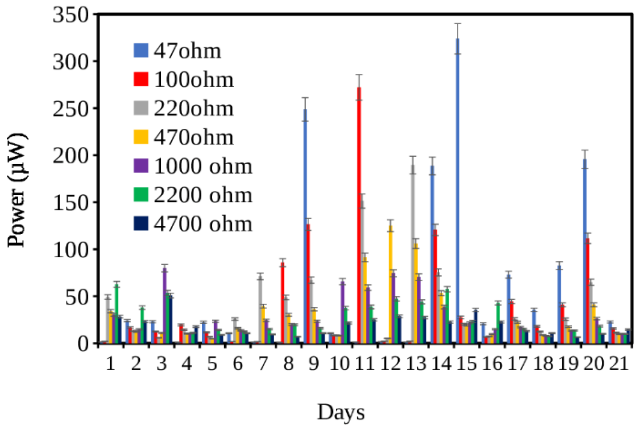

Fig. 5. Power versus time plot of the MFC with SAC electrode and HTC process water across the external loads. "Reproduced from *Musa Bishir, M. Tariq, D. Wüst, Andrea Kruse (2020). Comparative Performance of Two Different Locally Made Corncob Electrodes and Graphite for Electricity Generation in Microbial Fuel Cells (MFCs). 3rd Doctoral Colloquium Bioenergy: 17th/18th September 2020. Leipzig: DBFZ, 2020. Leipzig: DBFZ. 145 S. ISBN:978-3-946629-60-3. [3rd Doctoral Colloquium Bioenergy, [online], 17.-18.09.2020]* with permission from Deutsches Biomasseforschungszentrum gemeinnützige GmbH".

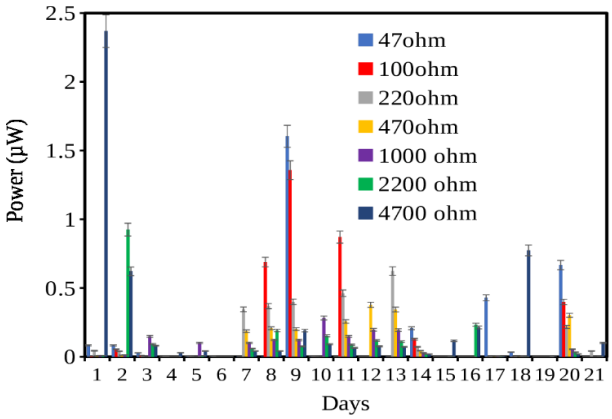

Fig. 6. Power versus time plot of MFC with graphite electrode and HTC process water across the external loads. "Reproduced from *Musa Bishir, M. Tariq, D. Wüst, Andrea Kruse (2020). Comparative Performance of Two Different Locally Made Corncob Electrodes and*

Graphite for Electricity Generation in Microbial Fuel Cells (MFCs). 3rd Doctoral Colloquium Bioenergy: 17th/18th September 2020. Leipzig: DBFZ, 2020. Leipzig: DBFZ. 145 S. ISBN:978-3-946629-60-3. [3rd Doctoral Colloquium Bioenergy, [online], 17.-18.09.2020] with permission from Deutsches Biomasseforschungszentrum gemeinnützige GmbH".

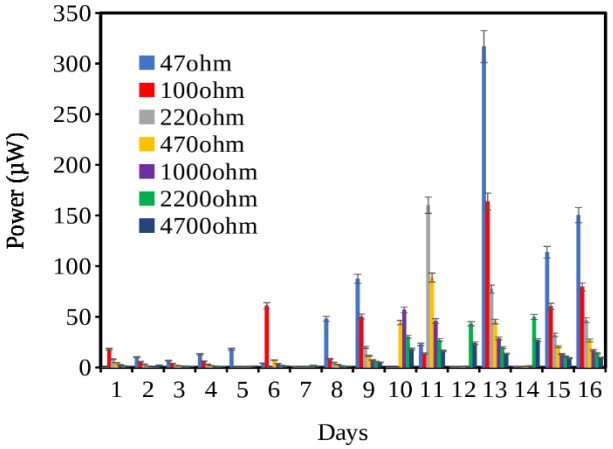

Fig. 7. Power versus time plot of the MFC with KAC electrode and treated biogas digestate across the external loads. "Reproduced from *Musa Bishir, M. Tariq, D. Wüst, Andrea Kruse (2020). Comparative Performance of Two Different Locally Made Corncob Electrodes and Graphite for Electricity Generation in Microbial Fuel Cells (MFCs). 3rd Doctoral Colloquium Bioenergy: 17th/18th September 2020. Leipzig: DBFZ, 2020. Leipzig: DBFZ. 145 S. ISBN:978-3-946629-60-3. [3rd Doctoral Colloquium Bioenergy, [online], 17.-18.09.2020]* with permission from Deutsches Biomasseforschungszentrum gemeinnützige GmbH".

The polarization curve was plotted describing the variation in current density over the power density and cell voltage under the function of external resistances (see Figure 8). At a voltage of 123.37 mV (at 47 Ω), the MFC with SAC electrode produced a maximum current density of 17.49 mA / m^2 and a power density of 2.15 mW / m^2. The maximum current obtained was 2.62 mA on the 15th day with one fresh substrate feeding. The current output obtained in this study almost matches the findings of Venkata Mohan *et al*. [43] at 50 Ω, but they obtained this result at a daily feeding of the fresh substrate (approx. 1 kg d^{-1}).

The OCV and the current obtained in this current study was higher than that of the findings of Mohanakrishna *et al* [56]. having OCV of 310 mV and current of 2.12 mA compared to the OCV of 459.63 mV and current of 2.62 mA in the present study only in 15th day of MFC operation. Probably due to the use of SAC pyrochar on the electrode with a higher surface area having more sites for microbial attachment [24], which was absent in the mentioned literature, although the used electrode size was bigger than that used in the study.

The highest power density achieved from the MFC with graphite electrode was 1.37mW/m^2 with highest current density of 106.75 mA m^{-2} at the lower voltage of 8.68 mV (see Figure 9). The study of the literature suggested that higher current density at a lower voltage makes the MFC systems becomes unstable. Internal losses during electron transport from bacteria to anode can be caused by a variety of factors, including activation losses, ohmic losses, and concentration losses [12].

The polarization curve for MFC with KAC electrode was plotted based on the variation in current density over the power density and cell voltage under the function of external resistances (Figure 10). The highest power density achieved was 2.11 mW / m^2 (0.41 W / m^3) having the current density of 17.31 mA / m^2 (3.25 A / m^3) and a voltage of 122.02 mV at 47 Ω on the 13th day of MFC operation. The MFC study of Li *et al* [57] obtained a current density of 4.84 A / m^3 and a volumetric power density of 1.14 W / m^3 which is higher than the findings of the present study. This is probably due to the use of raw animal carcass wastewater in the reported study with a higher COD value and higher nutrients concentration compared to the present study. In the current study, biogas digestate was used as an MFC substrate, with most of the nutrients being previously exhausted under the biogas production process. The second possible reason was the use of a bigger and commercially prepared anode electrode with graphite felt together with granulated activated carbon in the other study [57].

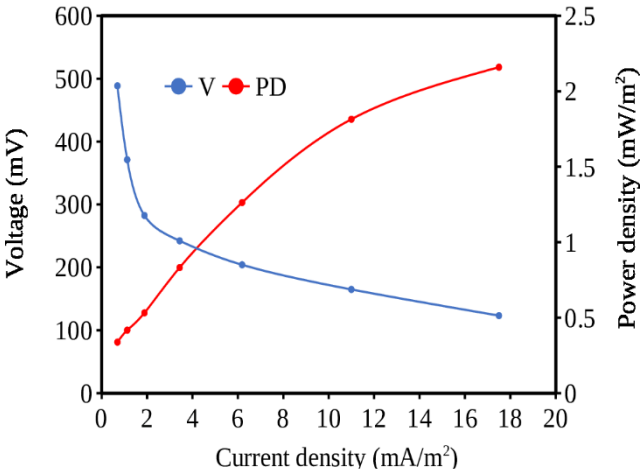

Fig. 8. Power density and polarization curves of the MFC with SAC electrode and HTC process water

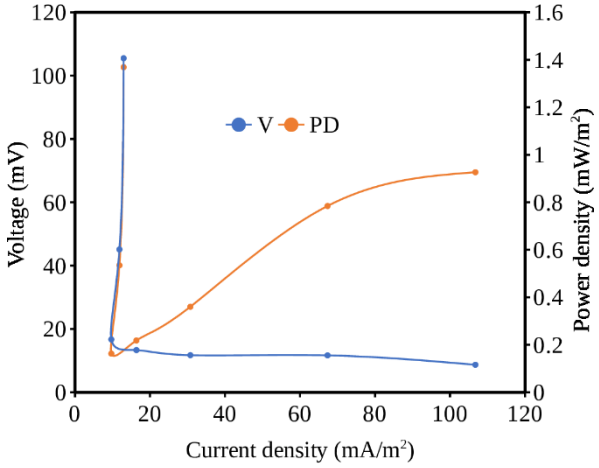

Fig. 9. Power density and polarization curves of the MFC with Graphite rod electrode and HTC process water

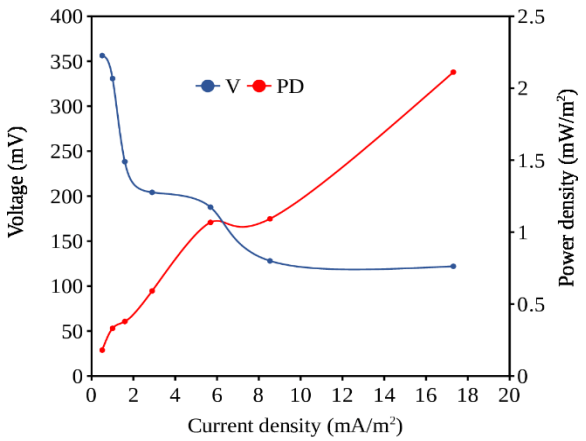

Fig. 10. Power density and polarization curves of the MFC with KAC electrode and treated biogas digestate

3.5 Coulombic Efficiency of the MFC Systems

The highest ε^c of 75 % was found with KAC electrode followed by the SAC electrode MFC with a ε^c of 64 % and lastly, ε^c of 48 % was found for graphite electrode MFC.

There were a few limiting factors to be kept in consideration of the MFC with KAC electrode. The substrate used was biogas digestate, in which the electrogenic bacteria were not the sole microbes present to utilize the available nutrients, others such as the methanogens were present since the biogas digestate was from a completed cycle of biogas production. This might have resulted in competition for nutrients between the desirable electroactive bacteria and the unwanted methanogens. Furthermore, the microbial density (concentration of the inoculum) used was lower than that used in the SAC reactor and the reactor with graphite electrode, yet the bacteria were able to multiply and produce stable biofilms in a shorter duration.

Figure 11 shows the SEM images of the corncob pyrochar without activation, and Figure 12 (a-b); graphite rod electrode before and after MFC operation, figure 13 (a-b); corncob pyrochar physically (steam) activated before and after MFC and figure 14 (a-b); chemically (KOH) activated pyrochar before and after MFC respectively. The transformation in surface morphology can be clearly observed with and without activation. Smaller pore size was observed in non-activated pyrochar. The image in figure 12(b) shows the microbial communities dispersed over the graphite electrode, which could be that the microbes were dispersed on top of the electrode, rather than forming biofilms due to lower surface area. Wider pores were observed with SAC pyrochar and microbial biofilms were observed after the MFC operation. The largest pores were observed with KAC pyrochar as depicted in the BET results and denser microbial biofilms were observed. The denser biofilms might be due to high degree of bacterial colonization on the KAC electrode, which is known to be greatly associated with the high surface area and porosity observed with this electrode. In general, the electrode surface morphology post MFC operation depends largely on the degree of biofilm formation on it, which in turn depends on the pore diameter and pore volume of the carbon material (pyrochar in this study) used in making the electrodes.

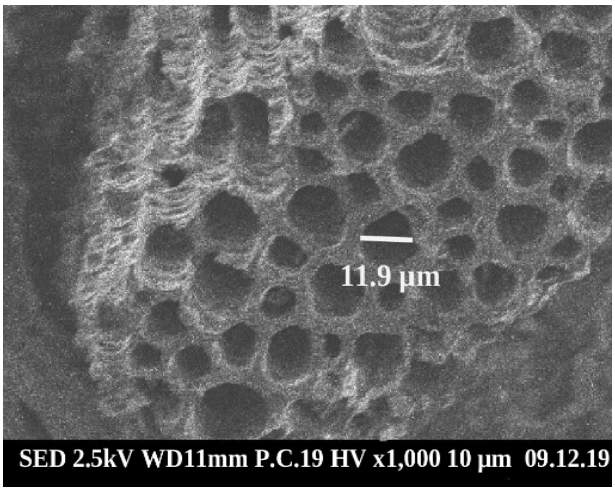

Fig. 11. Scanning Electron Micrograph of the non-activated corncob pyrochar

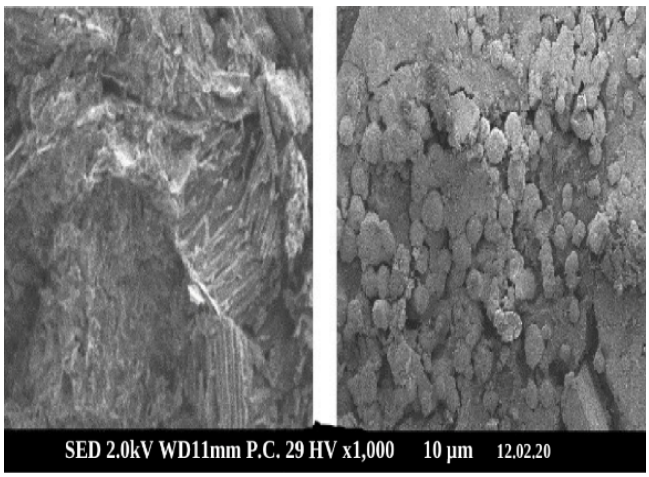

Fig. 12. Scanning Electron Micrographs of (a) graphite rod electrode before MFC and (b) after MFC

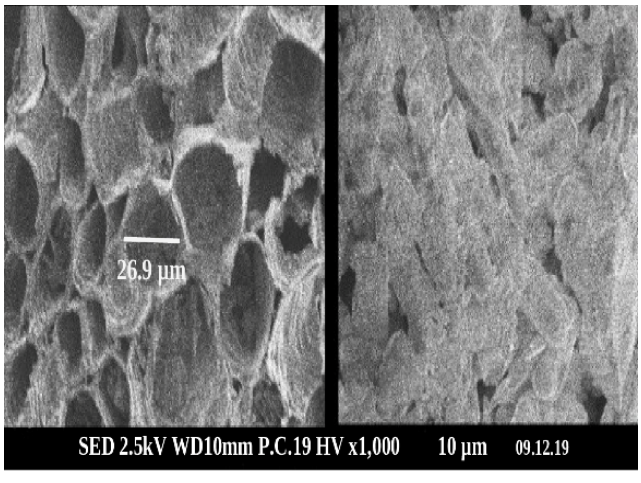

Fig. 13. Scanning Electron Micrographs of (a) Steam-activated pyrochar electrode before MFC and (b) after MFC

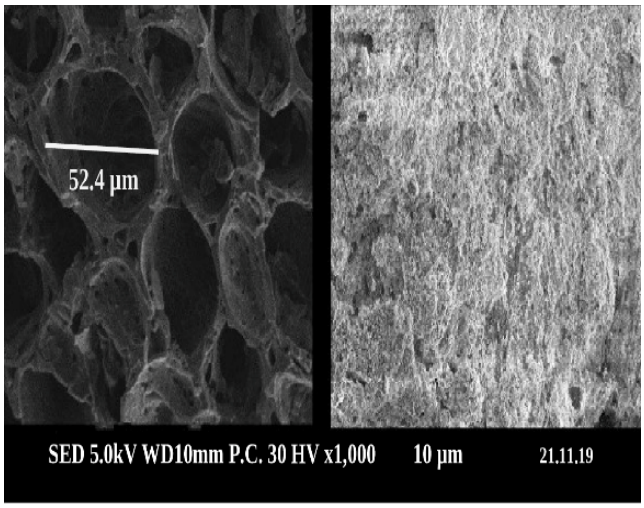

Fig. 14. Scanning Electron Micrograph of KAC electrode before (a) and (b) after MFC operation

4. Conclusion

It can be concluded that biobased electrode materials could be used in the generation of electricity from various wastewaters; HTC processes water and biogas digestate in a dual-chambered MFC. When compared to the other electrodes, the KOH activated corncob pyrochar (KAC) with the largest surface area of 1626 m^2 / g showed the best MFC performance. Graphite rod electrode, despite being an excellent conductor, could not perform competitively due to its low surface area and porosity, which are essential for the growth of microbial biofilms. The SAC electrode MFC performed comparatively best based on the rate of removal of COD (51 %), but the rate of COD removal alone does not suggest a better overall MFC performance. However, despite a lower COD removal rate of 25% in the MFC system with KAC electrode, its Coulombic Efficiency was the highest (75%) of all the MFCs, meaning that it had the best MFC performance than MFCs with SAC and graphite electrodes. Overall, the MFC containing KAC electrode relatively yielded the highest power output of 316.8 µW in a shorter duration of 13 d while the MFC system with SAC electrode yielded its highest power (323.8 µW) in 15 d which was only about 2% higher than the former. Future research will look at the possibilities of improving the performance of the KAC electrode by treating it with complex ions like manganese and iron, as well as analysing the stability of the locally made corncob electrode over a longer period, such as 3-6 months or more. The effects of adding a chemical electron mediator will also be examined in future studies.

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Conflict of Interests

The authors have no conflicting interests, and the funders were not involved in the study design, data collection, analysis, or interpretation, article preparation, or the decision to publish the findings.

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List of Abbreviations, Symbols & Units

MFC	Microbial Fuel Cell
HTC	Hydrothermal Carbonization
HPLC	High-Performance Liquid Chromatography
KOH	Potassium Hydroxide
BET	Brunauer-Emmett-Teller
COD	Chemical Oxygen Demand
DOC	Dissolved Organic Carbon
SAC	Steam-activated Corncob Pyrochar
KAC	KOH-activated Corncob Pyrochar
SA_{BET}	Specific BET Surface Area
PD	Power Density / mW / m^2
Ϭ	Electrical Conductivity / S
ε^c	Coulombic efficiency / %
Ω	Ohm
L	Litre
S	Siemens
µ	Micro
W	Watt
A	Ampere
V	Volt
Kg	Kilogramm
m	meter
s	second
%	Percent

Data Availability Statement

The data that support the findings of this study are openly available in "figshare" at https://doi.org/10.6084/m9.figshare.16540143.v1, reference number [16540143] [59].

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