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Performance Evaluation of Aquaponics-Waste-Based Biochar as a Cathode Catalyst in Sediment Microbial Fuel Cells for Integrated Multitrophic Aquaculture Systems

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Abstract: The sustainable development of aquaculture faces a significant challenge due to the need for the frequent treatment of aquacultural waste. This research presents a pioneering solution by concurrently utilizing aquacultural waste to produce biochar and enhancing a sediment microbial fuel cell (SMFC)'s treatment efficacy for waste generated from the integrated multitrophic aquaculture (IMTA) system. The water quality parameters of the aquacultural pond water were analyzed, and synthetic wastewater was prepared to validate the system's efficiency. Over a period of more than 50 days, the SMFC was operated and monitored, yielding an average chemical oxygen demand (COD) removal efficiency of 86.31 ± 2.18%. The maximum operating voltage of the SMFC reached 0.422 V on the 21st day of operation when connected to an external resistance of 975 Ω. A novel-activated aquacultural biochar catalyst was synthesized from aquaponics waste and used as a cathode catalyst, substantially improving the SMFC's performance. Characterization studies demonstrated that the aquacultural biochar catalyst was an active electrocatalyst, accelerating the oxygen reduction reaction rate and leading to increased power output and overall efficiency of the SMFC. The SMFC utilizing the aquacultural-waste-based biochar cathode catalyst showcased the highest maximum power density, with a range of 101.63 mW/m^2 (1693.83 mW/m^3), and the lowest internal resistance, indicating superior performance. These results validate the reliability of implementing SMFCs in actual aquaculture systems. A novel modular design for SMFC reactor-assisted small-scale integrated poultry–fish culture systems is proposed for further practical application in real-life aquaculture settings. This research contributes to finding sustainable and effective methods for waste treatment for aquaculture, promoting the development of environmentally friendly practices in the industry.

Keywords: aquaculture waste; biochar; electrocatalyst; integrated multitrophic aquaculture; sediment microbial fuel cells

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1. Introduction

The worldwide increase in the production of organic waste has increased the need for treating and using them as manures, fertilizers, nutritive feeds, or energy substrates. Integrated multitrophic aquaculture (IMTA) systems and wastewater treatment techniques represent a novel area of research that can be established as cutting-edge technology in the agriculture and aquaculture sectors. On the other hand, sediment microbial fuel cell (SMFC)-assisted wastewater treatment systems have the potential for simultaneous agriculture/livestock/synthetic wastewater and aquacultural wastewater treatment, along with promising power production for powering different low-energy sensors, such as water quality or environmental sensors.

Activated aquacultural biochar catalysts can be prepared from aquacultural waste, which can potentially improve the performance of SMFCs by acting as a cathode catalyst. Biochar-based catalysts are made of naturally available sources, mainly waste products, which make them a better sustainable alternative to metallic catalysts, making them more cost-effective for large-scale applications. Waste minimization, wastewater treatment, water utilization, and renewable energy production are the primary advantages of SMFC-assisted aquaculture systems. IMTA systems and wastewater treatment techniques represent a novel area of research that, in future, could be established as a cutting-edge technology in the fields of agriculture and aquaculture.

The fish meal was successfully replaced with chicken offal silage (made from chicken viscera only) in commercial Nile tilapia (*Oreochromis niloticus*) feed [1]. Organic fish feeds were also developed using pig feces and poultry droppings as a replacement for 30% of soybean meal in the diet of Nile tilapia, without any adverse effects on their growth [2]. Researchers recommend using livestock wastes from organic production systems as substitutes for expensive components in fish feed production. Higher survival and growth rates were observed for common carp and *Labio gonius* cultured in tanks manured with poultry droppings [3]. The fish market wastewater was treated using halophiles in an air-cathode microbial fuel cell (ACMFC) under saline conditions, and potential chemical oxygen demand (COD) removal coupled with energy production was achieved [4]. The integration of fish culture and poultry rearing in transplanted rice has shown efficacy in enhancing nutritional security [5].

The higher COD removal efficiency of the SMFC reactor significantly affects its performance related to the power output [6]. MFCs have shown the potential of nitrogen removal, including both nitrate and ammonium, from recirculating aquaculture systems with simultaneous energy recovery [7]. In microbial carbon capture cells (MCCs), algae consume CO_2 and produce oxygen, maintaining oxygen levels near the cathode surface. The regular MFC is a specific type of BES. with several promising potentials, such as wastewater treatment, electricity generation, and biosensor-based applications. MFCs have the considerable potential of acting as energy supply devices for sensors, thereby providing the required electric current to power the sensors.

The MFC itself can also act as a biosensor to detect refractory contaminants [8,9] and heavy metals [10,11] and determine the biochemical oxygen demand (BOD) of the wastewater sample [12]. Moreover, it has been observed that, in the medical field, specific deoxyribonucleic acid or DNA strands can be detected by MFCs without an external analytical device [13]. In addition to that, MFCs have proven their potential to supply power for several water quality, environmental, medical, and volatile fatty acid (VFA) sensors [14]. Nevertheless, MFCs have multiple advantages over a single system, making them a cost-efficient and renewable energy source. SMFCs produce energy from the electro-potential difference between aerobic water and anaerobic sediments [15].

Due to climatic and other environmental parameters, the system's performance may be diminished while being tested in actual field conditions [16–20]. SMFCs have the potential to act as power generators for different water quality, environmental, and even medical sensors, which, in fact, provide multiple advantages for assisting this type of reactor with IMTA systems. The whole SMFC treatment system, with an effective catalyst, must first be replicated at the laboratory scale to analyze the performance of COD removal and power generation before applying it on a large scale. SMFCs can be integrated into aquaculture systems so as to provide a complementary source of energy and improve water quality [15].

Integrated poultry–fish culture techniques are effective in extensive culturing methods but have a future scope for development in a small-scale, intensive form with an effective wastewater treatment technique. The use of poultry droppings as fish feed is itself a wide area of research and can effectively replace the supplementary fish feed diet. The extensive integrated poultry–fish culture system impacts an ecological risk in releasing the poultry droppings into the pond without proper maintenance, which a suitable mechanical poultry cage system can reduce.

In this research, a novel-activated aquacultural biochar catalyst was synthesized from aquacultural wastes and used as a cathode catalyst to improve the performance of SMFC. Material and electrochemical characterization studies were carried out to examine the aquacultural biochar catalyst's properties. Polarization studies were carried out by varying the external resistance from 10 kΩ to 10 Ω to compare the performance of SMFC reactors with and without activated aquacultural biochar-based cathode catalysts in treating synthetic wastewater (poultry–fish-based). A detailed design of an intensive poultry–fish culture system assisted with SMFC-based wastewater treatment was proposed after validating the data from the experimental SMFC reactor's performance for treating actual aquacultural wastewater.

2. Materials and Methods

2.1. Design and Fabrication of SMFC

An experimental SMFC reactor was designed and fabricated, made from a poly-acrylic cylindrical column having an internal diameter of 12.7 cm and a height of 120 cm. The design was plotted on AutoCAD software and shown in Figure 1. The total reactor volume is 15.19 L. Out of the entire reactor height of 120 cm, 15 cm is given as freeboard for headspace, 65 cm is filled with synthetic wastewater replicating the actual aquacultural wastewater (aquacultural wastes and poultry droppings) and 45 cm is filled with pond sediment collected from aquaculture pond. Aquacultural wastewater is collected from the experimental aquaculture pond behind the aquacultural engineering building at IIT Kharagpur and poultry droppings are collected from the tech market IIT Kharagpur and water quality analysis is carried out to estimate the COD value of the samples.

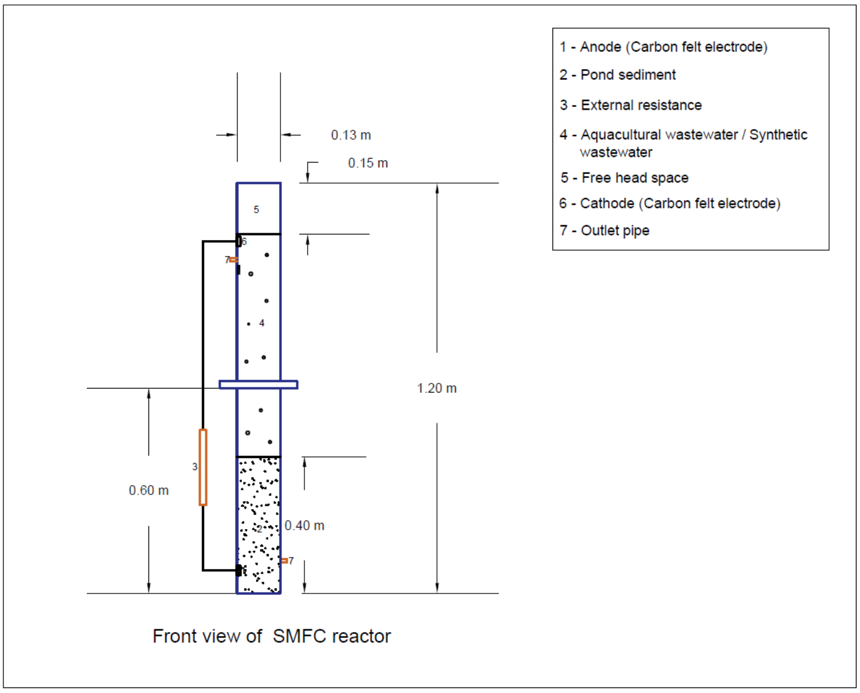
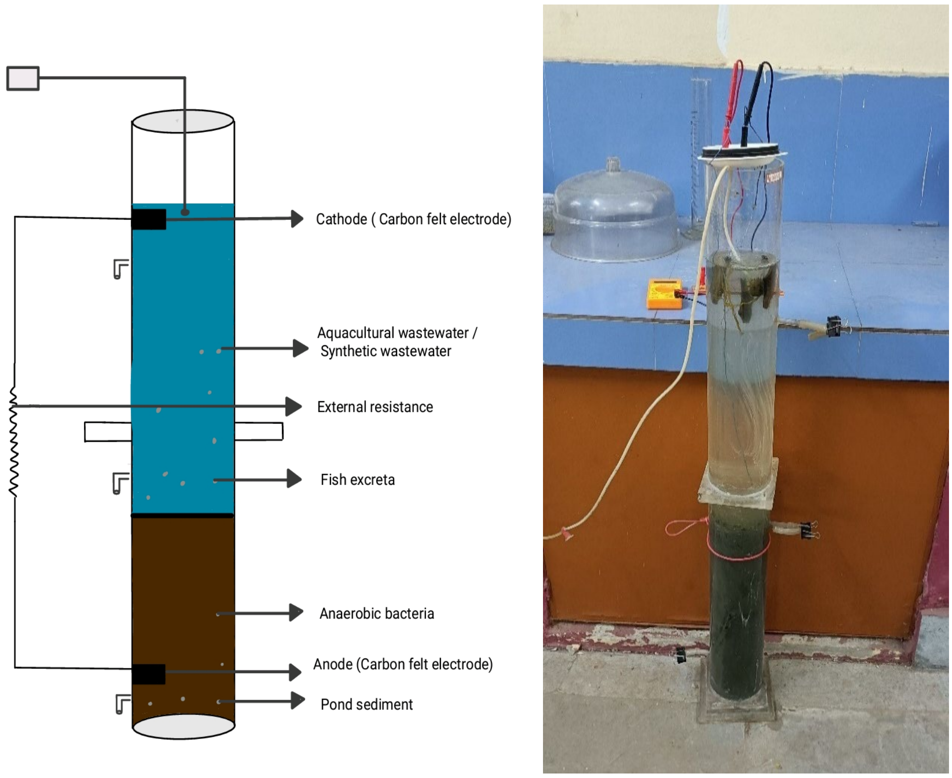


Figure 1. CAD design of SMFC reactor.

Three carbon felts, each of 6 cm × 3 cm × 0.5 cm, stacked parallel to a plastic rod was used as the cathode and carbon felt with the dimension of 18 cm × 3 cm × 0.5 cm was used as the anode. The carbon felt electrode and collected pond sediment are shown in Figure 2a,b. After filling up the pond sediment to act as the inoculum, an anode electrode was placed at the bottom attached to the pond sediment. A cathode electrode stacked parallelly was placed in the overlying water. The total projected surface area of the anode and cathode was 108 cm^2. The anode and cathode were electrically connected using a copper wire with an external resistance of 1000 Ω. The cathode and anode were placed at the top and bottom at a distance of 15 cm from both zones, respectively. The theoretical explanation of SMFC and the actual experimental reactor is shown in Figure 3a,b. Three inlet/outlet pipes were provided at the reactor to collect the water samples. The anodic zone was operated under anoxic conditions, and the cathodic zone was aerated [21]. Aeration is provided at the cathode layer using the commercially available aquarium aeration pump (SOBO WP3200, China) at a depth of 15 cm from the top [22].

(a) (b)

Figure 2. (**a**) Carbon felt electrode; (**b**) Pond sediment.



(a) (b)

Figure 3. (**a**) Theoretical explanation of SMFC; (**b**) Experimental SMFC reactor.

The closed reflux colorimetric method is commonly used for determining the COD of the water sample using a spectrophotometer. The water sample (10 mL) is mixed with a digestion reagent that consists of a mixture of sulfuric acid (H_2SO_4) and potassium dichromate ($K_2Cr_2O_7$). A blank solution is also prepared. The mixture is heated in a refluxing system until all of the organic matter in the sample is oxidized. The digestion vials are placed in a heating block and heated at 150 °C for 2 h to oxidize the organic matter in the solutions. The digestion vials are sealed and the solutions were thoroughly mixed. After digestion, the digestion vials are removed from the digester and allowed to cool to room temperature. The absorbance of the blank and water sample was measured at 600 nm using a spectrophotometer [23]. The COD of the sample (in mg/L or ppm) is calculated by comparing its absorbance to the standard curve generated from the absorbances of the standard solutions [24]. The closed reflux colorimetric method is a reliable and accurate method for determining the COD of a water sample and is widely used in water quality analysis. All the experiments were carried out in a batch mode where fresh feeding was performed after achieving the optimal water quality. The anodic zone was fed with synthetic wastewater with sucrose as the primary carbon source to achieve the optimal experimental COD value of a mixture of aquacultural and poultry wastewater.

2.2. Aquacultural Biochar Preparation and Activation

Aquacultural waste biomass was collected from aquacultural tanks of the aquaponic systems situated in the Aquacultural Engineering building at IIT Kharagpur, India, to synthesize the catalyst. Aquacultural waste biomass was first filtered through a 2 mm sieve at first to remove the large particles and floating matter. Then, it was filtered through filter paper to remove harmful substances. The biomass retained was rewashed to filter further impurities. The aquacultural biomass was then centrifuged at 4000 rpm to separate the supernatant liquid from the slurry biomass. The slurry biomass was then kept in an oven at 60 °C and oven-dried overnight. The biomass obtained was stored in a sealed container, weighed, and found to be 10 g. Then, it was pyrolyzed in a pyrolysis reactor in an anoxic condition at 400 °C for 2 h with an initial heating rate of 10 °C per minute. The pyrolyzed sample was rewashed with ethanol (15% *v/v*) and distilled water to filter out the impurities and non-pyrolyzed fraction, then kept in the oven at 60 °C [16,19,25].

The thermal activation of the aquacultural biochar was carried out using a tube furnace at a higher temperature of 900 °C for 1 h with an initial heating rate of 5 °C min^{-1} in the argon atmosphere. Biochar catalyst is thermally activated at 900 °C for 1 h so that this process helps activate the biochar's surface area and create a porous structure. The high temperature causes the carbon atoms in the biochar to rearrange themselves, creating a more reactive surface that can better adsorb and react for the catalytic reactions. Longer activation time may lead to the degradation of the biochar structure. The aquacultural biochar was powdered using mortar and pistil, closed in a tight seal container, and the final weight was noted down. It was again washed with ethanol and distilled water and dried in the oven at 60 °C overnight. The activated aquacultural biochar catalyst was stored in a sealed container for further analysis and experimentation (Figure 4).

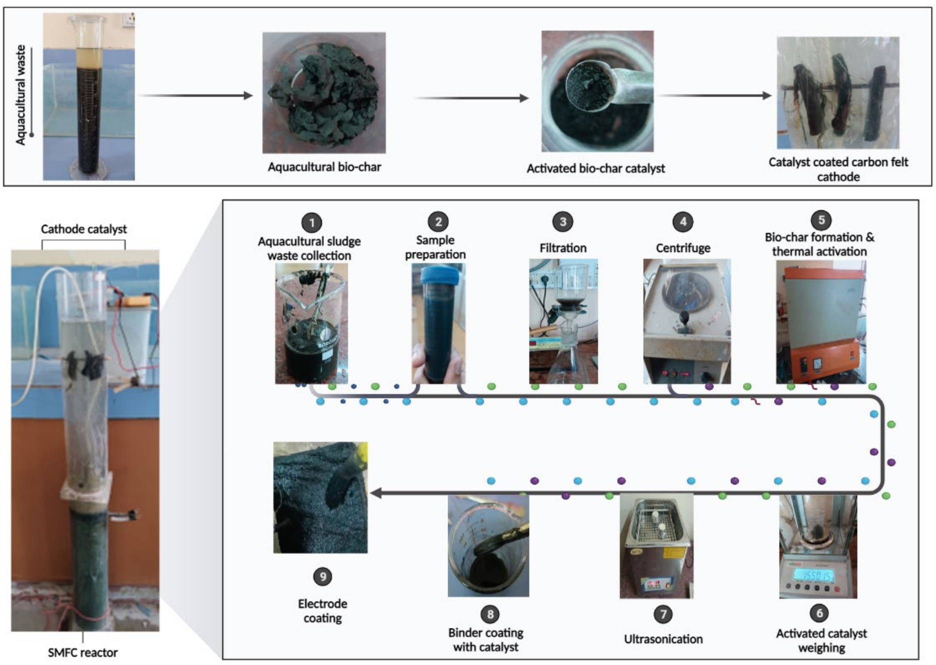


Figure 4. Process flowchart of preparation of cathode catalyst from aquacultural wastes for performance improvement of SMFC reactor.

2.3. Material and Electrochemical Characterization of Activated Catalyst

The synthesized aquacultural biochar catalyst was analyzed through different material and electrochemical characterization studies such as Raman spectroscopy, X-ray diffraction (XRD) analysis, cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), etc. SMFCs are the type of MFCs that use sediment as the electron donor. The cathode catalyst in an SMFC is an important component that helps improve the efficiency of the oxygen reduction reaction (ORR) and increase the cell's power output. Several characterization studies can be performed on a cathode catalyst in an SMFC. A polarization curve can measure the voltage and current generated by the SMFC under different loads. This curve can provide information about the power output and the efficiency of the cathode catalyst. EIS can be used to measure the impedance of the cathode catalyst at different frequencies. The cathode catalyst is coated with the carbon felt electrode, and the characterization studies of the catalyst are carried out. The impedance provides information about the resistance to electron transfer and oxygen diffusion through the cathode catalyst layer.

CV and EIS are two commonly used techniques for characterizing cathode catalysts in fuel cells. CV is a technique used to study the electrochemical behavior of a material. In CV, a voltage is applied to the cathode catalyst, and the resulting current is measured. The voltage is then swept in a cyclic pattern, and the current is measured at each voltage. The resulting current–voltage (I–V) curve provides information about the electrochemical properties of the cathode catalyst, including the potential of the ORR, the kinetic parameters, and the electroactive surface area. EIS is a technique used to study the impedance of a material at different frequencies. In EIS, a small AC voltage is applied to the cathode catalyst, and the resulting current is measured. The impedance of the material is then calculated based on the phase difference between the applied voltage and the measured current. EIS provides information about the resistance of the cathode catalyst to electron transfer, the diffusion of oxygen through the catalyst layer, and the double-layer capacitance. The EIS method was used to determine the electrochemical properties and the resistance values.

Both techniques provide complementary information about the electrochemical properties of the cathode catalyst. CV offers information about the electrochemical activity of the cathode catalyst and the reaction kinetics. In contrast, EIS provides information about the resistance to electron transfer and the diffusion of oxygen through the cathode catalyst layer. Together, these techniques can be used to optimize the cathode catalyst and improve the performance of the fuel cell.

XRD can be used to analyze the crystal structure of the cathode catalyst. This can provide information about the crystal structure and composition of the catalyst layer, affecting the ORR activity. These characterization studies can help to optimize the cathode catalyst in an SMFC and improve the efficiency and power output of the cell. Raman spectroscopy is a type of vibrational spectroscopy that involves using laser light to measure the vibrational modes of a material. Raman spectroscopy was carried out by using a Raman spectrometer. In the context of SMFCs, Raman spectroscopy is a characterization technique used to study the chemical and structural properties of the cathode catalyst. The method provides information about molecular vibrations and can be used to identify the presence of specific chemical bonds, functional groups, and other molecular features. Raman spectroscopy can be used to study the carbon structure of the catalyst, such as the degree of graphitization and the presence of defects, which can affect the performance of the cathode. Raman spectroscopy can also be used to study the microbial biofilm that forms on the cathode catalyst in SMFCs. The technique can provide information about the composition and structure of the biofilm, including the presence of extracellular polymeric substances and other biomolecules.

2.4. Performance Evaluation of SMFC with and without Catalyst

The SMFC obtained a stable phase after about 15 days of operation following the acclimatization phase, where the microbes got acclimatized to the environment. The SMFC reactor was operated continuously for 50 days to check its performance in terms of COD reduction and power generation. For the first few cycles, the COD of the water sample was monitored regularly in order to find out the hydraulic retention time (HRT) in order to know after how much time the feed or the organic matter is to be supplied to the microbes.

After synthesizing the cathode catalyst obtained from aquacultural wastes, the catalyst is mixed with a binder agent, PDMS (polydimethylsiloxane), then ultrasonicated it for half an hour and coated with the carbon felt cathode electrode. According to the optimal catalyst loading (2 mg/cm^2), carbon black (0.5 mg/cm^2) was proportionally mixed, and then dipped in acetone and kept for ultrasonication for half an hour. Then, the binder agent PDMS is added with an optimal load of 6.66 µL/mg, ultrasonicated simultaneously coated with cathode, and oven-dried at 90 °C overnight. For a single-carbon felt cathode electrode with an area of 6 cm × 3 cm, 72 mg of aquacultural biochar catalyst and 0.479 mL of binder agent (PDMS) were required for the uniform coating of the cathode electrode. For the three carbon felt electrodes stacked parallelly on the top of the SMFC reactor acting as the cathode, a total of 1.44 mL of binder agent was added, then ultrasonicated, coated on the cathode, and dried. Then, the same experimental cycle was carried out first with aquacultural pond water followed by synthetic wastewater.

In the cathodic part of the MFCs, the catalyst is used to improve the oxygen reduction reaction (ORR) performance. The cathode is the ORR site, and essential reaction in MFCs that produces water from oxygen and electrons. In MFCs, a catalyst such as platinum is often used at the cathode to facilitate the ORR. Even though platinum has a high catalytic activity for the ORR and can effectively reduce the energy required for the reaction to occur, it is expensive, so it is necessary to use other biocatalysts. Using a catalyst at the cathode of an MFC can improve the cell's performance in several ways. First, it can increase the efficiency of the ORR, which can lead to higher power output from the cell. Second, it can reduce the cathode's overpotential and the energy required to drive the ORR.

The electrical parameters, such as open-circuit voltage (OCV), operating voltage (OV), power production, power density, current density, etc., are determined to evaluate the performance of SMFC [26–29]. OCV is the maximum potential difference between electrodes in a system. Due to some internal losses, the OV will be less than OCV. OV is the operating voltage of the circuit when it is connected to an external resistance. OV and OCV of the SMFC system were determined using the multimeter. The polarization for the SMFC with and without cathode catalyst for both aquacultural and synthetic wastewater was performed using a reference electrode (Trulab, Thane, India), multimeters (DT-830D Unity, Delhi, India), and resistance box (GEC05R Decade Resistance Box, Bangalore, India). The voltage generated from the reactor setup is measured, and the total power production is estimated correspondingly. To determine the power output of the SMFC, the voltage and current were measured with a digital multimeter, and the power output was computed using Ohm's law. Polarization studies were conducted by varying the external resistance from 10 KΩ to 10 Ω after stabilizing the circuit for 5 to 10 min at each resistance. The SMFC's internal resistance was determined by analyzing the plot of voltage versus current using the slope of the line.

3. Results and Discussion

The characterization studies of the synthesized aquacultural biochar catalyst such as CV, and EIS exhibit an active electrocatalytic property of the catalyst, which can help in faster ORR rate, which ultimately leads to higher power output and overall efficiency of the system. Raman spectroscopy and XRD analysis suggest that the biochar has good electrochemical activity and catalytic properties.

The water quality parameters such as DO, pH, COD, turbidity, and conductivity were analyzed at the start after collecting and filling the aquacultural wastewater in the reactor. To study the performance of SMFC, parameters such as power generation, voltage, COD removal, pH, conductivity, dissolved oxygen (DO), nitrate, etc., were measured and monitored [30]. Since the wastewater quality was optimal enough and did not require any treatment, synthetic wastewater had to be synthesized (for more accurate analysis) considering the level of contaminants that can occur in actual large-scale aquacultural systems or integrated livestock aquacultural systems. The feed composition is estimated for both the laboratory SMFC reactor and SMFC-assisted integrated poultry–fish culture system using the standard feed composition with 1000 mg/L of COD [16–20].

3.1. Characterization Studies of Synthesized Activated Aquacultural Biochar Catalyst

The activated aquacultural biochar catalyst prepared from aquacultural wastes was used as the cathode catalyst in the SMFC reactor for enhancing ORR. The WE current (A) refers to the current measured at the working electrode (WE) during CV experiments. In CV experiments, a voltage waveform is applied to the working electrode, which causes an electrochemical reaction to occur at the surface of the electrode. The current flowing through the electrode is then measured as a function of the applied voltage. The representative CV profiles of aquacultural biochar catalyst-coated carbon felt and bare carbon felt are shown in Figure 5. There is a prominent reductive current output of 2.5 mA when applied at a potential of 1 V.

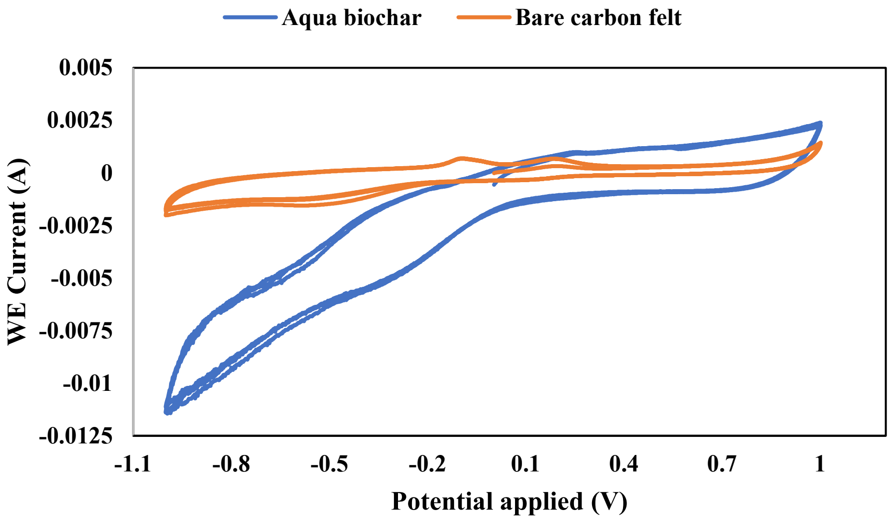


Figure 5. CV plots of aquacultural biochar catalyst coated carbon felt and bare carbon felt electrode without any catalyst.

In contrast, for bare carbon felt, the current produced is 1.5 mA when applied a 1 V potential which clearly shows aquacultural cathode catalyst has shown higher current output. Peaks in the CV correspond to the redox reactions associated with the biochar-based cathode catalyst. A current of the working electrode (*y*-axis) versus the potential applied (*x*-axis) is plotted in order to analyze CV data. The resulting graph is known as a cyclic voltammogram (Figure 5).

The CV data determine the peak potentials that correspond to electrochemical reactions occurring at the electrode surface. The peaks are typically characterized by a sharp increase in current. The potential at which the peaks occur is known as the peak potential. The CV curve of the aquacultural catalyst conducted under oxygen saturated medium showed a prominent reduction in the peak at −1 V, which depicts that the cathode catalyst can have a higher ORR. The CV profile of the aquacultural catalyst showed a current density peak of 0.0025 A at an applied 1V potential compared to the bare carbon felt without the catalyst. The current density of the biochar-based cathode catalyst-coated electrode is higher than the bare carbon felt, which justifies that the electrochemical activity of the cathode catalyst is efficient compared to the electrode without the catalyst. The CV data analysis of aquacultural biochar catalysts in SMFC provides valuable insights into the electrochemical behavior of the catalyst. It helps in understanding the more efficient performance of SMFC with this catalyst.

In order to analyze the data from an EIS experiment, impedance data are plotted as a Nyquist plot which is interpreted to determine the resistance and capacitance values (electrochemical behavior of the system). In the EIS experiment of a cathode catalyst used in SMFC, Z′ (Ω) and −Z″ (Ω) represent the real (resistive) and imaginary (capacitive or inductive) components of the impedance, respectively. In a Nyquist plot, a semicircle observed with its diameter corresponds to the charge transfer resistance (R_{ct}) of the cathode catalyst. R_{ct} value is the most crucial parameter in ORR kinetics. The lower value of R_{ct} corresponds to faster electron transfer, resulting in higher electrical conductivity and higher ORR activity. The diameter of the semicircle is related to the charge transfer resistance, which can be used to assess the performance of the cathode catalyst in SMFC. The charge transfer resistance value of aquacultural catalyst was found to be much lower than the bare carbon felt without a catalyst after EIS data analysis and interpretation, which is shown in Figure 6.

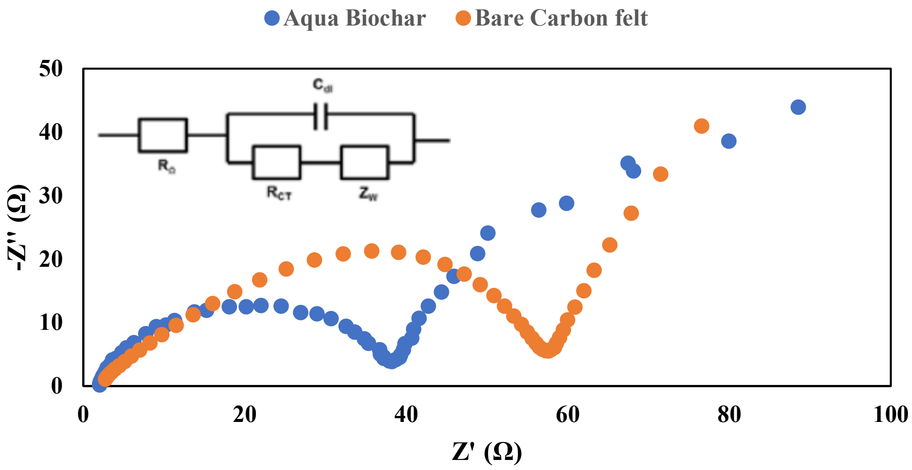


Figure 6. EIS plots of aquacultural biochar catalyst coated carbon felt and bare carbon felt along with its Randles circuit with Warburg element.

The charge transfer resistance (R_{ct}) represents the resistance to the flow of electrons at the cathode–electrolyte interface. The R_{ct} value of aquacultural biochar cathode catalyst was determined to be 39 Ω, whereas it shows around 58 Ω for bare carbon felt. The results imply lower charge transfer resistance to the carbon felt electrode coated with activated aquacultural catalyst than the electrode without catalyst. Thus, it was confirmed that the activated aquacultural biochar catalyst has the potential for higher electrical conductivity and faster ORR activity. A lower R_{ct} indicates a better electron transfer ability of the cathode catalyst, which can result in higher current generation and better performance of the SMFC. Therefore, minimizing the R_{ct} is a significant factor in designing and optimizing cathode catalysts for SMFC. In the SMFC reactor, a lower charge transfer resistance is generally better for a cathode catalyst, because it indicates less opposition to the flow of electrons in the circuit, which can result in a higher power output. Higher resistance can lead to a drop in the output voltage, which can limit the overall performance of the MFC. However, the optimal resistance range can vary depending on the specific design and operating conditions of the SMFC [31].

The XRD plot is a graph of intensity versus the angle of diffraction (2θ), where peaks in the intensity correspond to specific crystallographic planes of the material. The peaks between 20 and 30 degrees in the XRD plot typically indicate the presence of graphitic structures in the biochar catalyst, which can contribute to its catalytic activity (Figure 7). The smaller peaks between 30 and 40 degrees could correspond to the presence of crystalline phases of minerals or metals in the biochar, depending on the specific composition of the biochar. The disordered smaller peaks after 40 degrees indicate the presence of distinct crystalline phases in the biochar material in addition to amorphous carbon, which is also a desirable characteristic for catalytic applications. Amorphous carbon can provide additional surface-active sites for catalytic reactions and enhance the surface area of the biochar catalyst. Combining these characteristics can make the biochar catalyst an effective material for catalytic applications in various fields, such as wastewater treatment and energy conversion.

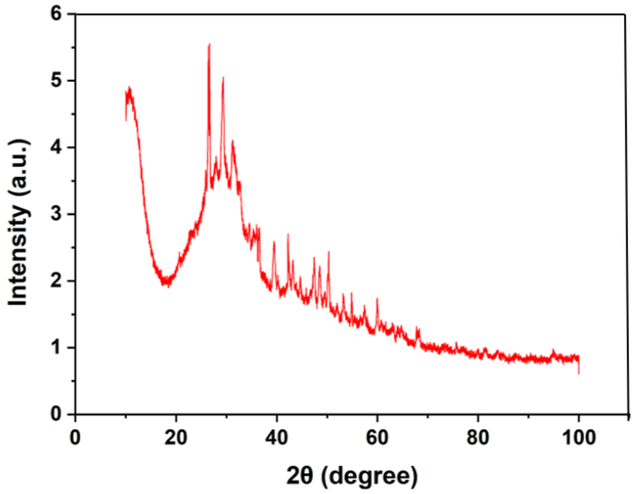


Figure 7. XRD plot for activated aquacultural biochar catalyst.

The results show that biochar has higher stability, higher catalytic activity, and higher surface area, allowing for more catalytic reactions. The exact interpretation of these peaks would require additional information, such as the intensity, shape, and location of the peaks and other characterization techniques. The presence of graphitic structures, amorphous carbon, and crystalline phases can contribute to the catalytic activity and selectivity of the biochar catalyst. Thus, the results clearly show the promising catalytic property of aquacultural biochar-based cathode catalysts.

Raman spectroscopy can identify functional groups in the biochar catalyst, such as carbon–carbon double bonds, carbon–nitrogen bonds, and oxygen-containing groups like hydroxyl and carboxyl, etc. Biochar catalysts with higher degrees of graphitization are generally more stable and have higher catalytic activity. The graph obtained from the Raman spectroscopy of a biochar catalyst represents the intensity of Raman scattering (y-axis) as a function of the Raman shift or wavenumber (x-axis). The Raman shift measures the energy difference between the incident and scattered photons and is expressed in units of inverse centimeters (cm^{-1}). The position, shape, and intensity of the peaks in the Raman spectrum provide information about the biochar catalyst's vibrational modes and chemical composition. By analyzing the Raman spectrum of the biochar catalyst, as shown in Figure 8, the identity and quantity of various functional groups and carbonaceous species present in the synthesized biochar were determined.

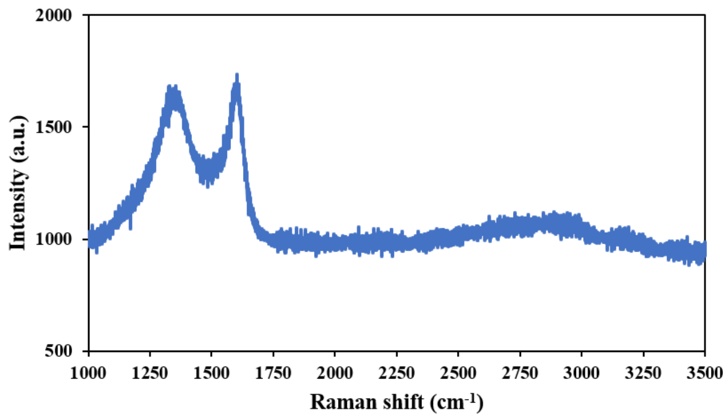


Figure 8. Raman spectroscopy plot for activated aquacultural biochar catalyst.

The Raman shift intensity peak of the synthesized biochar around 1575–1625 cm^{-1} is usually attributed to the presence of sp^2 hybridized carbon, which is commonly found in graphene-like structures. This peak is commonly referred to as the "G-band" in Raman spectroscopy and indicates graphitic carbon structures. This peak suggests that the biochar may have a high degree of graphitization, which can confer specific properties such as high electrical conductivity and increased stability. This peak is also associated with aromatic and double-bonded carbon groups in biochar, such as C=C, C=O, and C=N. Therefore, this peak indicates the presence of these functional groups in the biochar. The intensity of this peak is related to the concentration of these functional groups. Therefore, a higher intensity peak indicates a higher concentration of oxygen-containing functional groups in the biochar structure. The presence of these functional groups can potentially enhance the electrochemical activity of the biochar as a cathode catalyst in SMFCs.

The Raman shift intensity peak of a biochar in the range of 1575–1625 cm^{-1} is typically associated with the C=C stretching vibrations of aromatic ring structures. This peak is often used as an indicator of the degree of aromaticity in the biochar, as the intensity of the peak is proportional to the concentration of aromatic carbon in the material. Therefore, a higher intensity peak in this range indicates a higher degree of aromaticity in the biochar. The presence of a higher intensity peak suggests the degree of graphitization and the quality of the carbon material. In biochar analysis, the presence of the G band indicates that the biochar contains a significant amount of graphitic carbon, which can improve its electrical conductivity and catalytic properties [32]. Overall, this analysis suggests that the biochar has good electrochemical performance as a cathode catalyst in SMFCs.

3.2. Performance of SMFC

The performance of SMFCs with various configurations (PW, SW, PW-BIO, and SW-BIO) gave similar electrochemical performances. It started producing an OCV of 772 mV and an operating circuit current of 0.4 mA on the first day after its acclimatization phase. The stable operating voltage was generated until the COD value reached the optimal range. The hydraulic retention time (HRT) was determined to be 4 days after carefully considering the COD removal. SMFC generated a maximum OV of 0.422 V when connected to an external resistance of 975 Ω on the 21st day of operation.

SMFC was operated for 10 cycles with regular feeding, and the water samples' OV, OCV, and COD value range was regularly monitored. In the first five days, the COD value of the water sample decreased to a reasonable extent, ensuring the reactor system's proper functioning. The operating voltage decreased linearly with the amount of organic matter present inside the reactor. On the first day of operation, SMFC started producing an OCV of 772 mV and an operating circuit current of 0.4 mA. The stable operating voltage was generated until the COD value reached the optimal range and HRT was determined to be 4 days. The SMFC reactor showed the highest COD removal efficiency to the extent of 88.39% over the regular operational interval during SW-BIO configuration.

The COD of the water sample before and after SMFC-assisted treatment is monitored regularly over 10 cycles. During the 10 cycles, an average of 86.31 ± 2.18% COD removal efficiency was obtained. The water sample with a COD range of more than 1200 mg/L (which is the maximum COD range of integrated aquaculture systems such as fish poultry culture [33–35]) is mostly reduced to the permissible COD limit (250 mg/L) of the water sample within 36 h. Poultry chicken manure has a COD range of 450 mg/L and a BOD range of 480–500 mg/L [36]. Many researchers also found that the COD value of poultry litter and poultry droppings ranges up to 1200 mg/L [34].

The integrated MFC techniques with activated sludge wastewater treatment have achieved similar effluent water quality [6]. The COD removal efficiencies over the 10 experimental cycles were more than 80% as shown in Figure 9. An average COD removal efficiency of 86.31 ± 2.18% was obtained with the SMFC-assisted wastewater treatment. These results show the excellent performance of the SMFC reactor in assisting wastewater treatment with the multiple advantages of power production. The results validate the reliability of using SMFC or similar BES in actual aquacultural systems.

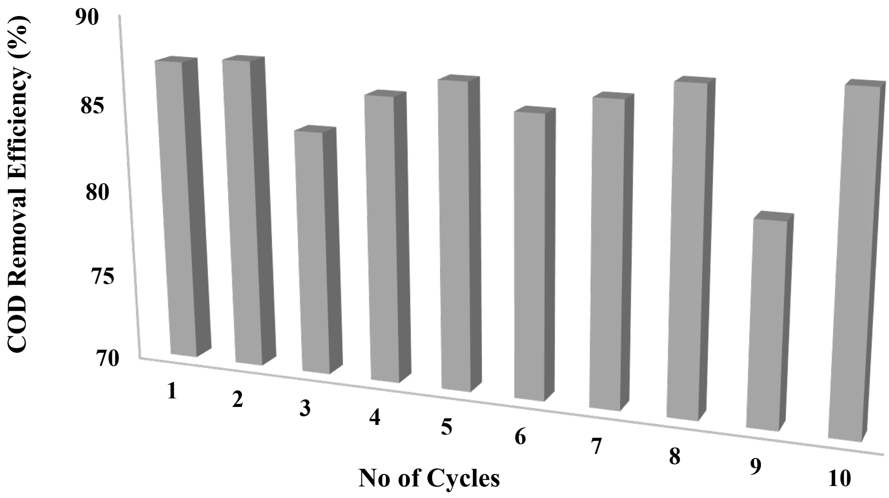


Figure 9. Stable COD removal efficiency range of wastewater to effluent over the 10 experimental cycles.

3.3. Comparative Performance Evaluation of SMFC with and without Catalyst

The performance of SMFC before and after using synthesized biochar catalyst was compared, and the results were analyzed. The polarization curve was used to calculate essential performance parameters of the SMFC, including the maximum power output, the internal resistance, power density, and current density. The polarization study characterizes the performance of SMFC. It provides valuable insights into the efficiency and power output of the MFC, which can be used to optimize the design and operation of the system.

Polarization curves were obtained for SMFC with and without catalyst in treating aquacultural and synthetic wastewater (replicating the actual integrated poultry–fish wastewater COD composition). The polarization was performed using a resistance box, multimeters, and a reference electrode. In the SMFC polarization experiment, PW represents the SMFC devoid of any cathode catalyst treating aquacultural wastewater, and SW means the SMFC devoid of cathode catalyst treating synthetic wastewater. Similarly, PW BIO and SW BIO represent the SMFC with aquacultural biochar cathode catalysts treating aquacultural and synthetic wastewater, respectively.

The SMFC without a cathode catalyst, which treats synthetic wastewater, showed a maximum power density (MPD) of 46.68 mW/m^2 (777.93 mW/m^3), which was found to be higher than SMFC treating aquacultural wastewater, which showed an MPD range of 39.18 mW/m^2 (653.09 mW/m^3). The SMFC with aquacultural waste-based biochar cathode catalyst treating the synthetic wastewater led the highest MPD among all the four, with the MPD range of 101.63 mW/m^2 (1693.83 mW/m^3), whereas the SMFC with biochar-based cathode catalyst, which treats aquacultural wastewater, showed less MPD than SMFC treating synthetic wastewater but demonstrated a much higher MPD than the 2 SMFCs without cathode catalyst. It showed an MPD range of 81.81 mW/m^2 (1363.58 mW/m^3). Therefore, the highest MPD was obtained by SMFC operated with aquacultural biochar cathode catalyst during polarization studies. The MPD value achieved by each SMFC with and without catalysts treating aquacultural and synthetic wastewater is shown in Figure 10a,b.

From the polarization curve, the MPD ranges of SMFCs without a catalyst and with a catalyst in treating aquacultural and synthetic wastewater was determined to be 39.18 mW/m^2 (653.09 mW/m^3) [PW], 46.68 mW/m^2 (777.93 mW/m^3) [SW], 81.81 mW/m^2 (1363.58 mW/m^3) [PW BIO], and 101.63 mW/m^2 (1693.83 mW/m^3) [SW BIO]. The internal resistance of the system arises due to the overpotential losses occurring at electrodes, which is determined from the slope of the straight-line portion of voltage vs. current [37]. The corresponding internal resistance for each system is shown in Figure 10c. From the

VI curve, the internal resistances of the respective SMFC without catalyst and with catalyst in treating aquacultural and synthetic wastewater were found to be 138.06 Ω (PW), 71.14 Ω (SW), 59.19 Ω (PW BIO), and 49.34 Ω (SW BIO) which demonstrates improved performance of SMFC by the employment of aquacultural biochar cathode catalyst.

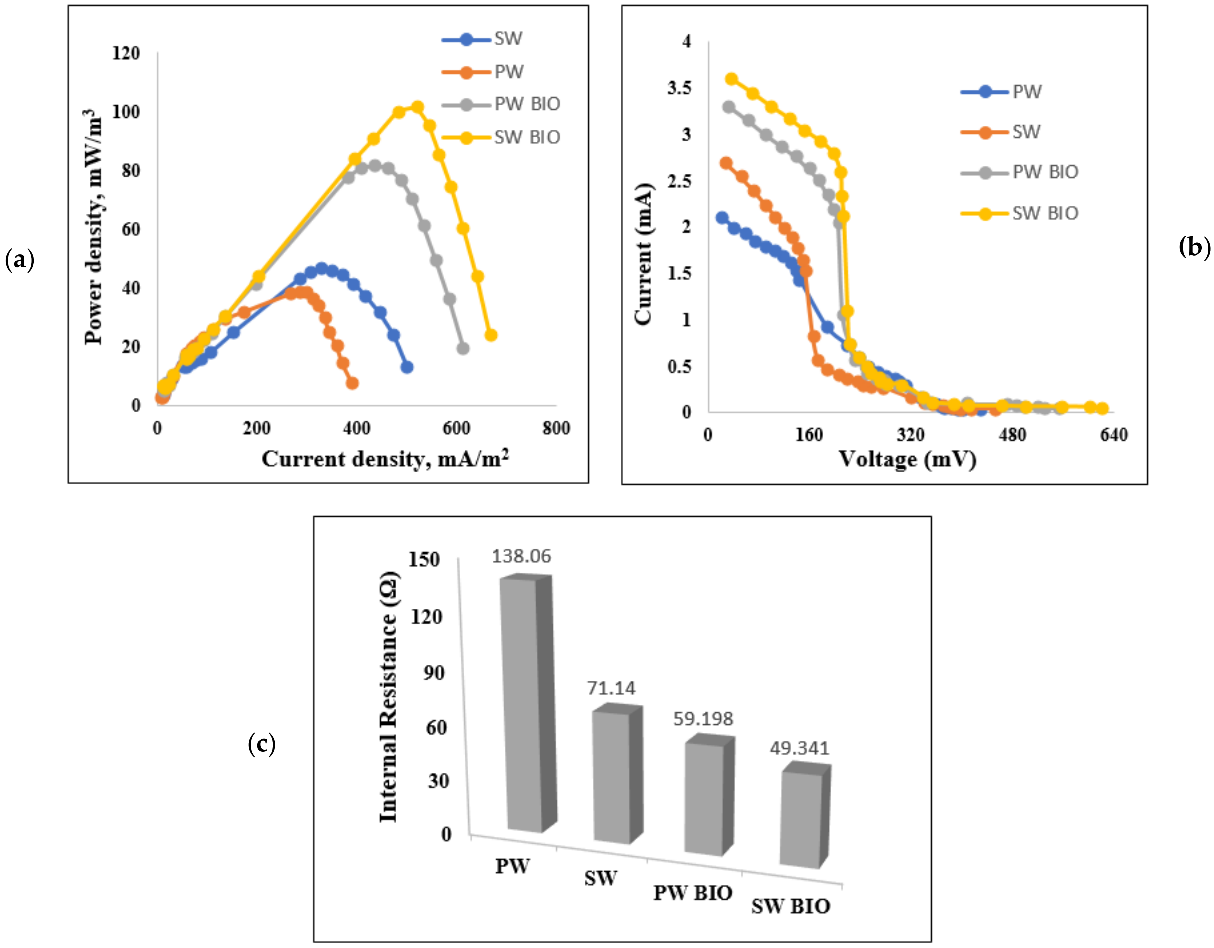


Figure 10. Polarization plots: (**a**) Power density vs. current density curve, (**b**) voltage vs. current curve, and (**c**) internal resistance value ranges.

Among all the systems, the SMFC with biochar catalyst-treating synthetic wastewater showed the lowest internal resistance, which depicts the system' improved performance by using a cathode catalyst and the higher organic matter of the water sample has a significant effect on the system's performance. Thus, the SMFCs with aquacultural waste-based biochar cathode catalyst showed a much higher MPD than SMFCs without the cathode catalyst, which proves the aquacultural biochar catalyst's potential in enhancing the SMFC performance. The results clearly indicated that SMFC operated with activated aquacultural biochar cathode catalyst showed efficacy in improving the operating voltage, thereby increasing the reactor's power generation and assisting in obtaining higher power density.

In this research, an SMFC reactor was designed, fabricated, and monitored for more than 50 days. The water quality parameters of the aquacultural water sample were analyzed, and respective to the final COD value, synthetic wastewater considering the maximum COD range that can occur in an integrated poultry–fish system was prepared for more accurate results.

3.4. Design Proposal for SMFC-Assisted Integrated Poultry–Fish Culture

Fish can be fed with grain and chicken feces as supplementary feed substrates. The poultry house will be built over the fish pond in an extensive integrated system, and the chicken droppings will fall into the pond. Higher survival and growth rates were observed for common carp and *Labio gonius* cultured in tanks manured with poultry droppings [3]. Poultry droppings can be used as manure and supplementary fish feed substrate. The waste can increase the growth of phytoplanktons, zooplanktons, and detritus, which can act as a substrate for microorganisms.

A design proposal for SMFC assisted integrated poultry–fish culture system was formulated by validating the data obtained from the SMFC reactor's promising performance in wastewater treatment and power generation. The design specifications for the integrated poultry–fish culture are proposed so that the poultry cage setup should be installed on the top of the fish tank, and SMFC equipped inside the fish tank will treat the wastewater and maintain the aquaculture water quality with simultaneous power production.

In the proposed intensive poultry–fish culture system, chickens are grown in vertically stacked layers of cages, and the fish is cultured in fish tanks. When the chickens produce excreta in the form of droppings, they fall through the wire meshes or are conveyed through mechanization, get into the tank, and act as the manure of supplementary fish feed. Fish eat the undigested food in excreta while digested foods are decomposed by bacteria, and they serve as nutrient cycling and phytoplankton growth takes place serving food indirectly. SMFC equipped inside the fish tank will treat the wastewater and maintain the aquaculture water quality. The design drawing of SMFC-assisted wastewater treatment for an integrated poultry–fish culture system as proposed is shown in Figure 11, and further research on it is still going on.

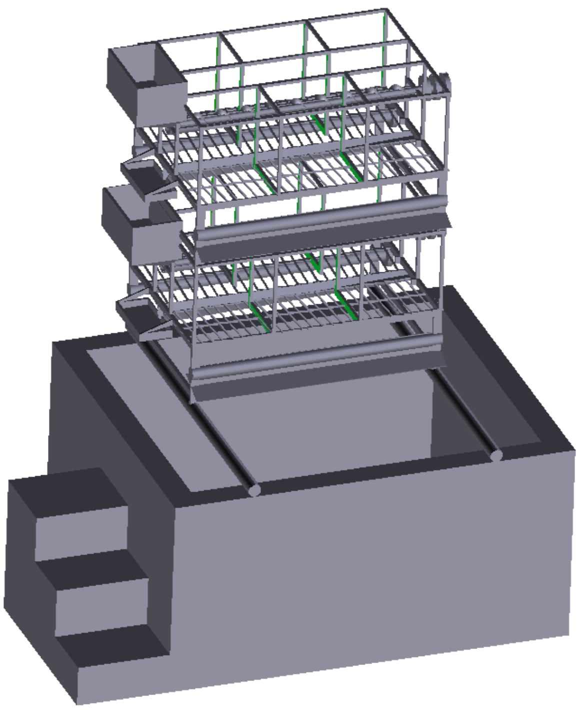


Figure 11. Design drawing of SMFC-assisted wastewater treatment for an integrated poultry–fish culture system.

4. Conclusions

This study represents the experimentation, analysis, and validation of incorporating wastewater treatment techniques like SMFC into IMTAs like integrated poultry–fish culture. An average COD-removal efficiency of 86.31 ± 2.18% and a maximum operating voltage of 0.422 V, when connected to an external resistance of 975 Ω, was obtained, showing the reliability and efficacy of using SMFC in actual aquacultural systems. A novel activated aquacultural biochar catalyst was synthesized from aquacultural wastes and coated on carbon felt electrodes to use as the cathode catalyst, which helps in ORR. The characterization studies proved that the biochar shows good electrochemical activity, higher stability, catalytic properties, and lower $R_{ct,}$ thus making it a practical material as a cathode catalyst in SMFC for wastewater treatment and energy conversion. From the polarization curve, the MPD ranges of SMFCs without a catalyst and with a catalyst in treating aquacultural and synthetic wastewater was determined to be 39.18 mW/m^2 (653.09 mW/m^3) [PW], 46.68 mW/m^2 (777.93 mW/m^3) [SW], 81.81 mW/m^2 (1363.58 mW/m^3) [PW BIO], and 101.63 mW/m^2 (1693.83 mW/m^3) [SW BIO]. The SMFC with aquacultural waste-based biochar cathode catalyst treating the synthetic wastewater showed the highest MPD and lowest internal resistance, which depicts the improved performance of the SMFC by using a cathode catalyst. In this research, a novel-activated aquacultural biochar catalyst was synthesized from aquacultural wastes and used as a cathode catalyst for improving the performance of SMFC, which in turn can effectively assist an intensive IMTA system. SMFC using aquacultural waste-based biochar catalysts can effectively be incorporated into IMTA systems resulting in a potentially valuable technology for sustainable IMTA systems. SMFC-based IMTA systems can thus drastically improve water quality, generate renewable energy, improve fish growth and survival, and maintain sustainability.

Author Contributions: K.K.J. conceptualized and ideated this research under the mentorship of G.D.B.; K.K.J. performed all the formal analyses and experiments; P.S. assisted in the lab investigations and assisted in the design of the SMFC reactor; G.D.B. arranged the funding and resources; K.K.J. wrote the full original manuscript draft, P.S. edited the necessary corrections, and G.D.B. completed the final edit. All authors have read and agreed to the published version of the manuscript.

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