JE&
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Journal of Engineering and Emerging Technologies
jeet.cspc.edu.ph
ISSN: 2782-9421
E-ISSN: 2984-8172
Published 30 December 2024

# Design and Development of Microbial Fuel Cell Graphite-Titanium Electrodes with Piggery Wastewater Substrate

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## RESEARCH ARTICLE

**Abstract**
Microbial fuel cell (MFC) is a bio-electrochemical system that converts biochemical energy to electricity. A design of MFC was developed consisting of graphite and titanium plates as anode and cathode, respectively, with piggery wastewater as organic substrate. The architectural design of MFC was a single-chamber air-cathode membrane-less, simple, and cheap design. A rectangular chamber plexiglass was fabricated to hold 500 mL piggery wastewater and electrodes with an anode with an active surface area of $0.0149\,m^2$. The piggery wastewater contained a mixed culture of *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, and *Proteus spp.*, which were incubated in a mediatorless system. The physicochemical characteristics of the piggery wastewater in terms of temperature, pH level, dissolved oxygen level, total dissolved solids, and electrical conductivity before the operation were 29.2°C, 6.54, 9.53 mg/L, 3,550 ppm, and 7,519 $\mu$S/cm, respectively, and after the one-day incubation and seven days operation at room temperature were 29.9°C, 5.55, 3.74 mg/L, 6,857 ppm, and 13,859 $\mu$S/cm, respectively. The measured electricity parameters of the MFC produced a maximum output, in terms of cell voltage, current output, and power density, which were 112.50 mV, 100.00 $\mu$A, and 570.47 $\mu W/m^2$, respectively.

**Keywords:** Electrode, Graphite, Microbial Fuel Cell, Mixed Culture, Piggery Wastewater, Titanium

**DOI:** http://doi.org/10.52631/jeet.v3i1.305



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***Submitted** 29 May 2024*

***Revised** 11 September 2024*

***Accepted** 30 November 2024*

***Citation***
*Esperas, N. (2024). Design and Development of Microbial Fuel Cell Graphite-Titanium Electrodes with Piggery Wastewater Substrate. Journal of Engineering and Emerging Technologies. 3(1). 37-49. doi: 10.52631/jeet.v3i1.305*

## 1 INTRODUCTION

The microbial fuel cell (MFC) is a bio-electrochemical system (BES) biocatalyst that converts biochemical energy into electrical energy. This self-sustaining device can eliminate pollutants and treat wastewater while generating electrical power. MFC is a promising technology for efficiently obtaining sustainable energy from wastewater. Generating electrical energy relies on microorganisms that can produce protons as a by-product of their metabolism [1], [2].

Various designs of MFCs have been developed for different applications. The common designs used for MFC are single-chamber, double-chamber, up-flow, continuous flow, etc. [3]. Among these, single-chamber air-cathode membrane-less MFC shows potential for it is a simpler and cost-effective application in producing electricity and wastewater treatment [1], [4], [5]. But despite this intensive research in the field of MFC, there's still a need to discover and explore to optimize the device's performance in power generation.

Another parameter that must be considered when designing the MFC is the materials for electrodes. The anode and cathode are the two terminals of the MFC where the oxidation-reduction reaction (ORR) and the extracellular electron transfer (EET) occur [6], [7]. Graphite is a carbon-based material commonly used as an anode material because of its good properties as an electron acceptor [8]. At the same time, titanium has good properties as a cathode electrode because it is a non-corrosive, highly stable, and biocompatible material [9]. Despite this, pairing up these two materials as anode-cathode electrodes for a single chamber MFC has not yet been explored.

Another thing to consider when designing an MFC is the substrate, which acts as the nutrient source of microbes. The source of the electricity in the MFC relies on the metabolism of microorganisms. The microorganisms' metabolism must be sustained in the MFC anodic chamber to secure electricity production. There are various substrate sources aside from carbohydrate-rich substances, such as domestic and industrial wastewater, sewage, leachate, etc. Using wastewater for the MFC is more suitable for its practical application [3], [5].

The Philippines is an agricultural country; the swine industry was the second-largest agricultural sector, according to the Swine Situation Report of the Philippine Statistical Authority (PSA) [10] in 2021. According to the article Pig Progress [11], it also became evident that backyard farms domesticating swine production has been increasing. In a 2016 census, 64 percent of the total swine population was accounted for from backyard farms. Without a proper system of wastewater treatment and disposal from this piggery farm, pollution to the environment and body of water, such as eutrophication, may occur [12]. Various methods were explored, but there's not yet a study in the local exploring this opportunity of power generation from wastewater of the agricultural sector, particularly from swine, using the technology of MFC.

Numerous studies have been conducted on harvesting electricity using MFC. However, there's still a need to explore a more convenient and conventional method of generating electrical power. Also, the opportunity to convert biological energy from piggery wastewater to electrical energy locally in the Philippines must be explored to combat the energy crisis. Thus, this study designed and developed a single-chamber air-cathode membrane-less MFC using graphite-titanium electrodes and a piggery wastewater substrate. Then, the performance of the turbine was evaluated.

This study generally aimed to design and develop a microbial fuel cell using graphite and titanium plates as anode and cathode electrodes with piggery wastewater substrate. It has also identified the microorganisms present in the piggery wastewater. Then, the determination of its physicochemical characteristics was conducted. Lastly, it measured the generated electricity regarding cell voltage, current output, and power density.

The output of the study was a significant contribution to the future development of MFC technology. This development is essential for the search for a sustainable source of electricity. It may also improve treatment methods of piggery and other agricultural wastewater. The data output of this study will serve as a benchmark for further improvement and development related to the microbial fuel cell, wastewater treatment, and piggery waste management system.

## 2 METHODOLOGY

This study followed a systematic process and procedure to carry out the objectives. The following discussion tackles the methodologies and step-by-step procedures to design and develop the device, identify the present microorganisms, determine the substrate's physicochemical characteristics, and measure the generated electricity.

### 2.1 Research Design

This study used the exploratory research design approach to attain its objective. Exploratory research is designed to discover and investigate a certain problem or topic. In this approach, the study starts with an idea, and through the method of collecting data, the hypothesis is tested [13].

Generally, this study explored the performance of a single-chamber air-cathode MFC using graphite-titanium electrodes powered by piggery wastewater. Specifically, it discussed the design and fabrication method implemented in prototyping a single-chamber air-cathode membrane-less MFC. Further, it identified the microorganisms present in the wastewater through biochemical testing. Moreover, it measured the physicochemical characteristics of wastewater before and after the operation in terms of temperature, pH level, dissolved oxygen, total dissolved solids, and electrical conductivity. Lastly, the electrical generation parameters of the device were measured in terms of cell voltage, current output, and power density.

## 2.2 Materials and Equipment

The materials used for the fabrication of the device were a 2-mm thick plexiglass for the chamber and a cover with a hinge attachment. It was cut using a laser cut machine and connected using a silicon adhesive. The graphite and titanium plates were commercially available and purchased through the online shop for the electrodes. An AWG #22 copper wire was used as a cell terminal. A sealant was used to seal and secure the device so it did not leak.

The following measuring devices were used to test the device. Two portable measuring devices were used to measure the physicochemical characteristics of piggery wastewater. One was the multifunctional digital water quality tester (EX-9909SP) for measuring pH level, TDS, and EC. The other was an RC Yago Digital DO meter to determine dissolved oxygen and temperature. These were manually calibrated before every use.

A data acquisition device programmed using an Arduino Mega (ATmega328P-8-bit AVR family) microcontroller was constructed for the electrical parameters. The voltage detector sensor module and INA219 current sensor module were used to measure the voltage and current output, respectively. All data recording was saved on a micro-SD card, and to secure the power supply of the data acquisition device, it was supplied by a universal power supply.

## 2.3 Collection of Piggery Wastewater

A substrate with a mixed culture of microorganisms has a greater potential for power generation [14]. Thus, this present study implemented a mixed culture for the substrate. The substrate acted as a source of nutrients for the microorganisms present in the inoculum. A backyard pigpen located at Brgy. Pinagwarasan, Basud, and Camarines Norte were the sources of piggery wastewater. The substrate comprised 400 mL of wastewater and 100 mL of manure mixed well before pouring into the device. Moreover, a glucose concentration of 5 g/L helps boost the substrate's nutrients for the microorganisms' metabolism [15]. Thus, 2.5 g of glucose was mixed with the substrate for the piggery wastewater.

## 2.4 Identification of Microorganisms

A 2 mL sample of collected piggery wastewater was brought to the laboratory of Leon D. Hernandez Hospital for biochemical testing to identify microorganisms present in the substrate. Upon receiving the wastewater sample, it was inoculated on a blood agar and MacConkey agar plate and was incubated for 48 hours at 37°C. Figure 1 shows the cultivated bacteria from the piggery wastewater prepared to identify species of microorganisms.

## 2.5 Testing of Physicochemical Characteristics

A 100 mL sample was separated from the collected piggery wastewater and poured into a sterilized container for the physicochemical characteristics testing. Two digital water quality testers were used for the testing procedure. One is the multifunctional tester (EX-9909SP) for pH level, TDS, and EC. The meter was placed on the container until the value stabilized before the measurement was recorded. Then the second one is an RC Yago Digital DO meter for DO and temperature.

**Figure 1.** **Biochemical Testing of Piggery Wastewater**

The device was opened after the seventh day of operation of the MFC. Another measurement of the substrate's physicochemical characteristics was conducted. From the device, 100 mL was poured into a separate sterilized container. It was mixed well before the testing. The same procedure was used for the measurement of physicochemical characteristics.

## 2.6 Testing of Electricity Generation

After the collected piggery wastewater was poured into the device, it was sealed using sealant to prevent the leakage of wastewater from the device, and it secured the anaerobic process. It is essential that the operation is anaerobic to ensure the conversion of biochemical energy to electrical energy [4].

Four sample replicates were placed in the engineering laboratory for monitoring at room temperature (27°C~29°C). The MFC was incubated for one day to secure biofilm formation in the anode [3], [16]. After the incubation, the data acquisition device was connected to the device, the wire from the graphite plate was connected to the positive terminal of the device, and the wire from the titanium plate was connected to the negative line, as shown in Figure 2. The MFC was connected to an external load of 200 ohms used [17]. The voltage and current output will be recorded every 5 minutes for seven days of device operation.

**Figure 2.** **Operating 4 Replicated MFCs connected to Data Acquisition Device**

The computation of the power density was based on the formula as seen in (1) [3]:

$$\text{Power Density} = \frac{I\,E_{\text{cell}}}{A_{\text{anode}}} \quad (1)$$

where I was the current output, $E_{cell}$ was the cell voltage, and $A_{anode}$ was the total surface area of the anode. The total surface area of the anode was average to 0.0149 $m^2$.

## 3 RESULTS AND DISCUSSIONS

The following is the discussion of the findings for every specific study objective. Data was presented using figures, tables, and graphs for efficient analysis of the results.

### 3.1 Design and Fabrication of MFC

The architecture used for the MFC was single chamber air-cathode membranes. This design consisted only of one compartment, the anodic chamber, which housed the piggery wastewater substrate and the electrodes. It was considered because of its simplicity and cost-effectiveness; it also performed well compared with other designs [1], [4], [5].

As shown in Figure 3, the operational process of a single chamber air-cathode membrane is less MFC. Inside the chamber, the microorganism present in the piggery wastewater is inoculated on the anode surface, where biofilm develops, and the electron exchange between the microorganisms and anode occurs. The substrate was the source of carbon and nutrients that the microorganisms consumed. Electricity production happens as a by-product of the microorganisms' metabolism [1], [2].

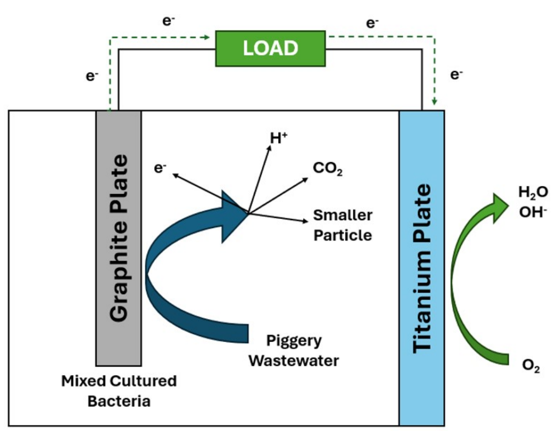


**Figure 3.** Schematic Diagram of the MFC

The design of the MFC must hold 500 mL of the substrate and include the anode electrodes. The chamber was constructed with dimensions of 10 x 11.7 x 6 cm using a plexiglass of 2 mm thickness, as shown in Figure 4. It has an internal volume of 607.50mL. Since the MFC's architecture was air-cathode, one side was opened with a rectangular shape of 7.5 x 7.5 cm centered 2.5 cm from the bottom. With this, the cathode plate was exposed to air and the other side to the substrate for the oxidation process and electron exchange.

The graphite plate acted as the anode electrode for the MFC. The graphite is placed in the chamber's center and submerged in the piggery wastewater. Since the total amount of the wastewater was 500 mL, the graphite plate was cut to a dimension of 10 x 9 cm with a thickness of 2 mm. Thus, the 8 cm x 9 cm x 2 mm surface area of the graphite plate was exposed to the substrate. Therefore, the total surface area for the biofilm formation available for the microorganism was calculated to be 0.0149 $m^2$.

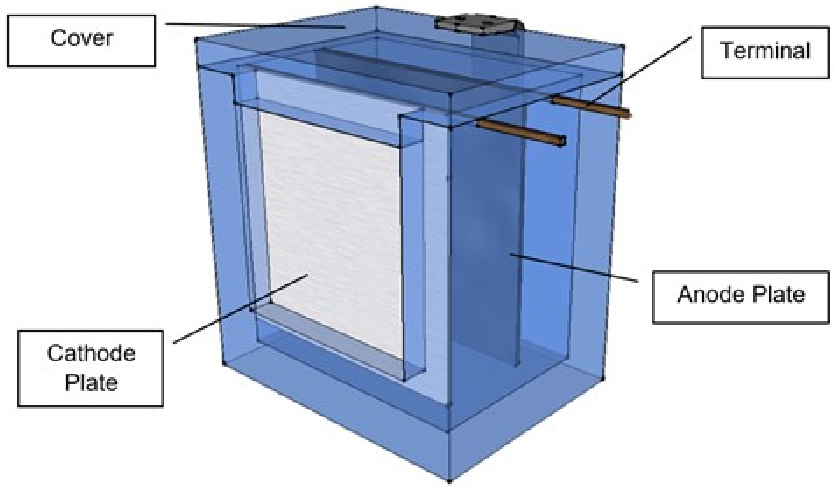


**Figure 4.** **Isometric View of the Single Chamber Air-Cathode Membraneless MFC**

The fabrication process of this MFC followed the steps presented in the following discussion. First, for the chamber, the material used for the MFC was plexiglass with a thickness of 2 mm and dimensions of 10 x 11.7 x 6 cm. The open side of the device was cut with a rectangular shape of 7.5 x 7.5 mm, which was 2 cm from the edge. Each device wall was cut using a laser cutter machine and connected using a silicon adhesive. A hinge was used to secure the opening of the cover. The container was constructed in a glassware fabrication shop.

For the electrodes, graphite and titanium plates were purchased through online stores. The electrode plates were cut to fit in the chamber with a 10 x 9 cm dimension. A hole was punched on each plate for the splicing of conductor wire, serving as a terminal for the device. An #22 AWG conductor wire with a cross-sectional area of 0.34 $mm^2$ was used for these terminal wires. Then, the titanium plate was attached to the open side of the MFC using a sealant. Meanwhile, the graphite plate was placed at the center of the MFC, 2 cm away from the titanium plate. Figure 5 shows the actual fabricated single-chamber air-cathode membrane-less MFC.

**Figure 5.** **Fabricated Single-Chamber Air-Cathode Membraneless MFC**

## 3.2 Microorganisms Present in Piggery Wastewater

The biochemical testing and bacterial identification results were released after three days. The piggery wastewater was identified to contain *Escherichia Coli, Salmonella spp., Shigella spp., and Proteus spp.* These microbes were the composition for the mixed-cultured bacteria introduced to the device.

Escherichia Coli is the most common microbe used for pure cultured MFC among these identified bacteria. This is a non-spore-forming, Gram-negative bacterium, usually motile by peritrichous flagella [18]. It is an active biocatalyst in electricity production in a mediator MFC [19], [20], [21]. However, another study stated that Escherichia coli and Shigella spp. are not exoelectrogens, meaning they cannot produce electricity independently [17].

Meanwhile, Salmonella spp. is a genus of Gram-negative, non-spore-forming prokaryotic rods, facultative anaerobe bacillus belonging to the Enterobacteriaceae family [22], [23]. These microbes are usually associated with a leading cause of infections, such as diarrhea [24]. This microbe is not commonly used for MFC application; some studies reported that Salmonella spp. They didn't grow biofilm and eventually died [17], [25].

Moreover, Shigella spp. is a Gram-negative, non-motile rod found on water's surface and within contaminated drinking waters. This bacteria's little-known ability is to form and survive in biofilms [26]. The study by Zhao et al. [27] identified shigella as an electrogenic bacteria related to electricity production in their MFC device. However, in the study report of Vasieva et al. [28], it has one of the highest losses after the operation of MFC using domestic wastewater.

Lastly, Proteus spp. is a Gram-negative, non-sporing, oxidase-negative, facultatively anaerobic bacilli known for its ability to assume different forms [29]. Proteus is considered a very special electroactive bacteria because of its swarming ability, and most of the genes have a strong survival ability [30].

The result of the microorganism's identification was that the present bacteria were a good indication of producing electricity from the piggery wastewater and being subjected to MFC application. The device employed a mediators system. Thus, it is important to notice the performance of these bacteria in generating electricity in the MFC. Therefore, a further study could be conducted to investigate individual performance and longevity when under operation. Isolation of the bacteria for pure culture can also be explored.

## 3.3 Physicochemical Characteristics of Piggery Wastewater

Physiological characterization was conducted to determine the quality of the piggery wastewater before and after the MFC operation. A 50 mL sample was put in a sterilized plastic container from the collected piggery wastewater. The temperature, pH level, total dissolved solids, electrical conductivity, and dissolved oxygen level were determined using a multifunctional water quality tester. The summary of results is presented in Table 1.

**Table 1. Physicochemical Characteristics of Piggery Wastewater Before & After Operation**

| Physicochemical Characteristics | Before Operation | After Operation |
|---|---|---|
| Temperature | 29.2°C | 29.9°C |
| pH Level | 6.54 | 5.55 |
| DO | 9.53 mg/L | 3.74 mg/L |
| TDS | 3,550 ppm | 6,857 ppm |
| EC | $7,159\,\mu$s/cm | $13,859\,\mu$s/cm |

The testing result showed that the temperature, pH level, and DO were on level enough to sustain the life and the food for the metabolic activity of the microorganisms. The MFC sustained its acidophilic environment [16]. There was a large decrease in DO; it may be interpreted that the microorganisms consumed the oxygen. At the same time, the decrease in pH level indicated an increase in H+, which is a by-product of the oxidation-reduction reaction in the MFC [2]. Further, the increase in DO concentration influenced the performance of the MFC. It increases in internal resistance and, thus, decreases in power density and volume [31]. Contrary to the result of the present study, with the decrease in DO, the performance of the MFC results in a decrease in voltage and power output.

For the TDS, it was observed that the number of organic matter dissolved in wastewater increased. This is a poor indication of treating the wastewater, but this may be related to the chemical changes in the water composition as an effect of oxidation and reduction reactions between the electrodes and substrate. After seven days of operation, the treated piggery wastewater was in a lighter shade of brown than the untreated piggery wastewater. Thus, it is recommended that other tests for water quality, such as BOD and COD, be conducted to verify the effect of MFC in treating wastewater.

The electrical conductivity of a substance is related to the amount of dissolved salt. This parameter is an indication of the capability of a substance to conduct electric current. The study of Tremouli et al. [32] indicated that the increase in salinity or dissolved salt in the electrolyte decreased the electrical performance of the MFC.

## 3.4 Electricity Generation of MFC

A data acquisition device was developed to ensure the continuous recording of output to evaluate the electrical power generation performance of the MFC. The device measured the voltage and current output every 5 seconds for seven days of operation. The recorded data was summarized in an Excel file and presented in the following graphs.

### *3.4.1 Cell Voltage*

The MFC with a graphite and titanium pair of electrodes powered by piggery wastewater was tested and evaluated for its performance in generating electricity. As shown in Figure 6, the initial cell voltage was 80 mV. It reaches the maximum voltage of 112.5 mV after three days. The depletion of voltage starts by four days, which indicates the depletion in the nutrients for microorganisms as reflected in the decrease in DO. However, after the depletion in cell voltage, a sudden spike maximum of 102.5 mW was observed after 157 hours. In another study, these voltage spikes were related to adding substrates like yeast and acetate [33], [34]. However, no new substrate was applied to the system in the present study. The spikes in voltage output may relate to the behavior of microbes inside the anodic chamber. A further study should be conducted to investigate the microbes' reactions closely as the chamber's environment changes.

The cell voltage averages 66.09 mV for the seven-day duration of operation. As compared with the theoretical standard cell potential of graphite and titanium, it can be calculated to be:

$$\text{Power Density} = \frac{I\,E_{\text{cell}}}{A_{\text{anode}}} \quad (1)$$

$$E_{\text{cell}} = E_{\text{cathode}} - E_{\text{anode}} \quad (2)$$

$$E_{\text{cell}} = (-1.63V) - (0.13V) = -1.73V$$

It suggests that the internal resistance affects the cell voltage of the MFC [35], [36].

### *3.4.2 Current Output*

The MFC was connected to an external load of 200 ohms to measure the current output. Due to the limitation of the knowledge of how the device will behave, the actual data tabulated shows a negative value, which indicates that the current flows reversed during the testing. However, for

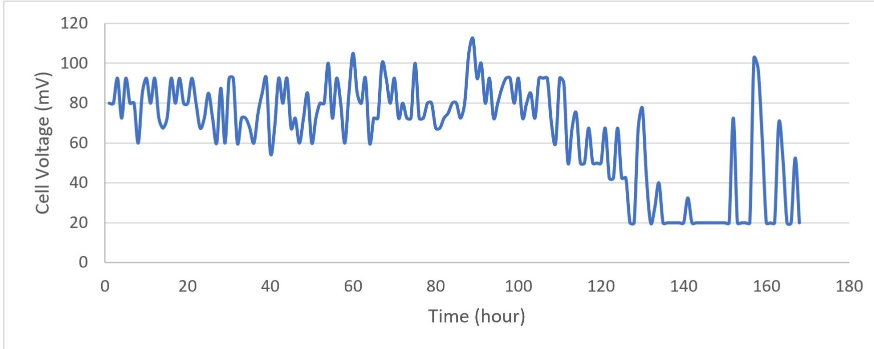


**Figure 6. Cell Voltage vs Time Performance of MFC in 7 Days Operation**

the simplicity of the analysis, the absolute values of the current output were used to analyze the data.

As shown in Figure 7, the initial current output of the MFC was 50 $\mu$A. The maximum current output recorded was 100 $\mu$A after 9 hours and repeated after 40 hours. Although there was a deletion in the cell voltage, the current has consistently flowed at approximately 50 $\mu$A. This almost sustained current output of the MFC can be related to the continuous metabolism activity of microorganisms. Thus, microorganisms can form biofilms on the surface of the anode.

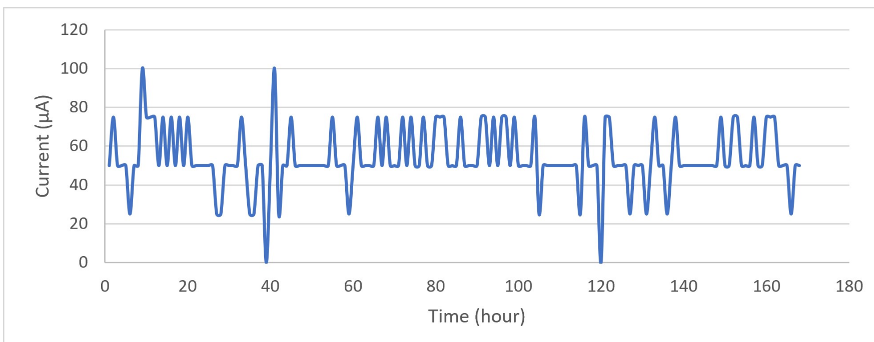


**Figure 7. Current Output vs Time Performance of MFC in 7 Days Operation**

On average, the current output was measured to be 54.02 $\mu$A. Using Ohm's law, the theoretical value of the current output was 330.43 $\mu$A, which is relevantly higher than the actual output.

#### *3.4.3 Power Density*

The performance of the MFC was analyzed according to its power density. It is the amount of power per surface area of the anode. The power density was computed using the Formula 1. The total surface area of the anode was 0.0149 $m^2$. Thus, the calculated power density result is presented in Figure 8.

The graph shows that the maximum power density was 570.47 $\mu W/m^2$ after 9 hours. The power density was uniform until four days, and a decrease in the output was observed. The theoretical power density value was calculated to be 4, 247.06 $\mu W/m^2$.

The study aims to design and develop an MFC that utilizes graphite-titanium as an electrode with piggery wastewater as a substrate. The design single-chamber air-cathode membrane-less MFC can generate electricity with a maximum output of 112.5 mV, 5100 $\mu$A, and 570.47 $\mu W/m^2$. Moreover, the present study showed that a mixed culture of microorganisms present in the

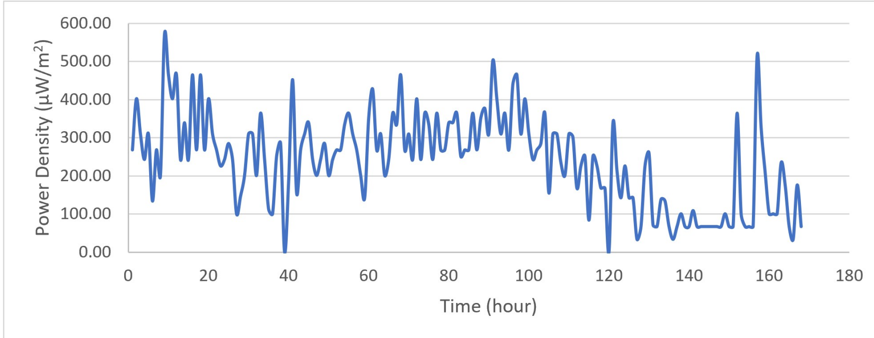


**Figure 8.** Power Density vs Time Performance of MFC in 7 Days Operation

piggery wastewater – Escherichia Coli, Salmonella spp., Shigella spp., and Proteus spp. were the main constituents in generating electricity from the wastewater in a mediators system of MFC.

## 4 CONCLUSION AND RECOMMENDATIONS

Generally, the study designed and developed a microbial fuel cell that utilized a conventional material – graphite and titanium plates as the anode and cathode, respectively. The device was fed with piggery wastewater as the substrate. This device can be a biocatalyst that converts biochemical energy into electrical energy.

The architectural design of the MFC was a single-chamber air-cathode membrane, which was simple to fabricate and cost-effective. The mixed culture of *Escherichia Coli*, *Salmonella spp.*, *Shigella spp.*, and *Proteus spp.* from the piggery wastewater converted biochemical energy into electrical energy in a mediators system. The physicochemical characteristics of the piggery wastewater, such as temperature, pH level, dissolved oxygen, total dissolved solids, and electrical conductivity, affect the metabolic activity of the microorganisms. The generation of electricity can be maintained in an acidophilic environment that causes the decrease of dissolved oxygen and, at the same time, an increase in temperature, total dissolved solids, and electrical conductivity. The generated electricity of the device was generally low for a practical application. However, a large-scale application of the device can be possible by properly designing the connections.

For further study, some of the applied methodologies could be improved. The device's design with a membrane, such as PEM and CEM, can be explored. This may improve the oxidation-reduction reaction between the electrodes and piggery wastewater. Pre-treatment of the graphite and titanium plate may also be introduced to enhance the reaction. The isolation of the bacteria may be considered and subject to injection on the anode plate. Additionally, lengthening the incubation of the anode may secure the growth of biofilms on the surface. Real-time monitoring of the physicochemical parameters of the piggery wastewater may be done to correlate the changes with the performance of the MFC. Lastly, conduct polarization and power curve tools to further characterize the MFC's performance. It is also recommended that the MFC be operated longer to observe the performance.

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