

Research Article

Domestic Organic Waste: A Potential Source to Produce the Energy via a Single-Chamber Microbial Fuel Cell

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Microbial fuel cell (MFC) is a method that is both effective and environmentally friendly for producing renewable electricity. Several studies have shown that one of the major challenges is the generation of electrons as a result of poor exploitation of organic substrates. One of the most talked about issues in modern molecular fusion is the reutilization of biological organic waste in an MFC. In this article, the effective utilization of domestic organic waste as an organic supply for bacterial species to generate energy was highlighted. The findings that were obtained corresponded to the one-of-a-kind MFC operation in which a voltage of 110 mV was generated in a time span of 12 days during operation with an external resistance of 500 Ω . With an internal resistance of 117 Ω , the maximum power density and the current density were recorded 0.1047 mW/m² and 21.84 mA/m², respectively. According to the results of the biological study, strains of bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter schindleri*, and *Pseudomonas nitroreducens* are the ones responsible for producing energy. In addition, final remarks with proposals for the future have been enclosed.

1. Introduction

The massive increase in global energy demand, combined with a constraint of energy supply, poses a serious threat to nonrenewable natural energy assets. A huge utilization of energy causes a worldwide problem by releasing CO₂ and other hazardous chemicals into the atmosphere. Various solutions are employed to minimize energy consumption and related difficulties [1]. However, there are still a few problems to overcome to solve the crisis; for example, one of the primary issues is reducing carbon. Scientists have been concentrating on renewable energy as a solution to ease environmental challenges for the past several decades. Solar

electricity, wind, tidal energy, and hydroelectric energy are all viable alternatives [2]. Bioenergy has emerged as a potential alternative to renewable resources. Since its demonstration in 1911 and renewable electric, the most effective alternative sustainable bioenergy technology is the microbial fuel cell (MFC) [3, 4]. The power of respiring bacteria is exploited by the MFC, which is a bioelectrochemical device that transforms organic substrates into electrical energy without the need of any external fuel sources. The MFC, at its core, is a fuel cell, which, via oxidation and reduction processes, generates power from organic sources [5]. The main distinction is evident in the name, in contrast to conventional fuel cells, which typically involve the oxidation

of a fuel at the anode and reduction at the cathode using chemical catalysts [6].

Microbial fuel cells use microorganisms to perform what they do best: oxidize and reduce organic molecules. Essentially, bacterial respiration is a large redox process in which electrons are transferred. Anywhere there are moving electrons, there is a chance of using an electromotive force for good. An MFC's anode and cathode are separated by a cation-specific membrane. Organic fuel is oxidized by microorganisms at the anode, generating protons that cross the membrane to the cathode and electrons that cross the anode to an external circuit, producing energy [7]. Of course, the secret is to capture the electrons that bacteria emit during respiration. Despite its many benefits, an MFC confronts obstacles including electron transport and generation. Bioenergy efficiency relies on electron production by the bacterial community which is directly associated with a provided organic substrate [8, 9]. According to the findings of a number of studies, carbon derivatives are capable of efficiently transporting electrons from the anode to the cathode but still have a relatively low generation rate. The high rate of electron production in bacterial species is hindered as a result of an inadequate supply of organic substrates [10, 11]. Recently, Fadzli et al. [12] carried out an in-depth literature review and came to the conclusion that it is essential to make use of organic substrates that are rich in carbohydrate content in order to improve energy performance. They also mentioned that the most effective method is to make use of organic substrates that were produced from waste products, such as local waste food, vegetables, bakery waste, trash from sugar industries, and other similar examples. The term "domestic organic waste" refers to organic matter that is produced at home and comes from a variety of different sources. The amount of food that was wasted in households continued to climb on a yearly basis, which was quite concerning [13]. As the process of managing waste gets more challenging, food waste is increasingly seen as a potential risk. Because more people live in well-developed nations, there is a greater volume of food waste to manage. This is because more people consume food, which results in more trash. It is estimated that around 33% of the food that is produced in Southeast Asia will be wasted [14]. It is estimated that, every day, Malaysians throw away between 0.5 and 0.8 kg of food that they have not consumed [15]. There is now a practice of disposing of all of this food waste in a landfill, which results in the attraction of rodents and the pollution of groundwater [16]. Food waste might lead to problems for both the environment and public health if it were not properly treated [17]. Even while humans consider these meals to be garbage, microbes such as bacteria, algae, and fungus see them as a valuable source of nutrients [18]. As a result, a number of academics began looking at ways to manage wasted food in a more effective manner while also providing advantages [19]. The use of domestic waste as an organic substrate, as recommended by earlier research, has the potential to result in a large increase in the amount of energy produced. The aim of this research was to examine

the electrogenic ability of bacteria by using household food waste as a substrate in an MFC system to determine its electron generation capacity. The research study also included electrochemical analyses.

2. Materials and Methods

2.1. Preparation of Organic Substrate. Domestic wastewater was used in this study as an inoculation source. It was collected from the campus cafeteria. On the other side, the domestic organic substrate was collected from the local house which contains mix vegetables, rice, bread, cake pieces, and fish pieces. The collected food waste (1 kg) was put into a plastic bag that could be sealed before being sent to the lab for further processes. The waste was separated from the plastic and bones before being processed for three minutes in an electric blender. For the electrical pulverize to grind the food waste to perfection, domestic wastewater (500 mL) was supplied. The coarse components were then removed from the food waste using a stainless-steel sieve to prevent clogging issues.

2.2. MFC Assembly and Operation. This project included a single-chamber MFC. A 500 mL water container which has two carbon rods served as an anode and a cathode. Each carbon rod was sized 10 cm in length and 1 cm in width. The distance between the cathode and anode was 5 cm. Before the food wastes were dumped into the container, the container was autoclaved in preparation to ensure that no other bacteria from different sources were involved. The container was filled with the prepared organic source which has a total amount of 350 mL. The anode rod was submerged in the bottom of the container, which served as a location for microbe adhesion as well as the generation of electrons. The cathode rod, on the other hand, was immersed in the upper layer of the container. Both the carbon rods had crocodile clips installed. A 500 Ω external resistor was connected to the crocodile clip. In Microbiology Teaching Laboratory A, USM, the MFC container was kept at 24°C at room temperature. A single-chamber MFC's schematic is shown in Figure 1. Organic substrate oxidation is the term given to this process that occurs inside the anode area. The domestic organic waste serves as a substrate for the bacteria to oxidize. To ensure that the outcomes were consistent each time, the experiment was also conducted three times.

2.3. Analytical Calculations. A digital multimeter made in China by UNI-T with the model number UT120B was used to measure the voltage during MFC operation. In addition, the following equations are used in order to provide an interpretation of the observed voltage in terms of power density (PD) and current density (CD). In order to determine the cell's internal resistance, equation (4) is used in the calculating process [20]:

$$V = IR, \quad (1)$$

$$PD = \frac{V}{RA}, \quad (2)$$

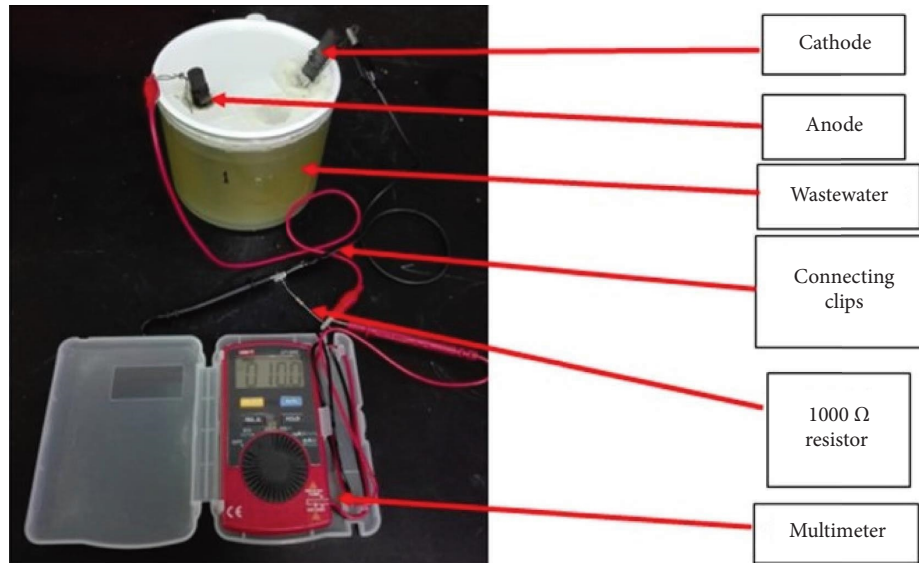


FIGURE 1: Basic used MFC setup in this study.

$$CD = \frac{1}{A}, \quad (3)$$

$$r = \left(\frac{E - V}{V} \right) R, \quad (4)$$

where A is the cross-sectional area, I is the current, R is the external resistance, r is the internal resistance, V is the voltage output, and E is the electromotive force (emf). The voltage of an open circuit was measured using a voltmeter with a high resistance connection, and the emf value was determined. The “Rext-variation” approach, which controlled the external resistance from 5000 to 100 Ω , was used to study the polarization curve. The polarization behaviour was observed when the operation achieved a pseudosteady state. Additionally, to examine the cell’s electrochemical performance, cyclic voltammetry (CV; Model BAS Epsilon Version 1.4; West Lafayette, IN, USA) was used. In the +0.8 V to –0.8 V potential range, CV curves were obtained on the 10th, 15th, and 20th days of the operation using a potentiostat device with a 10 mV/s scan rate. In CV, a Ag/AgCl reference electrode was used. The potential of the reference electrode was used to compare the potential of the electrodes. The specific capacitance denoted by C_p (F/g) is defined by integrating the whole set of data per unit area of the anode and the cathode. From the CV values, C_p is calculated using the following equation:

$$C_p = \frac{A}{2mk(V_2 - V_1)}, \quad (5)$$

where k is the CV scan rate in mV/s, A is the area of CV (AV), m is the number of loaded samples in the CV instrument, and $(V_2 - V_1)$ is the CV potential range (total voltage range).

2.4. Biological Analysis. Scanning electron microscopy (SEM) was used to examine the surface morphology of the electrode with biofilm at the end of operation. Using ethanol

and a phosphate buffer (pH 7), the sample was cleaned in preparation for SEM examination. After SEM examination, autoclaved blades were used to remove the anode electrode biofilm and subsequently isolate bacteria. The serial dilution approach was used to isolate bacteria in this experiment. A marked universal bottle was filled with 1 mL of MFC effluent, and then, it was filled with 9 mL of distilled water. The universal bottle was mixed by inverting the bottle carefully. This universal bottle was labelled as 10⁻¹. The 9 mL distilled water was piped into the next universal container. 1 mL of the first universal bottle’s solution was transferred to the second universal bottle. This procedure was repeated until dilution reached 10⁻⁵. The spread plate approach was used using a sterile nutrition agar plate. Three duplicate plates were plated for each of the successive dilutions. All of the plates were incubated for 24 hours at 37 \pm 2 $^\circ$ C in an incubator. After 24 hours, the plates were examined. The identified bacteria were then streaked on a marked agar plate to form a pure colony. The agar plate was incubated for 24 hours at 37 \pm 2 $^\circ$ C [21, 22].

2.4.1. Glycerol Stock. The isolated, pure bacterial colonies were used to create the glycerol stock. We inoculated 10 mL of nutrient-rich broth with a loopful of pure culture bacteria using a sterile inoculating loop. At 24 $^\circ$ C and 160 rpm, the nutrient broth was cultured for 24 hours. After 24 hours, we aseptically poured 500 μ L of overnight incubated cultures and 500 μ L of 40 percent glycerol into 1.5 mL Eppendorf tubes. The mixture was well combined before being frozen at 80 $^\circ$ C. To maintain bacterial cultures alive for extended periods of time, we use this glycerol stock [23].

2.4.2. Biochemical Analysis for Characterization of Bacteria. The biochemical tests were used to analyze bacterial isolates. All isolates were subjected to a Gram-staining technique prior to the biochemical test. The following biochemical tests were performed: oxidase, catalase, and motility tests.

(1) *Gram-Staining*. To begin, the glass slide was sterilized by placing a drop of distilled water on it. Loops of the bacterial culture from the nutrient agar plate were spread onto the glass slide. The smear was cured using heat. The smear was drenched with crystal violet for 1 minute before being gently rinsed under flowing tap water. The smear was then rinsed gently with flowing tap water after being saturated with iodine for 1 minute. The smear was decolorized for 30 seconds with 95% alcohol, depending on the thickness of the stain on the slide. Water was used to rinse the decolorized smear. Finally, the smear was soaked for 1 minute with safranin, which served as a counterstain, before the slide was softly wiped with water. Finally, the smear was dried before being examined under a 100x magnification light microscope. When seen under a microscope, Gram-positive bacteria look purple due to the thickness of the cell wall, which preserves the color of crystal violet. Gram-negative bacteria, on the other hand, look pinkish red when seen via a light microscope. When Gram-negative bacteria are decolorized with alcohol, they lose their crystal violet color but keep their safranin color.

(2) *Motility Test*. First, 50% nutrient agar was poured into culture tubes, and we waited until the agar solidified. Then, with a sterile straight needle, a colony of a fresh (18 to 24 hour) culture growing on the agar medium was taken, and a single stab was performed at the center of the tube to about 2 inches depth of the medium. Finally, the culture tubes were incubated at 35°–37°C and examined daily for up to 7 days.

2.4.3. *Polymerase Chain Reaction (PCR) and Gel Electrophoresis*. To begin with, PCR tubes were filled with 12.5 μL of master mix, 1 μL each of forward and reverse primers, 1 μL of DNA templates, and 9.5 μL of nuclease-free water. After that, the mixture was put into a PCR machine. Initial denaturation, denaturation, annealing, extension, final extension, and chilling are all phases in the PCR process. The PCR procedure is described in Table 1.

In a conical flask, 30 mL of Tris-acetate-EDTA (TAE) buffer was used to dissolve 0.3 g of agarose gel powder. After heating the mixture in the microwave until no visible powder remained in the glassware, 1.5 μL of Safe Red dye was added. After that, the gel was poured into the casting tray and let to set up. The comb was positioned right after the gel had been poured. A 0.5 M TAE buffer solution was used to flood the gel. 3 μL of the DNA ladder was pipetted into the first well from the left, followed by 2 μL of PCR products and 1 μL of dye, which were mixed and pipetted into the second well. Samples were placed in the following wells. Electrophoresis began at 80 V and lasted for 75 minutes. After removing the gel from the chamber, it was submerged in ethidium bromide (EtBr) solution for 10 minutes before being cooled for 30 minutes. Finally, the gel was submerged for 30 seconds before being exposed to UV radiation. The PCR result was then submitted for sequencing once the bands were produced. Following the identification of the sequencing results, bacterial species were identified using the National Center for Biotechnology Information (NCBI). The

TABLE 1: PCR protocol time.

Processes	Time (sec)	Temperature (°C)	Cycle
Initial denaturation	120	94	1
Denaturation	30	94	30
Annealing	30	52	30
Extension	90	72	30
Final extension	420	72	1
Cooling	∞	4	1

nucleotide BLAST feature was used as well [24]. All the data can be reproduced by following the same procedure.

3. Results and Discussion

3.1. *Voltage and Polarization Trend*. The experiment was carried out effectively in an MFC for a constant 20-day operation. According to Figure 2(a), to generate the maximum possible voltage, on day 12, the voltage was measured to have reached its maximum of 110 mV (0.110 mA). As soon as the first cycle was through, the voltage began to drop and continued until it reached 85 mV (0.85 mA). This could be the result of a certain kind of bacteria reaching the end of its life cycle [25]. Later, it began to increase the trend of voltage once again and eventually reached a maximum of 92 mV; nevertheless, it did not produce a value that was higher than the one recorded in the first time. After day 16 of the operation, the voltage began a steady decline, which served as a clear sign that the process had been successfully completed. The initial increase in voltage is due to the introduction of a new source of inoculum, whereas the voltage declines when certain exoelectrogens complete their life cycle. It entered the death phase, which indicates a diminishing tendency, but, as time passed, the provided substrate enabled the bacterial colony to produce electrons once again [26]. 200 mV was the voltage that was measured in the open circuit. In the subject of MFCs, the current findings are highly interesting when compared to the literature that has been conducted before. For example, by employing the plant-extract sap as an organic substrate, for instance, Yaqoob et al. [26], who come out of a similar trend, were able to obtain 200 mV in only 36 days. This finding suggests that the steady inoculation source is responsible for the greater voltage trend that was discovered in the current investigation.

Additionally, polarization experiments were conducted by varying the external resistance to examine the relationship between PD, CD, and voltage, as shown in Figure 2(b). During the operation of the MFC in a continuous fashion, 5000–100 Ω resistors were attached. Based on the data collected, it was determined that a combination of electronic resistance and a high degree of instability led to poor electron transit when the external resistance was high. In spite of the fact that the rapid transfer of electrons accounted for the low external resistance's demonstration of less stability in the electrical movement, the two cannot be considered independent of one another [27]. Equal internal and external resistances are required for ohm-free electrical mobility inside the cell. A fixed external resistance sequence

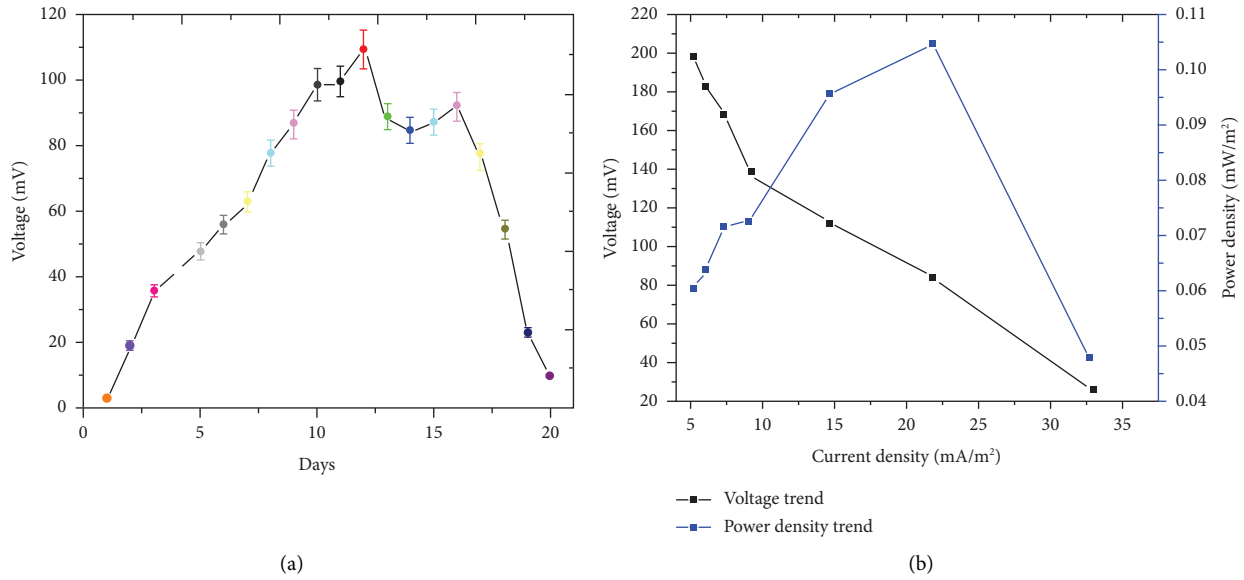


FIGURE 2: Recorded (a) voltage trend and (b) polarization behaviour.

was applied before commencing the method to find the best possible value for the fixed external resistance. Due to the increased external resistance, the voltage fell from its open-circuit value (OCV) but then slowly rose again. However, 500 Ω was recorded to be the cell design point during external resistance modulation. The highest PD was 0.1047 mW/m², and the maximum CD was 21.8441 mA/m². In this case, the calculated internal resistance was 117 Ω . Furthermore, it suggests that an external resistance of greater than 500 Ω may impede electron movement. Since a higher resistance reduces energy production, an external resistance of 500 Ω is optimal. It was found that the highest PD at 100 was 0.047 mW/m², whereas at 5000 Ω , the PD was 0.060 mW/m². This implies that electronic resistance must be considered to maintain stability over the electron's resistivity. Bringing the anode and cathode anode closer together reduces the internal resistance. Previous research favored using a single-chamber-based MFC rather than a double-chamber-based MFC [28–30].

3.2. Cyclic Voltammetry and Specific Capacitance. The measured CV curves at different times during MFC operation are shown in Figure 3 to facilitate analysis of electronic mobility. The CV curves illustrated the current in both the forward scan (FS) and the reverse scan (RS) on various days and at different times. The FS showed the rate of oxidation of organic precursors, whereas the RS indicated the rate of reduction. On day 10, the FS was 3.8×10^{-6} mA, on day 15, it was 4.0×10^{-6} mA, and on day 20, it was 6.1×10^{-6} mA, while the RS was -4.2×10^{-6} mA, 5.0×10^{-6} mA, and -6.5×10^{-6} mA on day 10, 15, and 20, respectively. The FS and RS were both determined to be at their peak levels on day 20. It shows that the rate of organic substrate oxidation and reduction was high and that this rate increased gradually until it peaked on the 20th day of the experiment. In the existing research, the rate of oxidation that reached its

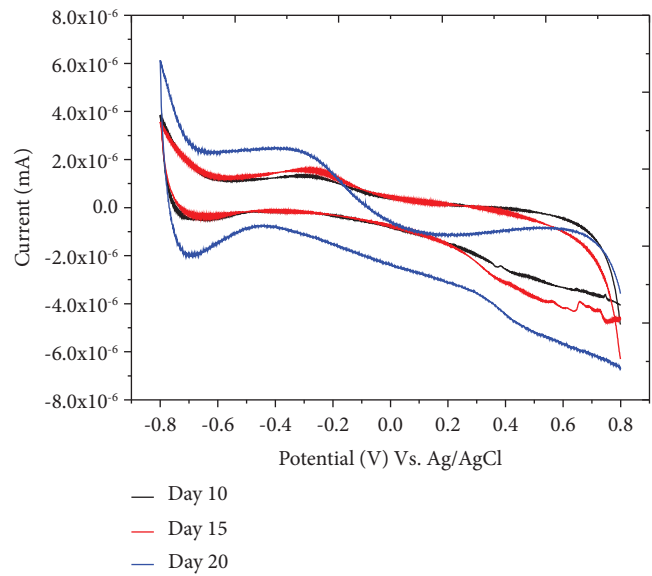


FIGURE 3: Recorded CV curves.

highest point was 0.8 mA, whereas the rate of reduction was -0.7 mA. The CV displayed the greatest overall oxidation and reduction rates throughout the operation. The response recorded relatively quickly in comparison to the previous literature, since food waste was used as inoculation [26, 31].

To get C_p values, CV curves are also evaluated. The C_p values reflected the rate of biofilm development and its consistency throughout the procedure. When we used organic waste from our homes as an inoculant, it was observed that the biofilm formed gradually and was quite stable. By examining the CV curves, we were able to reach this conclusion. In most circumstances, a low C_p value indicates that the biofilm is currently undergoing development, while a value that is less steady but increasingly grows indicates that the biofilm on the anode is stable. The C_p value, as

shown in Table 2, reflected the excellent performance of the biofilm in the present study. Hong et al. [32] observed a similar idea to characterize the biofilm's rate of production and stability using the CV curves.

3.3. Biological Characterization

3.3.1. Isolation of Bacteria from MFC. Through biochemical testing and a molecular technique, the bacterial isolates from the MFC were identified. The bacteria were isolated and purified from the wastewater sample using the streak plate technique. All the isolated bacteria had unique characteristics, and distinct morphologies in specific features were observed. From the wastewater sample, fifteen (15) identified bacteria were isolated and cultured on nutrient agar (NA) (Table 3). Wastewater bacteria are often referred to as nonfastidious bacteria since they use NA. Prior to biochemical testing, all of the isolates were cultured on NA. Using a compound microscope, the morphological characters and shapes were examined. All isolates were labelled as in "P" series.

According to the results which are described in Table 3, all isolated bacterial colonies appeared to have white or cream color. However, P1 appeared brownish, and P2 and P26 appeared greenish. The three different colony forms were observed, which were irregular, round, and punctiform. Only two types of colony elevations were observed which were raised and convex. Raised elevation was observed only for P1, P2, and P26. The remaining isolates that were found had convex elevation besides raised elevation. In addition, the shape of bacteria was identified when it was inspected under a light microscope. The observed bacterial shape was round-shaped or cocci, rod-shaped or bacilli, and between round- and rod-shaped called coccobacilli. Only P5, P8, and P26 were observed as coccobacilli, and other isolates were observed as either bacilli or cocci. The margin of the isolates was observed either smooth or wavy. Among all the isolates, only P1, P2, P17, and P26 appeared as a wavy margin, and the rest of them appeared as a smooth margin. Based on the texture, bacterial isolates were divided into three categories, which are watery, creamy, and sticky.

3.3.2. Biochemical Characterization. To properly define the isolates, the biochemical tests were carried out. Gram-staining, oxidase, catalase, and motility biochemical tests were performed. The biochemistry of bacteria varies depending on the species. The isolates were classified according to how they reacted to the substrates with the use of biochemical characterization.

(1) Gram-Stain. A Gram-staining technique was performed to know which isolated bacteria were Gram-negative or Gram-positive. At the time, the shape of the bacteria was observed. Among the fifteen isolates, only P26 (isolated bacteria) was shown to be Gram-positive, and others were Gram-negative, which is described in Table 4 and Figure 4. Gram-positive bacteria have a simpler biochemical component, consisting of around 90% peptidoglycan and 10%

TABLE 2: Recorded Cp values.

Measurement time interval	Capacitance (F/g)
10 th	0.00006
15 th	0.00006
20 th	0.00011

teichoic acid. Numerous Gram-positive bacteria may have increased surface zeta potential (negative surface charge) due to the covalent interaction between peptidoglycan and teichoic acid [33, 34]. The cell wall of Gram-negative bacteria, on the other hand, is made up of periplasm, an outer membrane protein, and about 10% peptidoglycan, which consists of lipopolysaccharide, lipophosphate, and lipoprotein. As a result, the surface zeta potential of most Gram-negative bacteria seems to be lower. It is probable that Gram-positive and Gram-negative bacteria's different cell surface charges have an impact on how electrogenic they are in MFCs. Therefore, based on the result, we can say that chemical components of the Gram-negative bacterial cell wall and the lower cell surface allow the bacteria to produce higher electricity than Gram-positive bacteria.

(2) Motility Test. For the motility test, 50% nutrient agar was used. This medium has a very soft consistency that makes it easy for bacteria to move through it, which results in cloudiness [35]. Agar that is somewhat solid is poked in the middle with the inoculum. A widespread growth zone that extends from the line of inoculation provides evidence of bacterial motility [35]. While some organisms spread out throughout the whole media, others only exhibit as discrete nodules or regions along the inoculation line. Only the region where the nonmotile bacteria were inoculated in the soft agar tube thrived. Based on the results, all the isolates were negative for the motility test which is described in Table 4 and Figure 5.

(3) 16S rRNA Analysis for Bacterial Identification. By using 16S rRNA sequencing analyses, a total of 5 isolates were identified. The isolates were chosen according to the results obtained in Tables 3 and 4. The isolates identified were P1, P2, P3, P8, and P10. The isolates were identified as *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* (with different strains), *Acinetobacter schindleri*, *Enterobacter sp.*, and *Pseudomonas nitroreducens*. Among the 5 isolates (Table 5), isolates P3, P8, and P10 were observed to have the highest query percentage (99%), and isolate P1 was observed to have the lowest query percentage (91%). Table 5 shows the isolated information from the National Center for Biotechnology Information (NCBI). The found bacterial isolates are well known in MFCs according to the previous literature [36–39].

3.4. Biofilm Study. As a means of assessing the biological aspect of the MFC process, SEM analysis was performed after the operation was completed. The anode and cathode electrodes underwent SEM analysis after the process. Figure 6(a) displays SEM images of both an untreated and a treated anode. The presence of several distinct species of

TABLE 3: General morphology and characteristics of 15 isolated bacteria.

Isolates	Colony color	Form	Elevation	Texture	Margin	Shape
P1	Brownish	Irregular	Raised	Sticky	Wavy	Bacilli
P2	Greenish	Irregular	Raised	Sticky	Wavy	Bacilli
P3	White	Punctiform	Convex	Creamy	Smooth	Cocci
P4	Cream	Punctiform	Convex	Watery	Smooth	Cocci
P5	Cream	Punctiform	Convex	Watery	Smooth	Coccobacilli
P6	White	Punctiform	Convex	Watery	Smooth	Bacilli
P7	White	Round	Convex	Creamy	Smooth	Cocci
P8	White	Round	Convex	Creamy	Smooth	Coccobacilli
P10	White	Punctiform	Convex	Sticky	Smooth	Bacilli
P11	Cream	Round	Convex	Creamy	Smooth	Bacilli
P12	Cream	Punctiform	Convex	Watery	Smooth	Bacilli
P17	Cream	Irregular	Convex	Creamy	Wavy	Bacilli
P19	White	Punctiform	Convex	Creamy	Smooth	Cocci
P22	Cream	Round	Convex	Creamy	Smooth	Bacilli
P26	Greenish	Irregular	Raised	Sticky	Wavy	Coccobacilli

TABLE 4: Summary of biochemical test results of 15 isolated bacteria.

Isolates	Gram-stain	Oxidase test	Catalase test	Motility test
P1	Negative	Positive	Positive	Negative
P2	Negative	Positive	Positive	Negative
P3	Negative	Negative	Positive	Negative
P4	Negative	Positive	Positive	Negative
P5	Negative	Positive	Positive	Negative
P6	Negative	Positive	Negative	Negative
P7	Negative	Negative	Positive	Negative
P8	Negative	Negative	Positive	Negative
P10	Negative	Positive	Positive	Negative
P11	Negative	Positive	Positive	Negative
P12	Negative	Positive	Negative	Negative
P17	Negative	Negative	Positive	Negative
P19	Negative	Negative	Positive	Negative
P22	Negative	Positive	Positive	Negative
P26	Positive	Positive	Positive	Negative

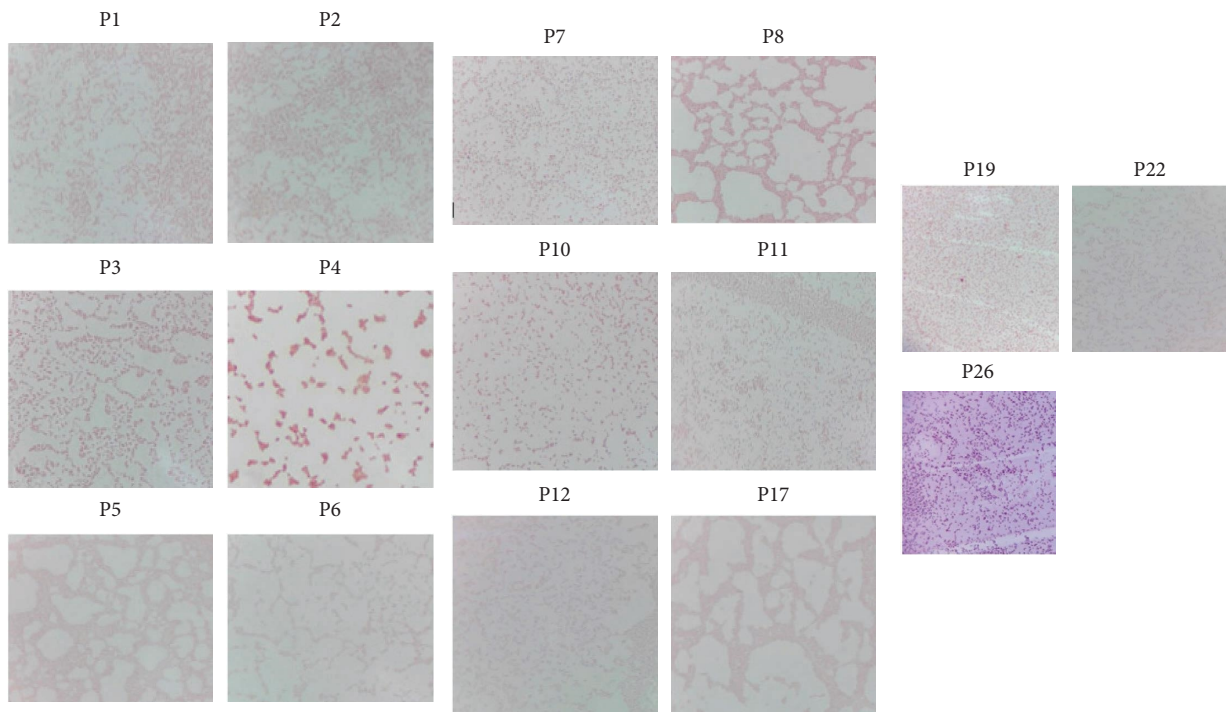


FIGURE 4: Gram-staining of 15 isolates obtained.

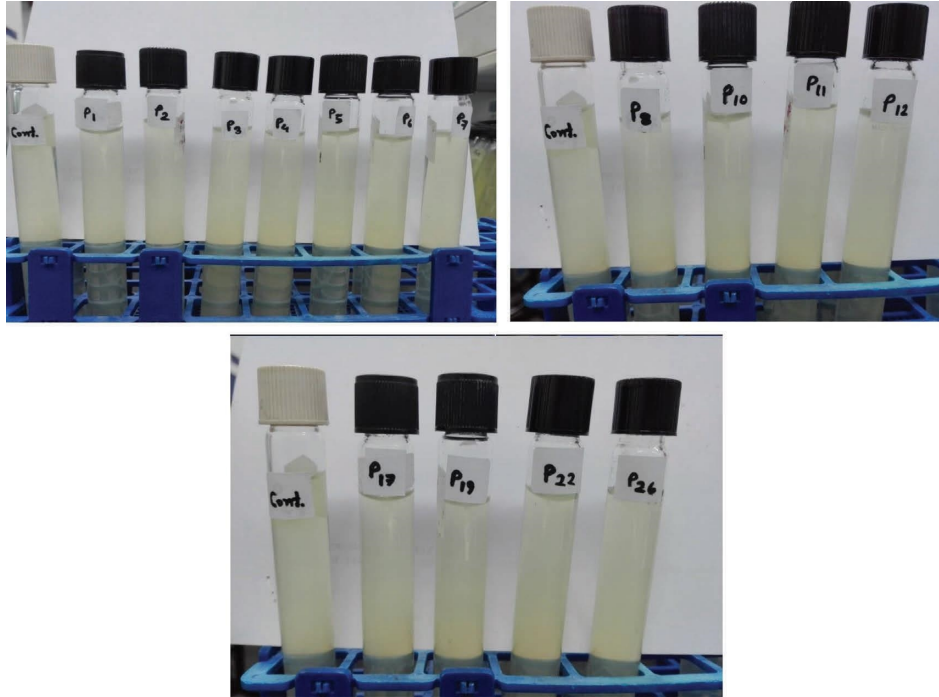


FIGURE 5: Motility test of different isolates.

TABLE 5: Isolate information from the NCBI database.

Isolates	Description	Scientific name	Query cover (%)	Percent identify	Accession number
P1	<i>Pseudomonas aeruginosa</i> strain PDW1018	<i>Pseudomonas aeruginosa</i>	91	97.1%	MZ642711.1
P2	<i>Pseudomonas aeruginosa</i> strain PDW764	<i>Pseudomonas aeruginosa</i>	95	98.27	MZ642721.1
P3	<i>Acinetobacter schindleri</i> strain BL AcIso69	<i>Acinetobacter schindleri</i>	99	99%	FJ860880.1
P8	<i>Enterobacter sp.</i> 18A13	<i>Enterobacter sp.</i>	99	99.26	AP019634.1
P10	<i>Pseudomonas nitroreducens</i> strain HBP1	<i>Pseudomonas nitroreducens</i>	99	96.70	CP049140.1

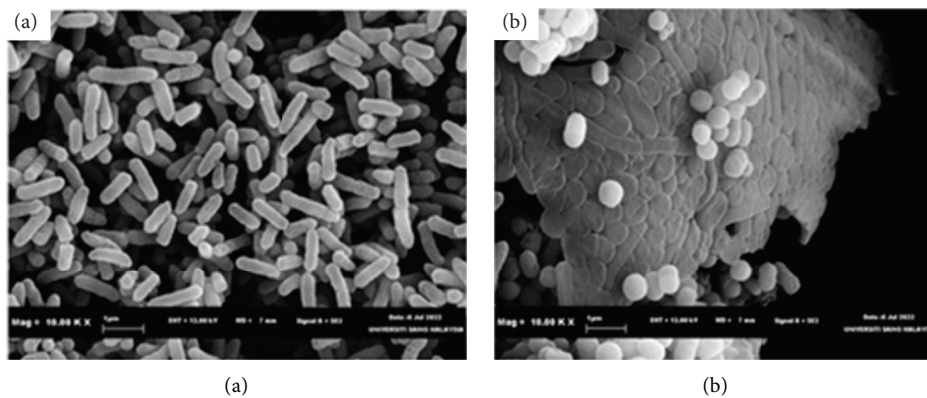


FIGURE 6: SEM images of treated (a) anode and (b) cathode electrodes.

bacteria, as shown in the SEM images, indicates that the procedure was not hampered by toxicity. The inoculum supply supplied was enough to sustain the development of the bacteria, as shown by the abundance and clarity of the bacterial colonies. The organic substrate is critical to the success of an MFC because it provides a constant atmosphere for bacterial growth. The findings of the current

investigation are novel. Figure 6(b), an SEM view of a cathode, also reveals a microbial community composed of different types of bacteria. The SEM analysis revealed that the filaments' appendages had a very similar shape, consisting of tubes or rods. Several studies in the field of MFCs have reported that the presence of conductive pili-based species like *Lysinibacillus* species, *Klebsiella pneumoniae*,

Acinetobacter species, *Bacillus* species, *Escherichia* species, and *Proteus* species is indicated by the presence of filamentous appendages/rod-shaped morphology [26, 40].

4. Concluding Remarks and Future Suggestions

This research revealed the suitability of using domestic food waste as a substrate in MFCs to produce energy in the presence of a common organic substrate found in home. The findings from using waste as a substrate in MFCs were different from those of another research. In only 12 days, we were able to generate 110 mV of voltage. The current study's biological characterizations demonstrated the consistency and quality of the biofilm activities created. The discovered bacterial species are also encouraging news for a practical electron source. However, an MFC has yet to realize its full potential in practical, industrial contexts because of a number of remaining problems. The most significant problem with an MFC is the sluggish rate at which electrons may be transferred from the anode to the cathode. The most important part of an MFC is the electrode material, which must allow electrons to move efficiently and be stable. There is currently no material suitable for MFC applications that can offer extremely efficient electron transportation. There are a number of ongoing initiatives to create reliable electrode material. Graphene-based derivatives generated from waste and metal oxide composites have lately shown promise as a game-changer in electron transport. Preparing electrodes from recycled materials may provide MFCs with a viable long-term strategy for survival. In addition, in the future, it may be possible to increase the shelf life of the substrate in MFCs by using waste products as a source, such as sugar waste and fruit waste based on carbohydrates.

Data Availability

All the data have been included in the text.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Amira Suriaty Yaakop and Fida Hussain contributed equally to this work.

References

- [1] C. Acar and I. Dincer, "Comparative assessment of hydrogen production methods from renewable and non-renewable sources," *International Journal of Hydrogen Energy*, vol. 39, no. 1, pp. 1–12, 2014.
- [2] P. D'Odorico, K. F. Davis, L. Rosa et al., "The global food-energy-water nexus," *Reviews of Geophysics*, vol. 56, no. 3, pp. 456–531, 2018.
- [3] M. C. Potter, "Electrical effects accompanying the decomposition of organic compounds," *Proceedings of the Royal Society of London - Series B: Containing Papers of a Biological Character*, vol. 84, no. 571, pp. 260–276, 1911.
- [4] B. E. Logan and J. M. Regan, "Microbial fuel cells-challenges and applications," *Environmental Science & Technology*, vol. 40, no. 17, pp. 5172–5180, 2006.
- [5] A. A. Yaqoob, M. N. M. Ibrahim, A. S. Yaakop, and A. Ahmad, "Application of microbial fuel cells energized by oil palm trunk sap (OPTS) to remove the toxic metal from synthetic wastewater with generation of electricity," *Applied Nano-science*, vol. 11, no. 6, pp. 1949–1961, 2021.
- [6] S. Arun, A. Sinharoy, K. Pakshirajan, and P. N. Lens, "Algae based microbial fuel cells for wastewater treatment and recovery of value-added products," *Renewable and Sustainable Energy Reviews*, vol. 132, Article ID 110041, 2020.
- [7] M. O. Idris, H. C. Kim, A. A. Yaqoob, and M. N. M. Ibrahim, "Exploring the effectiveness of microbial fuel cell for the degradation of organic pollutants coupled with bio-energy generation," *Sustainable Energy Technologies and Assessments*, vol. 52, Article ID 102183, 2022.
- [8] G. Palanisamy, H. Y. Jung, T. Sadhasivam, M. D. Kurkuri, S. C. Kim, and S.-H. Roh, "A comprehensive review on microbial fuel cell technologies: processes, utilization, and advanced developments in electrodes and membranes," *Journal of Cleaner Production*, vol. 221, pp. 598–621, 2019.
- [9] F. S. Fadzli and Y. A. Suriaty, "Benthic microbial fuel cells: a sustainable approach for metal remediation and electricity generation from saponin waste," *International journal of Environmental Science and Technology*, vol. 10, pp. 1–14, 2022.
- [10] S. Li, C. Cheng, and A. Thomas, "Carbon-based microbial-fuel-cell electrodes: from conductive supports to active catalysts," *Advanced Materials*, vol. 29, no. 8, Article ID 1602547, 2017.
- [11] A. A. Yaqoob, M. N. Mohamad Ibrahim, M. Rafatullah, Y. S. Chua, A. Ahmad, and K. Umar, "Recent advances in anodes for microbial fuel cells: an overview," *Materials*, vol. 13, no. 9, p. 2078, 2020.
- [12] F. S. Fadzli, S. A. Bhawani, and R. E. Adam Mohammad, "Microbial fuel cell: recent developments in organic substrate use and bacterial electrode interaction," *Journal of Chemistry*, vol. 2021, Article ID 4570388, 16 pages, 2021.
- [13] K. Umar and A. S. Yaakop, "Local fruit wastes driven benthic microbial fuel cell: a sustainable approach to toxic metal removal and bioelectricity generation," *Environmental Science and Pollution Research*, vol. 29, pp. 32913–32928, 2022.
- [14] Z. Yang, S. K. Koh, W. C. Ng et al., "Potential application of gasification to recycle food waste and rehabilitate acidic soil from secondary forests on degraded land in Southeast Asia," *Journal of Environmental Management*, vol. 172, pp. 40–48, 2016.
- [15] S. W. Abd Ghafar, *Food Waste in Malaysia: Trends, Current Practices and Key Challenges*, vol. 36, Centre of Promotion Technology, MARDI, Persiaran MARDI-UPM, Serdang, Malaysia, 2017.
- [16] H. Li, Y. Tian, W. Zuo et al., "Electricity generation from food wastes and characteristics of organic matters in microbial fuel cell," *Bioresource Technology*, vol. 205, pp. 104–110, 2016.
- [17] Y. Ahn, M. C. Hatzell, F. Zhang, and B. E. Logan, "Different electrode configurations to optimize performance of multi-electrode microbial fuel cells for generating power or treating domestic wastewater," *Journal of Power Sources*, vol. 249, pp. 440–445, 2014.
- [18] K. Palaniveloo, M. A. Amran, N. A. Norhashim et al., "Food waste composting and microbial community structure profiling," *Processes*, vol. 8, no. 6, p. 723, 2020.
- [19] N. N. M. Daud, A. Ahmad, A. A. Yaqoob, and M. N. M. Ibrahim, "Application of rotten rice as a substrate

- for bacterial species to generate energy and the removal of toxic metals from wastewater through microbial fuel cells,” *Environmental Science and Pollution Research*, vol. 28, no. 44, pp. 62816–62827, 2021.
- [20] A. A. Yaqoob, M. N. Mohamad Ibrahim, and A. S. Yaakop, “Application of oil palm lignocellulosic derived material as an efficient anode to boost the toxic metal remediation trend and energy generation through microbial fuel cells,” *Journal of Cleaner Production*, vol. 314, Article ID 128062, 2021.
- [21] A. Ali Yaqoob, N. Al-Zaqri, A. Suriaty Yaakop, and K. Umar, “Potato waste as an effective source of electron generation and bioremediation of pollutant through benthic microbial fuel cell,” *Sustainable Energy Technologies and Assessments*, vol. 53, Article ID 102560, 2022.
- [22] A. A. Yaqoob, M. A. Bin Abu Bakar, H. C. Kim, A. Ahmad, M. B. Alshammari, and A. S. Yaakop, “Oxidation of food waste as an organic substrate in a single chamber microbial fuel cell to remove the pollutant with energy generation,” *Sustainable Energy Technologies and Assessments*, vol. 52, Article ID 102282, 2022.
- [23] E. Lin, “Glycerol dissimilation and its regulation in bacteria,” *Annual Review of Microbiology*, vol. 30, no. 1, pp. 535–578, 1976.
- [24] A. A. Yaqoob, A. Serrà, S. A. Bhawani et al., “Utilizing biomass-based graphene oxide–polyaniline–Ag electrodes in microbial fuel cells to boost energy generation and heavy metal removal,” *Polymers*, vol. 14, no. 4, p. 845, 2022.
- [25] M. Schaechter, “A brief history of bacterial growth physiology,” *Frontiers in Microbiology*, vol. 6, p. 289, 2015.
- [26] A. A. Yaqoob, M. N. M. Ibrahim, A. S. Yaakop, K. Umar, and A. Ahmad, “Modified graphene oxide anode: a bioinspired waste material for bioremediation of Pb²⁺ with energy generation through microbial fuel cells,” *Chemical Engineering Journal*, vol. 417, Article ID 128052, 2021.
- [27] M. O. Idris, C. Guerrero–Barajas, H. C. Kim, A. A. Yaqoob, and M. N. M. Ibrahim, “Scalability of biomass-derived graphene derivative materials as viable anode electrode for a commercialized microbial fuel cell: a systematic review,” *Chinese Journal of Chemical Engineering*, vol. 55, pp. 277–292, 2023.
- [28] Z. Du, H. Li, and T. Gu, “A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy,” *Biotechnology Advances*, vol. 25, no. 5, pp. 464–482, 2007.
- [29] M. Do, H. Ngo, W. Guo et al., “Challenges in the application of microbial fuel cells to wastewater treatment and energy production: a mini review,” *Science of the Total Environment*, vol. 639, pp. 910–920, 2018.
- [30] K.-J. Chae, M. J. Choi, K.-Y. Kim et al., “Methanogenesis control by employing various environmental stress conditions in two-chambered microbial fuel cells,” *Bioresource Technology*, vol. 101, no. 14, pp. 5350–5357, 2010.
- [31] A. Khan, A. M. Asiri, M. R. Khan, M. Azam, and A. M. AlAmmari, “Cellulose derived graphene/polyaniline nanocomposite anode for energy generation and bioremediation of toxic metals via benthic microbial fuel cells,” *Polymers*, vol. 13, no. 1, p. 135, 2021.
- [32] Y. Hong, D. F. Call, C. M. Werner, and B. E. Logan, “Adaptation to high current using low external resistances eliminates power overshoot in microbial fuel cells,” *Biosensors and Bioelectronics*, vol. 28, no. 1, pp. 71–76, 2011.
- [33] E. R. Caudill, R. T. Hernandez, K. P. Johnson et al., “Wall teichoic acids govern cationic gold nanoparticle interaction with Gram-positive bacterial cell walls,” *Chemical Science*, vol. 11, no. 16, pp. 4106–4118, 2020.
- [34] G. Pankratova, L. Hederstedt, and L. Gorton, “Extracellular electron transfer features of Gram-positive bacteria,” *Analytica Chimica Acta*, vol. 1076, pp. 32–47, 2019.
- [35] S. Aryal, “Motility test – principle, procedure, uses and interpretation,” 2019, <https://microbiologyinfo.com/motility-test/>.
- [36] S. J. Forsythe, S. L. Abbott, and J. Pitout, “Klebsiella, enterobacter, citrobacter, cronobacter, serratia, plesiomonas, and other enterobacteriaceae,” *Manual of clinical microbiology*, pp. 714–737, 2015.
- [37] N. Yasri, E. P. Roberts, and S. Gunasekaran, “The electrochemical perspective of bioelectrocatalytic activities in microbial electrolysis and microbial fuel cells,” *Energy Reports*, vol. 5, pp. 1116–1136, 2019.
- [38] B. Saba, M. Khan, A. D. Christy, and B. V. Kjellerup, “Microbial phyto-power systems—A sustainable integration of phytoremediation and microbial fuel cells,” *Bioelectrochemistry*, vol. 127, pp. 1–11, 2019.
- [39] A. A. Yaqoob, A. Serrà, M. N. M. Ibrahim, and A. S. Yaakop, “Self-assembled oil palm biomass-derived modified graphene oxide anode: an efficient medium for energy transportation and bioremediating Cd (II) via microbial fuel cells,” *Arabian Journal of Chemistry*, vol. 14, no. 5, Article ID 103121, 2021.
- [40] A. A. Yaqoob, M. N. Mohamad Ibrahim, S. Rodríguez-Couto, and A. Ahmad, “Preparation, characterization, and application of modified carbonized lignin as an anode for sustainable microbial fuel cell,” *Process Safety and Environmental Protection*, vol. 155, pp. 49–60, 2021.