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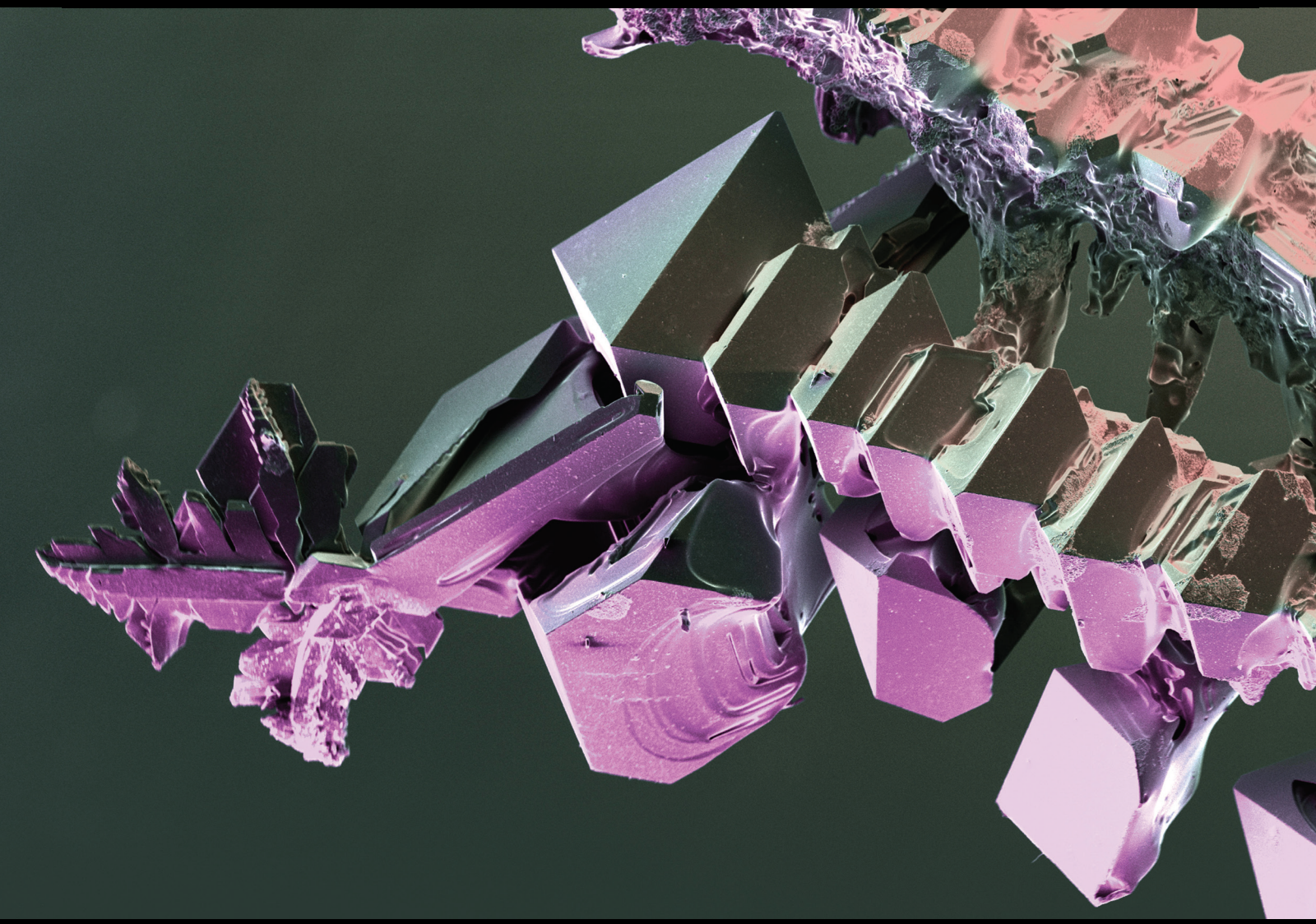

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Research Article

Impact of Commercial Sugar as a Substrate in Single-Chamber Microbial Fuel Cells to Improve the Energy Production with Bioremediation of Metals

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Microbial fuel cells (MFCs) have emerged as a viable method for bioremediation of toxic metals while also producing energy. In this paper, we examine the issue of organic substrate as a source of metabolism for microbe growth in MFC, as well as its significance for metal ion degradation in tandem with energy production. This study focused on the use of commercial sugar as an organic substrate in a single-chamber MFC. The MFC was operated for 27 days, with the highest voltage of 150 mV achieved on day 12, and toxic metal bioremediation efficiencies of 89%, 76.45%, and 89.45% for Pb^{2+}, Cd^{2+}, and Hg^{2+}, respectively. Every 24 hours, the organic substrate (sugar solution) was fed into the cell. This study's mechanism of metal ion degradation and electron transport is also thoroughly described. In addition, some future views have been highlighted.

1. Introduction

Due to increased industrial and human activity, hazardous waste, including toxic metals, is released into the environment. A scientific technique to limit this harmful metal leak has become vital. Most industrial effluents include this hazardous metal, which causes water pollution. As a result, wastewater hazardous metal removal is critical [1]. However, wastewater treatment technology must address a number of issues. Traditional methods of removing toxic metals from wastewater have high operational costs and are not environmentally sustainable [2–4]. Furthermore, several toxic metal treatment approaches, such as solvent extraction, adsorption, ion exchange, chemical precipitation, membrane filtration, and photocatalytic degradation, are highly energy-intensive, with very expensive control and handling procedures [5–7]. As access to portable water becomes an increasingly important need today, some focus is being given to the development of new perspectives on wastewater treatment that are both viable and cost-effective [8]. Furthermore, there is an increase in demand for clean energy that addresses the issue of carbon emissions in the environment [9, 10]. Microbial fuel cells (MFCs) have been identified as a promising emerging technique for recovering toxic metals from wastewater while also producing bioelectricity [11]. Because of its low operating cost and ecofriendliness, the MFC approach to toxic metal removal from wastewater could address the drawbacks of conventional methods of treatment [12]. MFC is a bioelectrochemical technique that uses chemical energy in the form of microbial organic substrates like carbon

sources to generate electrical energy while removing pollutants [13]. The most basic type of MFC is composed of an anodic region and a cathodic region separated by a proton exchange membrane to allow protons to pass through while restricting electrolyte movement from one region to the other [14]. The electroactive microbes facilitate electron transfer to the anode by utilizing the organic substrate, which is coupled with the oxygen reduction process in the cathodic region [15]. Various organic substrates have been used in MFC studies, including conventional glucose, acetates, and carbon sources derived from organic waste. These substrates have proven to provide a carbon source for bacterial species; however, they are yet to be efficient enough in terms of enhancing the system's overall performance [16]. The microbial species in the MFC derived their metabolism from the organic substrates, which aided in their growth and population, thereby improving electron generation and proton mobility in the MFC. Its poor performance as a carbon source for the community of bacterial species is primarily due to its instability [17]. This issue is a major setback for the MFC system, necessitating additional research to find an efficient and most suitable material capable of providing sufficient carbon and facilitating efficient metabolism for the microbes to support their enhanced electrogenic activities. The ability of microbial species to interact with the anode in the most efficient way possible during the electron transfer process is the maximum value of the MFC system [18]. In this study, commercial sugar is used as an organic substrate for MFC. Commercial sugar, also known as table sugar, is one of the most used ingredients in the production of foods and beverages. Wikipedia reports that white commercial sugar has 97% to almost 100% carbohydrates, less than 2% water, and no nutritional fat, protein, or fiber. Appropriate sugar concentrations stimulate bacterial growth, but at high concentrations, they may act as an antimicrobial agent [19]. As a result, for the bioremediation of Pb^{2+}, Cd^{2+}, and Hg^{2+} supplemented wastewater, this study employs an appropriate concentration of commercial sugar as a carbon source for the MFC system bacterial species. The substrate's efficiency in facilitating toxic metal recovery with simultaneous power generation via a single chamber MFC was investigated.

2. Experimental Details

2.1. Materials and Reagents. Commercial sugar (table sugar) obtained from a local market, tap water, lead nitrate (R&M chemicals), cadmium nitrate tetrahydrate (R&M chemicals), mercury nitrate (Sigma Aldrich), and distilled water were used in this study.

2.2. Inoculation Source. The wastewater was collected from a pond and treated with toxic metal ions at a concentration of 100 ppm. In the current study, the metal-supplemented wastewater was designated as synthetic wastewater and then used as a source of inoculation for the single chamber MFC. Table 1 displays various physicochemical parameters for the fresh and synthetic wastewaters. About 50 g of commercial sugar was dissolved in 500 mL of distilled water, and 10 mL of the sugar solution was supplied to the MFC daily. The thermometer (ZEAL LTD; UK), pH metre (EUTECH inst. USA), and electrical conductivity metre (Alpha/800, USA) were used to measure temperature, pH, and conductivity, respectively.

Table 1: The fresh and synthetic wastewater parameters as used in the present study.

Parameters	Fresh wastewater	Synthetic wastewater
Colour	Yellowish	Light yellow
Odour	Bad odour	Bad odour
Temperature	Room temperature	Room temperature
pH	6.93	6.23
Electrical conductivity	30 μs/cm	143 μs/cm
Pb^{2+}	0 ppm	50 ppm
Cd^{2+}	0 ppm	50 ppm
Hg^{2+}	0 ppm	50 ppm

2.3. MFC Setup and Operation. In the current study, a single-chamber MFC was used, with dimensions of 23 cm × 11 cm in length and diameter, respectively. The chamber tank had a capacity of around 700 mL, but 500 mL of toxic metal-supplemented wastewater was inoculated with the prepared sugar solution that is supplied to the system daily. The commercial graphite rods, measuring 9.0 cm × 1 cm ($h \times r$), were then used as the anode and cathode electrodes in the MFC, which were vertically placed at the edges. Copper wire was used to connect the electrodes, and a 1 kΩ external resistance was used to connect them. The external resistance was selected according to an external resistance selection procedure, as explained in previous literature [17]. The MFC was operated at room temperature for 27 days while voltage output was recorded.

2.4. Electrochemical Calculations. The voltage generated by the electronic interactions of the anode and cathode was measured using a digital multimeter every 24 hours. The current value in amperes was calculated using Ohm's basic law. To calculate the current density (CD), power density (PD), and internal resistance (r), equations (1)–(4) were used, where V denotes the voltage output, I denotes the current, A denotes the electrode area, r denotes the internal resistance, R denotes the external resistance, and E denotes the electromotive force. The OCV was used to measure the electromotive force. The internal resistance of MFC was determined using the polarization curve slope with a resistive load ranging from 5000 Ω to 100 Ω

$$V = \mathrm{IR}, \tag{1}$$

$$\mathrm{PD} = \frac{V^2}{\mathrm{RA}}, \tag{2}$$

$$\mathrm{CD} = \frac{I}{A}, \tag{3}$$

$$r = \frac{E - V}{V} R. \tag{4}$$

Furthermore, to characterise the redox events involved on the anode surface, cyclic voltammetry (CV) was used. The CV parameters were set to a scanning rate of 30 mV/s and a potential range of +0.8 V to 0.8 V. The analysis was carried out at days 10, 15, 20, and 27 (final day) of operational intervals. While platinum wire was used as the counter electrode, the reference electrode was Ag/AgCl. The specific capacitance (Cp) is defined as the sum of the anode and cathode integrations over the complete sequence of data per unit area of the cathode and anode. The Cp of each day's intervals was calculated from the CV accordingly using the following equation:

$$C_P = \frac{A}{2mk\,(V_2 - V_1)}. \tag{5}$$

2.5. Bioremediation Efficiency Calculation and Biofilm Studies. To evaluate the toxic metal bioremediation efficiency of the system, atomic absorption spectroscopy (AAS) for heavy metal analysis was used. Briefly, about 5 mL of the synthetic wastewater was collected from the MFC every 5 days to analyze its residual metal contents. The bioremediation efficiency (BE) of each toxic metal after the AAS analysis was calculated following equation (6), where C_1 denotes the initial metal ion concentration and C_2 denotes the final metal ion concentration. Furthermore, scanning electron microscopy (SEM) was used to investigate the biofilm community around the electrode surface at the end of the operation. The SEM analysis was performed on the treated anode and cathode, which were considered to contain stable biofilms. Furthermore, the elemental composition and morphology of the anodic biofilm were examined using electron dispersive X-ray (EDX).

$$\mathrm{BE} = \frac{C_1 - C_2}{C_1} \times 100. \tag{6}$$

3. Results and Discussion

3.1. Voltage Distribution, Polarization, and Internal Resistance Studies. The experiment was carried out successfully for a total of 27 days by supplying 10 mL of sugar solution as an inoculum source in the MFC. According to Figure 1(a), the operation was completed in a single cycle stage with maximum voltage generation. On day 12, the maximum obtained voltage output was 150 mV. The voltage was observed to drastically decrease to 0.0 mV on the 25th, 26th, and 27th days when the operation was finally stopped. This could be because the bacterial species has completed its life cycle and can no longer engage in electrogenic activities. According to the findings, the voltage output began low and steadily increased until it reached its peak on the 12th day. Thereafter, the voltage trend begins to decline. The decreasing voltage output value indicates that the electroactive bacteria were approaching the end of their life cycles, and as a result, their performance and stability became very low. This pattern was observed until the voltage output reached zero on day 25 and could not be restored until the operation was halted on the 27th day. Furthermore, as the voltage output decreases, the exoelectrogens are unable to derive their metabolism from the oxidation process of the continuous supply of sugar solution; hence, the process progresses towards completion. Although the maximum voltage was recorded on the 12th day, a few study results have revealed that the point at which the voltage is the maximum is also a direct indication of a significant change in the metal's state from soluble to insoluble [20–22].

The polarization experiment was carried out by comparing voltage output, PD, and CD across a varying external resistance range of 5000 Ω to 100 Ω. This is presented in Figure 1(b). During the continuous MFC operation, the 5000 Ω to 100 Ω resistors were connected separately at every test. Due to electronic resistance and high voltage destabilization, high external resistance demonstrated low electron transportation. Due to the rapid transfer of electrons, the low external resistance showed less stability in the electronic movement. When the voltage drops during the polarization experiment, the CD rises. The highest value of PD obtained at 100 Ω was 0.108 mW/m^2, but at 5000 Ω, this only offered 0.069 mW/m^2. The voltage output was steadily increasing but did not stabilize at low external resistance; however, quick stability was observed at higher resistance even though electrons were generated and flowed at a high enough level. The high electron flow causes voltage instability. The external supply of oxygen increased the cathodic reaction rate, which helped to stabilize the potential despite the increased resistance. The internal resistance of the cell was calculated to be 545.0 Ω. This polarization approach scenario and voltage generation trends have been described in a few previous studies [23–25].

3.2. Cyclic Voltammetry (CV) and Specific Capacitance. The CV study was performed at various operational intervals, as shown in Figure 2(a). In this study, the CV curves were taken at various time intervals to investigate the electronic mobility and redox potentials of the system during MFC operation [26]. The CV curves displayed the current values in the forward scan (FS) and reverse scan (RS) at various days, corresponding to the oxidation and reduction processes, respectively. The FS was 1.9×10^{-4} mA on day 10, 2.2×10^{-4} mA on day 15, 2.6×10^{-4} mA on day 20, and 3.2×10^{-4} mA on day 27, while the RS on day 10 was 2.8×10^{-4} mA, -4.0×10^{-4} mA on day 15, -4.6×10^{-4} mA on day 20, and 4.80×10^{-4} mA on day 27. It means that the rate of oxidation and reduction of organic substrate was high, increasing gradually and reaching a maximum on the 27th day. Overall, the CV study demonstrated that adequate oxidation and reduction processes occurred throughout the MFC operation. In comparison to previous studies [27, 28], the reaction was quite fast due to the sugar solution serving as an inoculation.

Furthermore, the CV curves provide information for calculating the Cp values. The Cp values demonstrated the rate of biofilm formation and stability throughout the

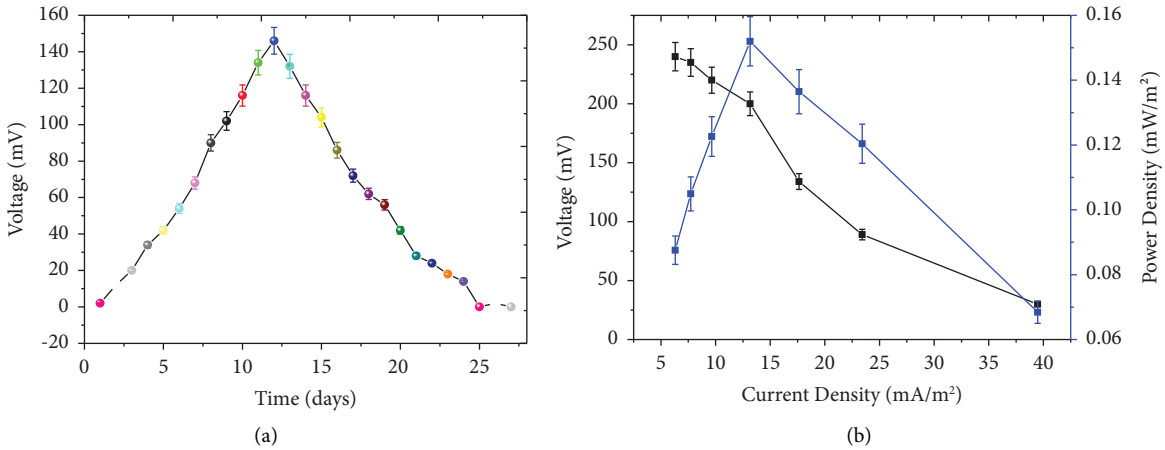


Figure 1: (a) Voltage output distribution; (b) polarization curve.

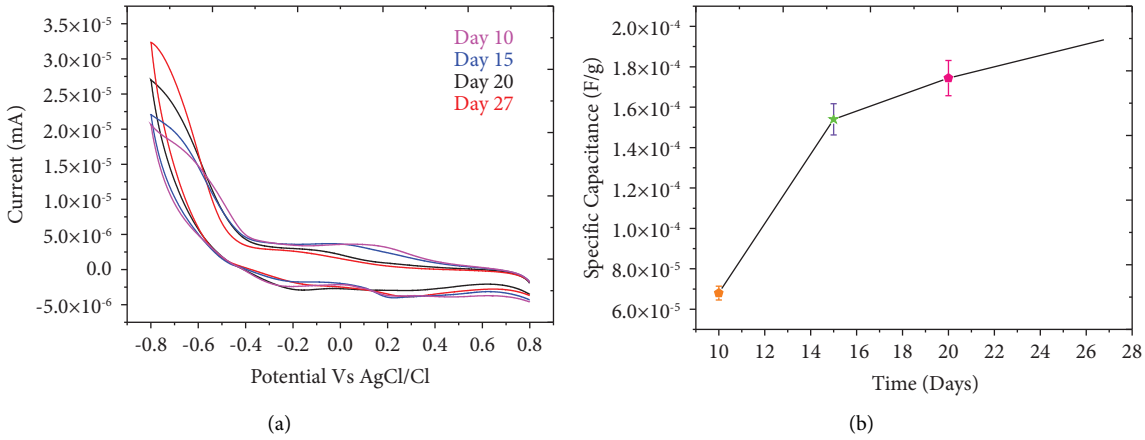
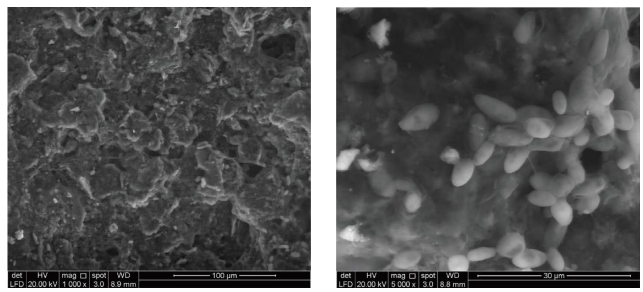


Figure 2: (a) Plot of CV at various time intervals; (b) specific capacitance pattern.

operation. The Cp value at each stage demonstrates that the biofilm was gradually produced and demonstrated good stability with the sugar solution inoculation source. Typically, a low Cp value indicates that biofilm growth is in progress, while a less stable but gradually increasing value indicates high biofilm development stability. Figure 2(b) depicts the Cp value of the current study at different study intervals, demonstrating the high performance of biofilm. Hong et al. [29] used a similar concept to describe the biofilm formation rate and stability using CV curves.

3.3. SEM and EDX Biofilm Studies of the Anode. On reaching the end of the MFC operation, the SEM-EDX investigation was conducted to analyze the microbial aspect of the process. Figure 3 depicts the anode and cathode SEM images at the end of the reaction. There is a diverse population of different bacterial species visible in the SEM images, which could provide proof of the absence of toxicity in the system. Because of the abundance and distinct spread of species of bacteria, it is possible to conclude that the supply of organic substrate was sufficient for strains of bacteria to develop and function [30]. During MFC operation, the organic substrate is critical to the growth and stability of bacterial populations. The current investigation's SEM observations revealed that there is a noticeable growth of microbes with rod-like appendages on the surfaces. Several studies in the field of MFC have found that the presence of rod-shaped filaments and appendages on SEM images reveals the existence of conductive-pili species. In accordance with the literature, these conductive pili-based species include *Acinetobacter* sp., *Lysinibacillus* sp., *Escherichia* sp., and *Klebsiella* sp. [31].

In addition, an EDX analysis was performed to examine the biofilms and observe any adsorption effects on the anode surface. There was no harmful material detected on the surface of the biofilm. Besides, no toxic metal was discovered, indicating that there was no adsorption effect in place. Figure 4 depicts the EDX spectra of the anode electrode following the MFC process. This also implies that the bacterial community grew rapidly and continuously until the substrate was completely oxidized [32].



(a) (b)

Figure 3: Electrodes SEM image at the final day of MFC operation: (a) anode electrode; (b) cathode electrode.

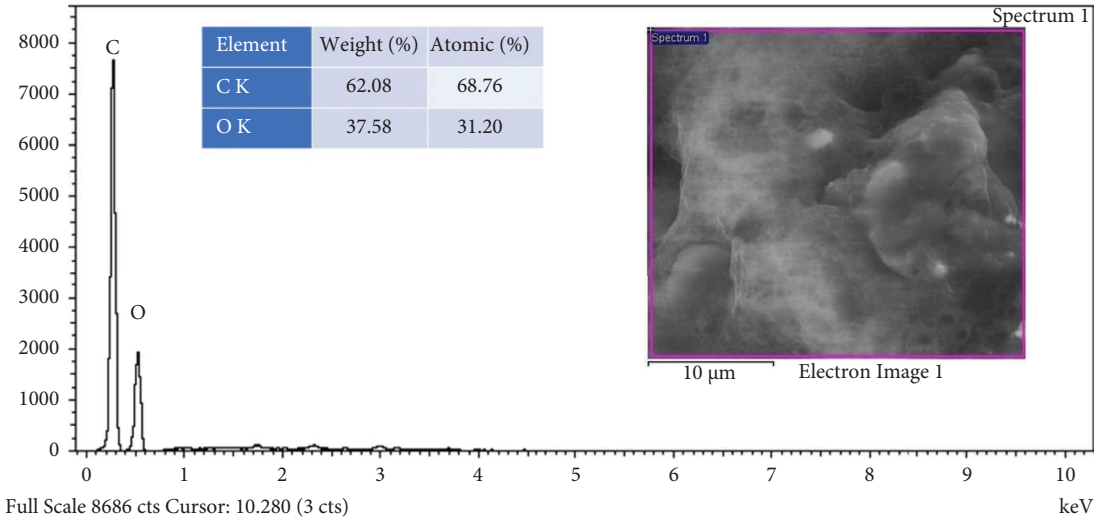


Figure 4: The EDX spectra of the anode biofilm at the completion of the MFC process.

3.4. Conductivity Studies. Figure 5 depicts the study of the conductivity trend at various time intervals. During the 27 days of MFC operation, day intervals were set to measure the cell's conductivity value. The conductivity value on the first day was 143 μS/cm, which gradually increased until the 25th day (1500 μS/cm). After the 25th day, they gradually decreased until the final day of the operation (820 μS/cm). This also implies that the conductivity was high on the 25th day, implying that the voltage output was higher at that time. The system's efficacy then declines due to a variety of factors such as the redox process, pH, organic substrate, temperature, and bacterial instability [33, 34]. Rojas-Flores et al. [35] recently reported a similar conductivity impact in MFC operation.

3.5. Toxic Metal Degradation and Its Mechanism. The MFC degradation of toxic metal ions is a promising trend nowadays, as the most recent area of a study recently conceived of the idea of using a large sugar-based substrate to produce energy while reducing metal ion concentration. Table 2 displays the heavy metal remediation data from the current study. Metal remediation via bio-electrochemical systems is the most recent and promising approach, particularly for MFC. The concentration of 50 ppm for every metal was preferred because previous research indicated that it was the most beneficial in MFC. For example, Li et al. [36] studied various Pb^{2+} and Cr^{6+} concentrations and discovered that 50 ppm provided the highest percentage of removal while possessing no toxic effect on the microbial community. Overall, 89.00% removal efficiency for Pb^{2+} was achieved in this study, while 76.45% Cd^{2+} and 89.45% Hg^{2+} were removed within the 27-day operation. Based on the developments and data, the metal ion concentration gradually decreases as the reaction progresses. It was not initially very high, but with the passage of time, it increased the remediation efficiency to more than 70%. The remediation efficiency was calculated using equation (6). The AAS is only used to determine the concentration of metal ions in the cell; it is not used to calculate the remediation efficiency. Because of the steady inoculum source, a high level of toxic metal remediation was achieved. Commercial sugar has been shown to be an excellent substrate for microorganisms' extracellular electron transport.

In addition, the mechanism of metal degradation and electron transfer was investigated. For performance evaluation, the MFC approach is primarily dependent on the electroactiveness of bacterial species. A few well-known exoelectrogen and metal-degrading bacterial species were

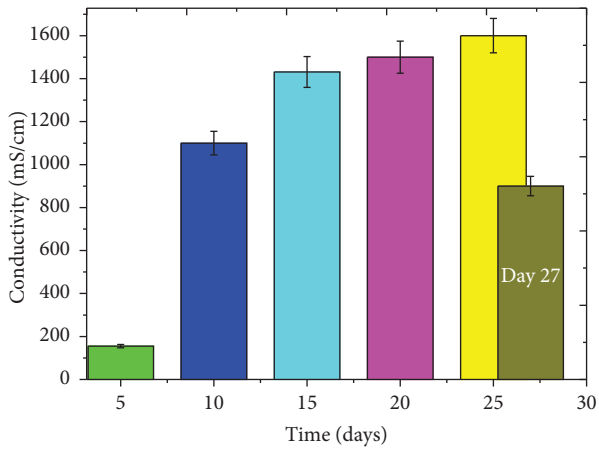


Figure 5: Conductivity value at various intervals of days.

Table 2: Percentage remediation trends of the toxic metal ion supplemented in the MFC system.

Toxic metal	Metal initial conc. (ppm)	Intervals (days)	Metal final conc. (ppm)	Remediation efficiency (%)
Pb^{2+}	50	10	32.63	34.74
		20	15.27	69.47
		27	5.50	89.00
Cd^{2+}	50	10	29.91	40.18
		20	15.27	69.47
		27	11.78	76.45
Hg^{2+}	50	10	23.22	53.57
		20	14.62	70.77
		27	5.28	89.45

responsible for this study. In MFC, bacterial species oxidize the organic substrate, allowing electrons and protons to be generated [37]. In the current study, bacterial species initiate the oxidation process for sugar solutions as organic substrates to generate and mobilize electrons and protons. The oxidation and reduction reactions can be written in the following way (equations (7) to (9)):

$$\text{Anodic reaction: Sugar} \longrightarrow C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 24H^+ + 24e^-, \quad (7)$$

$$\text{Cathodic reaction: } 24H^+ + 24e^- + 6O_2 \longrightarrow 12H_2O, \quad (8)$$

$$\text{Overall reaction: } C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy} + \text{Biomass}. \quad (9)$$

The electrons and protons produced are transferred to the anode electrode and subsequently to the cathode. The proton is usually transferred directly from the anode to the cathode, whereas the electrons are carried along the connecting outer circuit to the cathode [38]. Furthermore, before electron transport to the cathode, there is a phase of interaction between the bacterial cells and the anode electrode that results in electron transfer from the bacteria to the anode. The biofilm-covered round anode produced is a collection of bacterial electrogenic activities. Figure 6 depicts the most reported mechanism for electron transference from bacterial species to anode electrodes.

The soluble metal ions, on the one hand, are converted into insoluble states. Besides, AAS outcomes only reveal the residual metal ion concentrations. Metal ions that have been removed from MFC are converted to an oxide form and form a sludge-like paste. In several reports, the removed metal ions are converted directly to the oxide state, and the

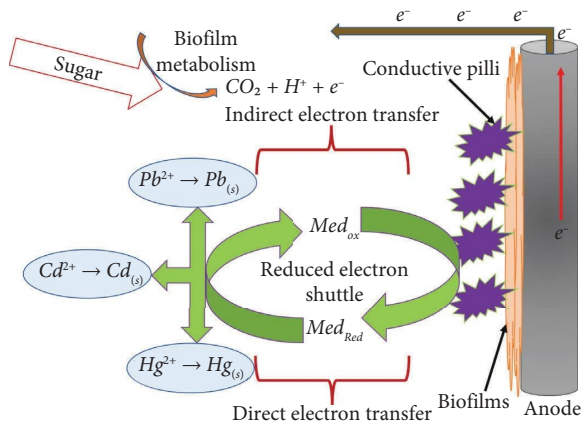


Figure 6: Proposed mechanism of the toxic metal degradation with the electron transfer mechanism.

subsequent sludge contains metals in the oxide form [39]. On the other hand, the metal ion biochemical reaction that occurs in the cell can be written as follows:

$$\begin{aligned} &Pb^{2+} \text{ reduction: } Pb^{2+} + 2e^- \longrightarrow Pb_{(s)}, \\ &Cd^{2+} \text{ reduction: } Cd^{2+} + 2e^- \longrightarrow Cd_{(s)}, \\ &Hg^{2+} \text{ reduction: } Hg^{2+} + 2e^- \longrightarrow Hg_{(s)}. \end{aligned} \tag{10}$$

4. Challenges and Future Perspective

MFC has opened new research avenues and is managed in an environmentally friendly and ecologically stable manner for power generation and wastewater bioremediation. MFCs are becoming more popular, and they can be used in a wide range of applications, including wastewater treatment, which includes the bioremediation of toxic metals and organic contaminants as well as the use of biological and oxygen demand sensors. Furthermore, researchers have identified two types of MFC: benthic and sedimentary MFC, which could offer a wide range of possibilities for enabling sea-bred gadgets, tracking and monitoring systems, and so on. As a result of the development of high-conductivity electrodes and their modification with metallic elements or conducting polymers, MFCs are becoming more common and important in electrical applications. MFC is a more advanced and promising bioelectrochemical cell that has proven to be a safe and sustainable source of energy for humans while also preserving the earth's clean environment. Furthermore, MFC is a developing scientific topic, and commercialization will require significant effort and time. Currently, one of the emerging issues in MFC is the instability of organic substrates and electrode materials [40]. Although the current study produced positive results, they could not last longer than 25 to 27 days. A stable, long-term organic substrate is required for commercial-scale MFC practice. Recently, oil palm trunk sap was used as a substrate in MFC and demonstrated 90-day stability. To address this issue, a high level of stability with a high content of the sugar-based organic substrate is required. Another common issue in MFC is the use of electrode material. Due to the electrode material, the energy was still insufficient via MFC. The electrode material, particularly the anode, should transport electrons more efficiently while also providing a biocompatible environment for bacteria to form a biofilm around the anode surface. Recently, the waste-derived electrode material has received the most attention due to its low cost and high performance [41]. The conversion of biowaste materials into electrode forms, such as anodes made from agro-waste biomass, has previously been well studied. Now, significant efforts are required in this field.

5. Conclusion

The current study focused on the use of commercial sugar (table sugar) in MFC as an organic substrate for bacterial species to bioremediate the toxic metal in the metal-supplemented wastewater while also generating bioenergy. The present investigation produced interesting results, such as a 150-mV voltage recorded in 12 days of MFC operation and a maximum remediation efficiency of highly toxic metals of more than 70%. When compared to several other organic substrates that have recently been studied, the commercial sugar source demonstrated easy oxidation as an organic substrate. SEM/EDX analysis revealed the presence of clusters of bacterial biofilms on the anode surface, which were responsible for the enhanced toxic metal degradation process. Furthermore, well-known species of exoelectrogen microbes were discovered in the MFC operation, which is currently underway, according to the data. The beneficial bacterial activities that occur as a result of sugar oxidation are contributing to the high performance with which metals are bioremediated. It implies that the organic substrate was subjected to an intense oxidation process, resulting in an abundance of mobilized electrons as a byproduct. Similarly, the CV analysis results revealed that oxidation was steadily increasing and a growing biofilm was forming with no harmful effect on the anode surface. The MFC can be extracted from the instability of the organic substrate factor by using a high-carbohydrate and microbially suitable organic substrate. However, efforts to commercialize MFC are still ongoing. The problems that must be overcome to bring MFC to the level of commercial viability may be addressed with the collaboration of professionals from diverse fields such as material sciences, physics, microbiology, and electrochemistry.

Data Availability

All the data have been included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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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/m^2 and 21.84 mA/m^2, 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 CO_2 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]:

$$V = \mathrm{IR}, \tag{1}$$

$$\mathrm{PD} = \frac{V}{\mathrm{RA}}, \tag{2}$$

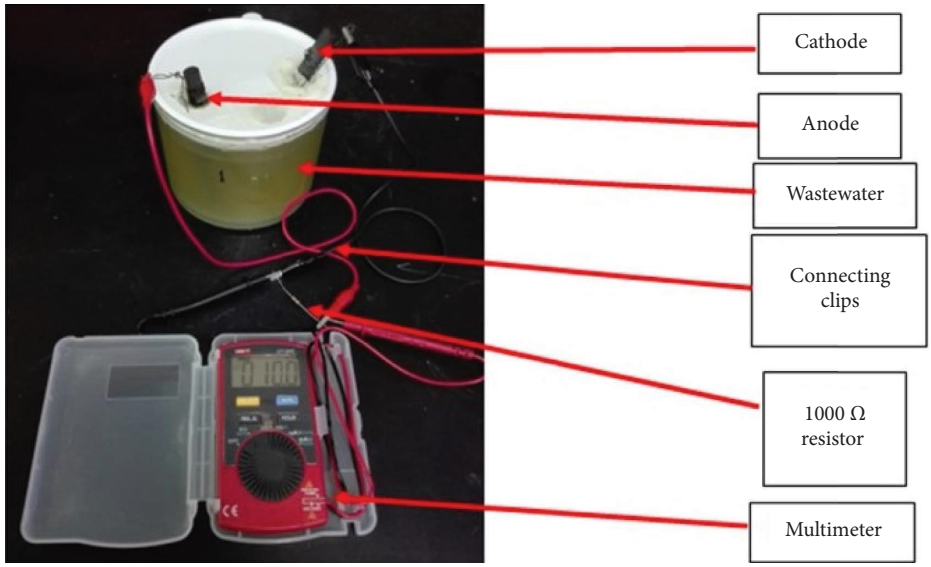


Figure 1: Basic used MFC setup in this study.

$$\mathrm{CD} = \frac{1}{A}, \tag{3}$$

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

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 10^{th}, 15^{th}, and 20^{th} 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 Cp (F/g) is defined by integrating the whole set of data per unit area of the anode and the cathode. From the CV values, Cp is calculated using the following equation:

$$\mathrm{Cp} = \frac{A}{2mk(V2 - V1)}, \tag{5}$$

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 $(V2 - V1)$ 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 ± 2°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 ± 2°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°C and 160 rpm, the nutrient broth was cultured for 24 hours. After 24 hours, we aseptically poured 500 μL of overnight incubated cultures and 500 μL of 40 percent glycerol into 1.5 mL Eppendorf tubes. The mixture was well combined before being frozen at 80°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 nucleotide BLAST feature was used as well [24]. All the data can be reproduced by following the same procedure.

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

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

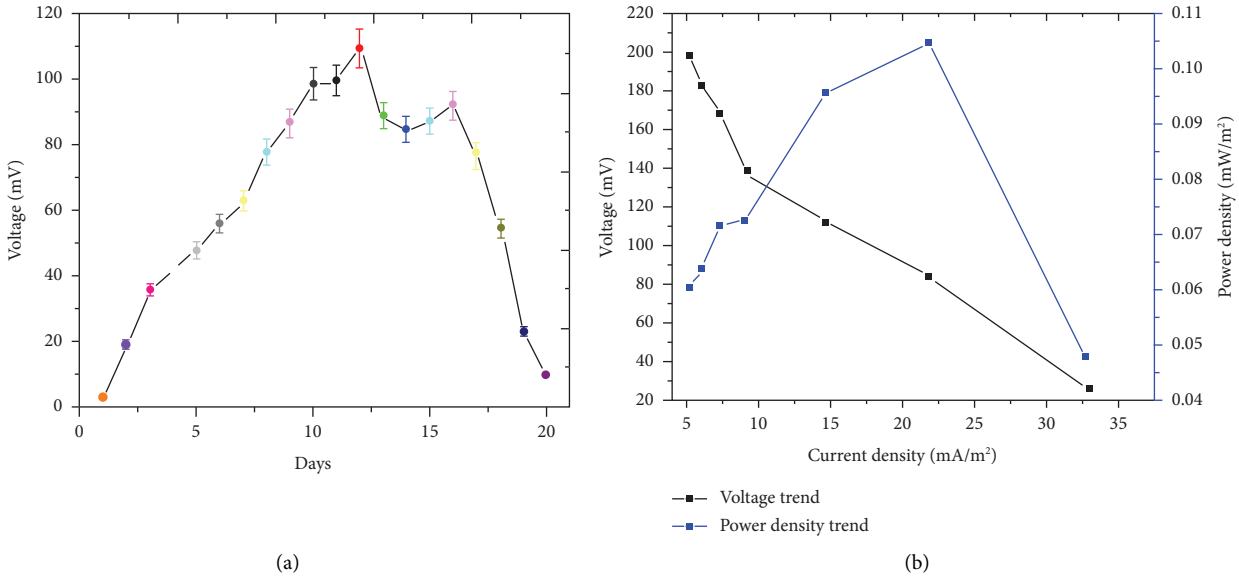


Figure 2: Recorded (a) voltage trend and (b) polarization behaviour.

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/m^2, and the maximum CD was 21.8441 mA/m^2. 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/m^2, whereas at 5000 Ω, the PD was 0.060 mW/m^2. 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].

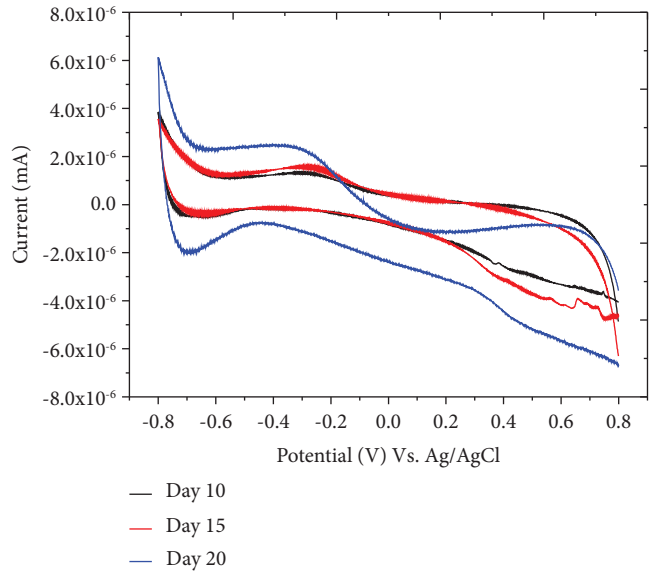


Figure 3: Recorded CV curves.

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 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 Cp values, CV curves are also evaluated. The Cp 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 Cp 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 Cp 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.

Table 2: Recorded Cp values.

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

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% 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

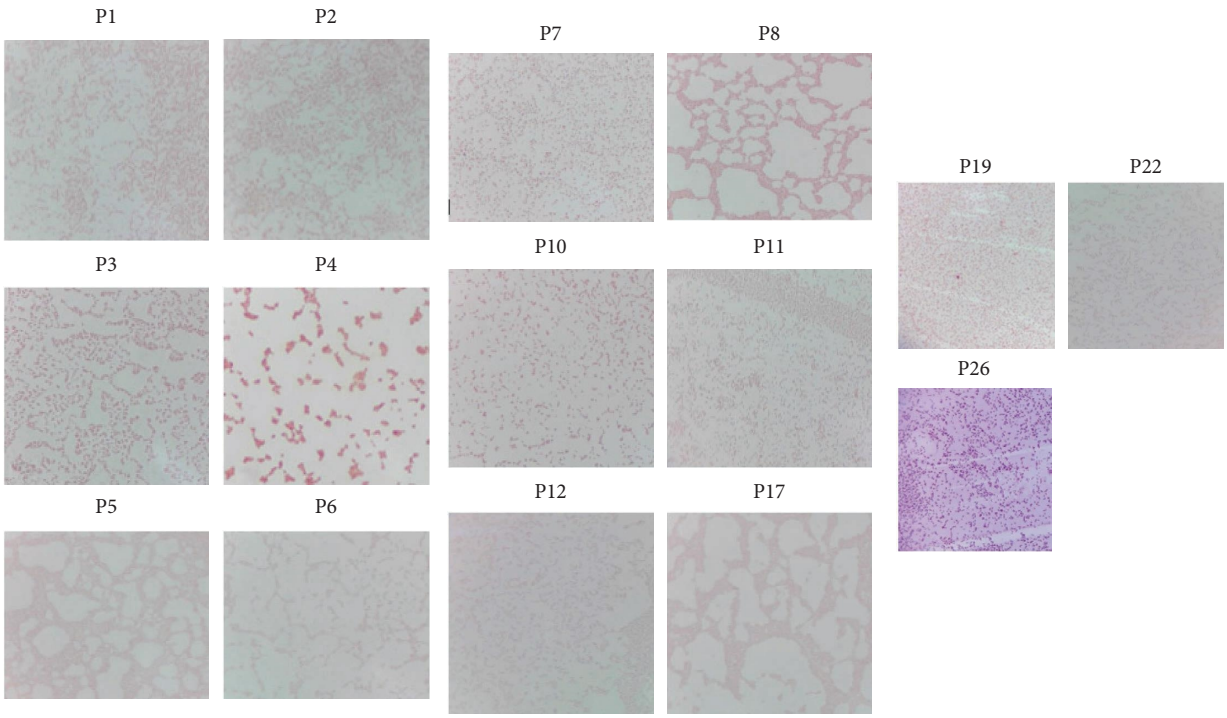


Figure 4: Gram-staining of 15 isolates obtained.

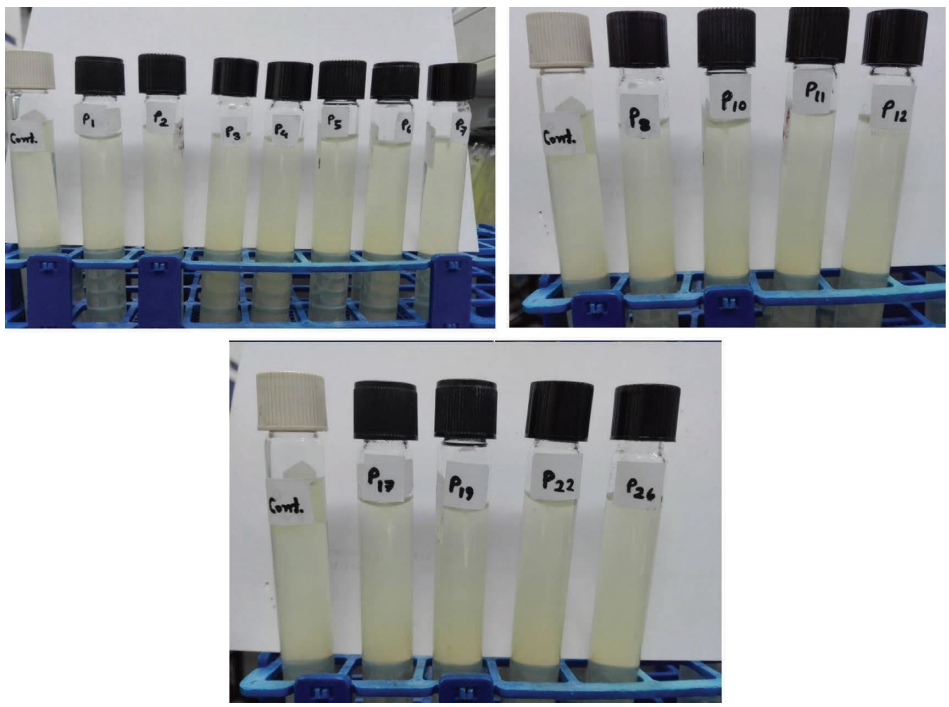
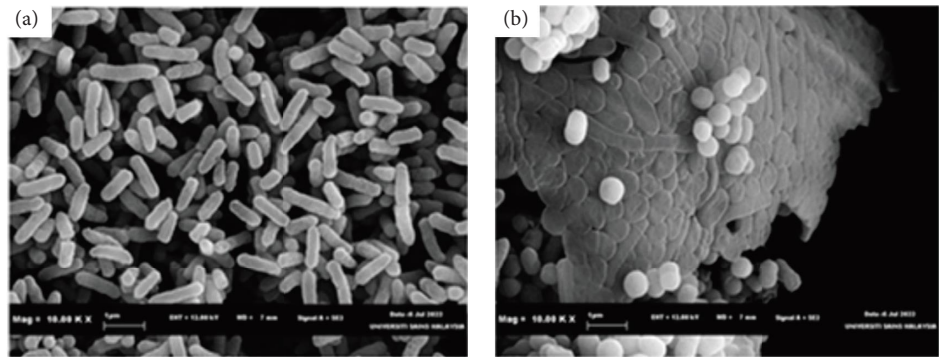


Figure 5: Motility test of different isolates.

Table 5: Isolate information from the NCBI database.

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



(a) (b)

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.

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Research Article

Degradation of Metal Ions with Electricity Generation by Using Fruit Waste as an Organic Substrate in the Microbial Fuel Cell

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A potential and developing green technology for producing renewable energy and treating wastewater is the microbial fuel cell (MFC). Despite several advancements, there are still several serious problems with this approach. In the present work, we addressed the problem of the organic substrate in MFC, which is necessary for the degradation of metal ions in conjunction with the production of energy. The utilization of fruit waste as a carbon source was strongly suggested in earlier research. Hence, the mango peel was used as a substrate in the current study. Within 25 days of operation, a 102-mV voltage was achieved in 13 days, while the degradation efficiency of Cr^{3+} was 69.21%, Co^{2+} was 72%, and Ni^{2+} was 70.11%. The procedure is carried out in the batch mode, and there is no continuous feeding of the organic substrate. In addition, a detailed explanation of the hypothesized mechanism for this investigation is provided, which focuses on the process of metal ion degradation. Lastly, future and concluding remarks are also enclosed.

1. Introduction

Heavy metals endanger the environment. Heavy metals' extremely toxic and nonbiodegradable qualities affect human health and ecosystems, particularly because they are used in industries and produce harmful effluent [1, 2]. When these effluents bioaccumulate in the food chain, humans will be exposed to the toxic materials, and subsequently, the effects can be grave [3, 4]. The discharge of these metals through contaminated effluent into bodies of water causes water pollution. Thus, treatment of heavy metal-based wastewater is required [5]. However, the technology of wastewater treatment also needs to overcome certain issues. Conventional methods of remediating heavy metals contained in waste require high operating costs and are not environment friendly [6–8]. As clean water is becoming an increasingly crucial need of today, the development of new approaches to wastewater treatment that are both sustainable and economical is receiving some attention [9]. A technique that is displaying promising results in recovering heavy metals from wastewater and generating energy simultaneously is called a microbial fuel cell (MFC) [10, 11]. The MFC technology could overcome the weaknesses of conventional treatment methods owing to its requirement for a low operating cost and eco-friendly nature [12]. An MFC is a technique that can convert the chemical energy of basic organic compounds such as sodium acetate and glucose into electrical energy while removing pollutants at the

same time [13]. The simplest MFC has anode and cathode compartments, normally separated by a membrane for proton exchange to limit the electrolyte movement [14]. The bacteria in the anode chamber assist the activity of electron transportation to an electrode in the organic , and this is paired with the reduction of oxygen at the cathodic chamber, resulting in power production [15, 16]. The presence of waste-based materials from plants aids in the formation of electrons and protons in MFC. However, there is an issue regarding the poor performance of the organic substrate used in the fuel cell towards the microbial community because of its instability [17]. This problem is a major setback for the system and thus requires further attention to provide an efficient organic-based waste that can facilitate the efficient supply of energy for the bacteria present in cells for the process of electrogenesis. The greatest value of a MFC system is the ability of the microbes associated with the electrode to degrade wastes and harmful chemicals [18]. In this study, the plant-based waste used for the MFC system is collected from the mango (*Mangifera indica*) fruit, specifically its peels. Based on the previous research by Ajila et al. [19], the content of carbohydrates in the mango peel ranged from 20.8% to 28.2%. The percentage of the fiber substance in the peel is in the range of 3.28% to 7.40%, with a higher amount found in ripe peels. The peel has a moisture content of 66% to 75%, a protein content of 1.76% to 2.05%, and an ash content ranging from 1.16% to 3.0% [20]. The mango peel has the potential to be a beneficial organic waste with a high nutrient content. This study utilizes mango peels as plant-based organic waste in the MFC system in domestic wastewater (with Cr^{3+}, Co^{2+}, and Ni^{2+}). The efficiency of organic waste in facilitating the recovery of heavy metals and the simultaneous generation of energy using MFC.

2. Experiment Details

2.1. Materials and Reagents. The mango peel waste (obtained from a local fruit market), tape water, chromium nitrate nonahydrate, cobalt nitrate hexahydrate, nickel nitrate hexahydrate (all chemicals were purchased from Sigma–Aldrich), and distilled water (DI) was used in this research.

2.2. Microbial Fuel Cell Assembly and Operation. Tap water was received, and 100 ppm of chromium, cobalt, and nickel ions were introduced into the wastewater. After the supplementation, the tap water is now recognized as synthetic water for this study. The physicochemical properties of both the tap water and the synthetic water are summarized in Table 1. The instruments utilized for the analysis of the temperature, pH, and conductivity of the tap and synthetic waters were a thermometer, a pH meter, and an electrical meter, respectively. The mango peels were obtained from a nearby regional fruit marketplace and were then cut into small pieces after washing. A 500-mL mixture of synthetic water and 0.5 kg of mango peel waste was placed in the single-chamber MFC. The chamber volume used was 600 mL. This study used one electrode to act as the cathode, with a height of 10 cm and a radius of 1 cm. Two electrodes

Table 1: Measured characteristics of the tap and synthetic water.

Parameters	Tap water	Synthetic water
pH	6.97	6.20
Odour	Unpleasant smell	Unpleasant smell
Electrical conductivity	110 μs	459 μs
Temperature	Room	Room
Cr^{3+}	0 ppm	100 ppm
Co^{2+}	0 ppm	100 ppm
Ni^{2+}	0 ppm	100 ppm

with a dimension of 10 cm × 1 cm (h × r) each to act as multiple anodes. The calculated surface area of the electrode was 69.13 cm^2. The distance between the anode and cathode electrodes was 12 cm. The electrodes were linked to each other using copper wire to help in electron transportation from anode to cathode, and 500 Ω of external resistance was provided after 3 days of open-circuit voltage. A decrease in the voltage value was observed after external resistance was implemented, but it slowly recovered. A large resistance should be used when there is an absence of voltage recovery, whereas smaller values of external resistance should be chosen when the value of the voltage shows no significant change [21]. For this work, the MFC was operated at room temperature for 25 days.

2.3. Electrochemical Test. Every day, the voltage of the cell was assessed using a multimeter. The efficiency of the current was measured through calculation of power density (PD) and current density (CD). Calculations of CD, PD, and internal resistance were carried out using the following equations [22]:

$$V = IR, \tag{1}$$

$$PD = \frac{V^2}{RA}, \tag{2}$$

$$CD = \frac{1}{A}, \tag{3}$$

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

where r denotes internal resistance, I denotes current, A denotes surface area, V denotes voltage output, R denotes external resistance, and E denotes electromotive force (emf). The emf value was obtained from the calculation through the measurement of the voltage for the open circuit by connecting with a voltmeter in the presence of resistance [23]. The redox reaction was examined using cyclic voltammetry. For every 10 days of the reaction, CV was recorded using a potentiostat, and the scan rate used was 10 mV/s with potential ranging from +0.8 V to −0.8 V. By using the "Rext-variation" scheme, the polarization behavior of the setup was studied, where a variable resistance box of 5000-100 Ω was applied to the external resistance control. Polarization curve analysis was carried out after the pseudosteady state of the operation. Approximately 30 min of variation time was required to observe the polarization behavior of the system.

The impact of anode resistance on voltage was additionally examined using electrochemical impedance spectroscopy (EIS). The frequency used ranged from 100 kHz to 100 mHz.

2.4. Metal Degradation and the Biofilm Study. The atomic absorption spectrometer (AAS) instrument is used to investigate metal recovery from wastewater. This instrument was used for the determination of the concentration of metal ions. After every 5 days, about 2 mL of the sample was collected from the MFC setup for analysis. Based on the AAS analysis results, the degradation efficiency (DE%) was calculated using the following equation:

$$DE\,\% = \frac{Ti - Tf}{Ti} \times 100, \tag{5}$$

where *Ti* denotes the initial concentration and *Tf* denotes the final concentration. The growth of a biofilm on the electrode indicates effective metal reprocessing and energy production. A scanning electron microscope (SEM) was used to investigate the morphology of electrode biofilms.

3. Results and Discussion

3.1. Voltage Trend. The generation of voltage that occurred during the metal degradation process that took place in the MFC operation is shown in Figure 1. The operation lasted 25 days and used the same external resistance (500 Ω). On day 13 of the operation, a voltage reading of 102 mV was reported as having reached its peak point. The findings indicated that the voltage production started out at a low value and steadily climbed until it reached its peak point on the 13th day. After 14 days of continuous operation, a decreasing trend in the voltage begins to manifest itself. The decrease in voltage production is a sign that the bacterial species are reaching the phase of death. After a few days had passed, the observation revealed that there was still a tendency toward a lower voltage. It indicates that the exoelectrogens are unable to regain control of the oxidation process of the organic substrate, and as a result, the process is moving forward to its conclusion. According to this research, the highest voltage was attained on day 13. Despite this, a few studies have shown that the point where the voltage is the highest is also a good indicator of a significant change in the state of the metal from a soluble to an insoluble state [21, 23, 24].

3.2. Polarization Behavior. In addition, the polarization performance was investigated by using various external resistances to compare the CD, PD, and voltage relations. It has been determined, using Figure 2, that the voltage and CD have a relationship that is inversely proportional. When the voltage drops, the current density (CD) goes up. At 500 Ω, the PD was at a maximum of 0.099 mW/m^2, but at 5000 Ω, it only provided 0.060 mW/m^2. It is important for successful electron transfer that the internal resistance and the external resistance should be equal. The greater the resistance of the external environment, the lower the electron transit. When the external resistance is low, the potential does not stabilize as quickly as it would otherwise, but electrons are still made and moved at a high enough level. The high electron movement causes an instability in the voltage. The maximum CD that was ever recorded was 31.57 mA/m^2. The cathodic reaction rate was further boosted by the external supply of oxygen, which contributed to the stabilization of voltage generation despite the increased resistance. The internal resistance was 734.0 Ω. Few studies used comparable methodologies to describe how energy is produced in electrochemical fuel systems [24–27].

3.3. Electrochemical Impedance Spectroscopy. AnEIS-Nyquist curve plot was drawn to assess the charge transfer resistance provided by the fuel circuit model. The EIS measurement that was performed after the procedure was finished and can be seen in Figure 3. This measurement was included in this research. According to the research, a straight line with a high Z'_{image} (Ohm) suggests a low electron transportation rate, whereas a semicircle or semibent line shows a high rate despite a lower Z'_{image} (Ohm) [28]. The current research demonstrates that the semibent lines at the start of the Z'_{real} (Ohm) suggest that there was electronic movement, but afterwards the straight line indicates that there is no high electronic movement. This was found by comparing the two types of lines. In most cases, a high level of electron mobility may be inferred from a reduced internal resistance, as well as from the form of a semicircle. There is a possibility that poor electronic mobility will result from higher internal resistance than external resistance.

3.4. Cyclic Voltammetry and Specific Capacitance. During the operation, the CV study was taped at a variety of different times. The charts illustrate the propensity of the metals to undergo oxidation and reduction at a variety of different time periods. As can be seen in Figure 4(a), the MFC operation displayed the maximum current throughout the forward and reverse scan speeds. The forward scan revealed that the current was 0.00001 mA on day 5, 0.00002 mA on day 10, and 0.000045 mA on day 25 of the cycle. In a manner analogous, the reverse scan revealed that the current was −0.0000 mA on day 5, −0.00001 mA on day 10, and −0.000025 mA on day 25 of the cycle. On day 25, both the forward and backward scans showed that the current was at its highest possible level. Both the forward scan and the reverse scan provide information on the rate at which the metal ions in the wastewater are being oxidized or reduced, respectively. Both the oxidation and reduction peaks reached their highest point on day 25, with the oxidation peak reaching 0.08 V and the reduction peak reaching −0.8 V. Additionally, the CV curves are utilized to calculate the Cp values at various time intervals during the process. During the operation of the MFC, the Cp values demonstrate the rate of biofilm production as well as the stability rate. This is shown in Figure 4(b). The high Cp rates demonstrate that the maturation of the biofilm development is becoming nearer on a step-by-step basis. The Cp readings have been on a decreasing trend, which indicates that the formation of the

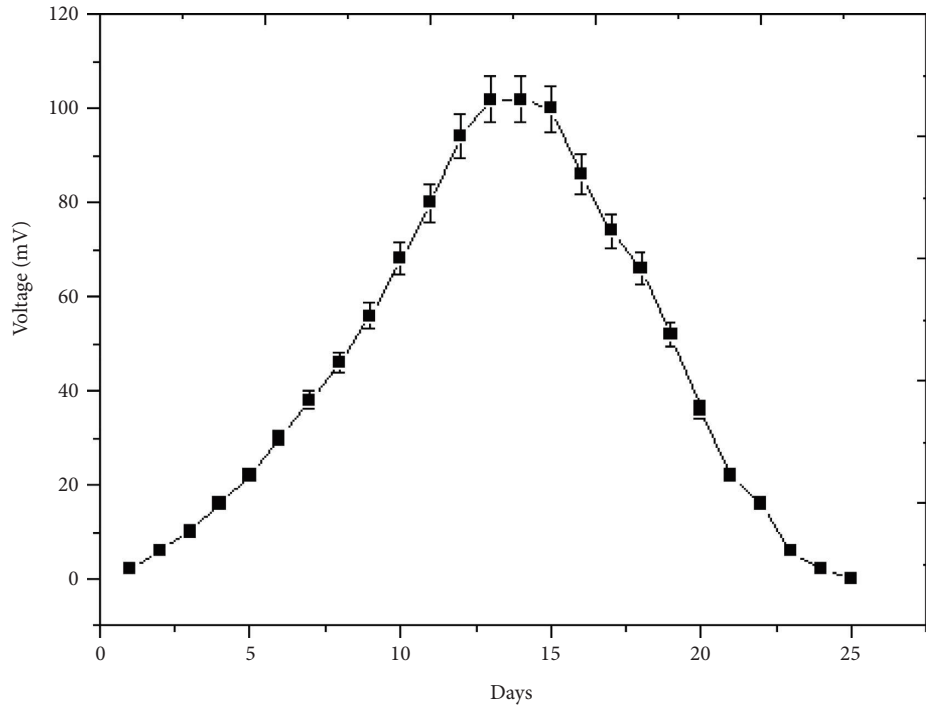


Figure 1: Voltage trend during the MFC operation for 25 days.

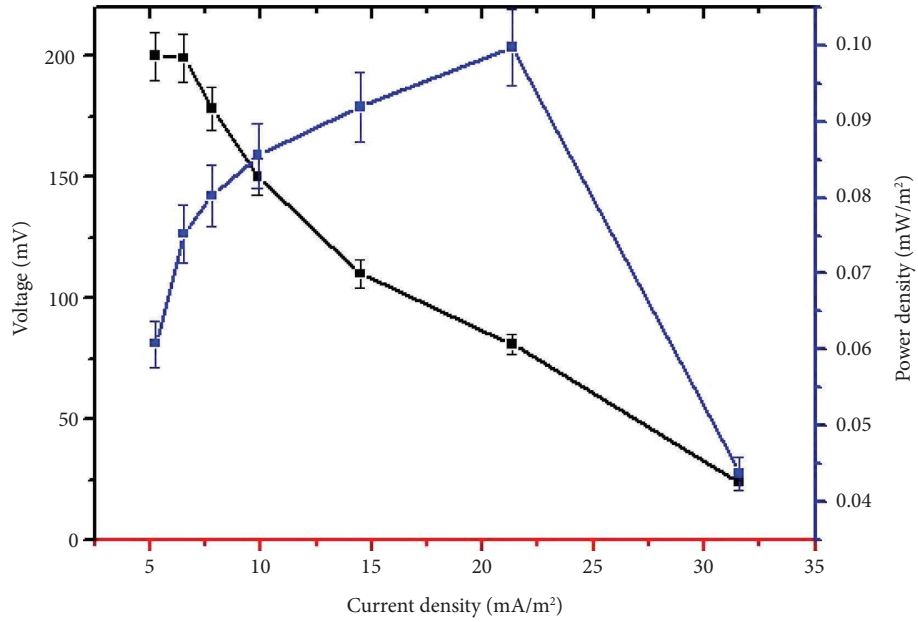


Figure 2: Polarization behaviour of operation.

biofilm was disrupted, and thus, the biofilm is not functioning as it should. According to the findings of this investigation, the concentration of Cp increased from 0.00002 on day 5 to 0.00014 on the following day 25. It was evident that the biofilm, after some time had passed, had achieved stability and had developed without being disrupted on the anode surface. A similar line of reasoning was followed by Hong et al. [29] in order to justify the production and the constancy rate of biofilm.

3.5. Degradation of Metals. The results of the investigation concerning the degradation of metal ions are shown in Table 2. The degradation of metals via the use of a bioelectrochemical system is emerging as one of the most promising and cutting-edge methods, especially in relation to MFC. The current research presents novel findings about the degradation of metal ions in synthetic water. As the process gets closer to being finished, there is a progressive improvement in the efficiency of the metal degradation.

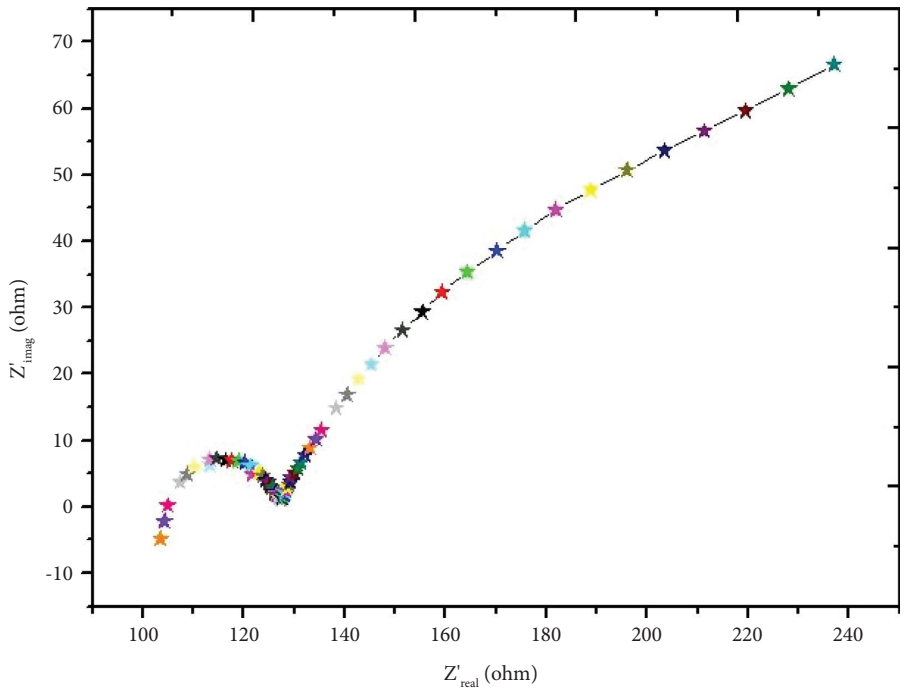
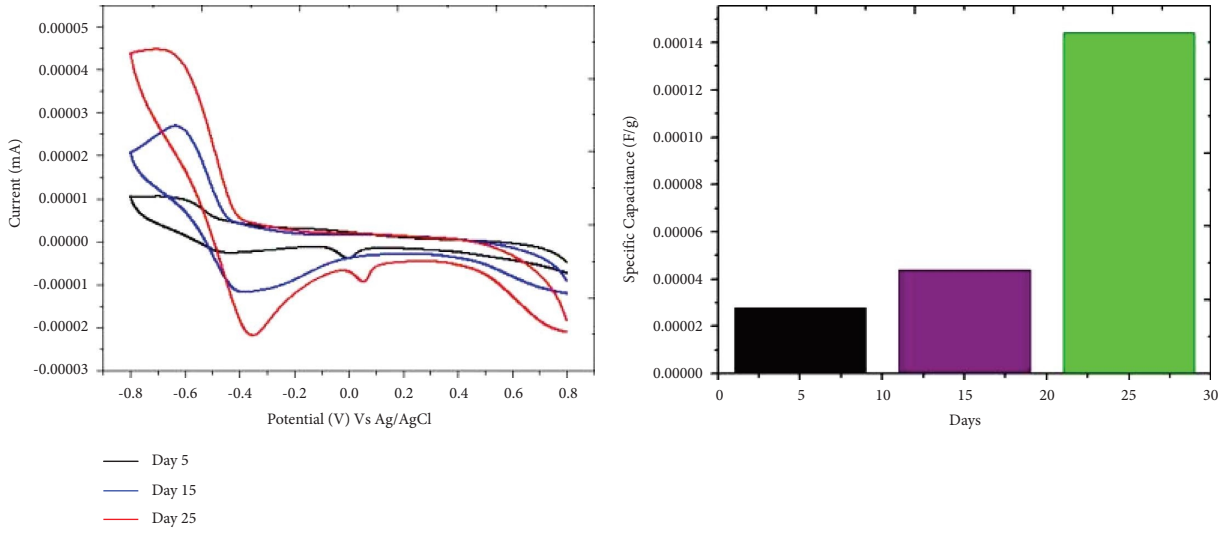


Figure 3: Electrochemical impedance spectroscopy study of the system.



(a) (b)

Figure 4: (a) CV at different days (b) specific capacitance values.

Table 2: Recorded degradation efficiency of metal ions in MFC.

Organic substrate	Concentration of metal in chamber (ppm)	Operational days	Degradation percentage of Cr^{3+}	Degradation percentage of Co^{2+}	Degradation percentage of Ni^{2+}
Mango peel extract	100	0	0.00	0.00	0.00
		5	13.53	17.20	15.84
		10	33.45	36.89	35.27
		15	51.00	57.10	58.35
		20	64.82	66.90	67.49
		25	69.21	72.00	70.11

When compared to the findings of the previous research, the ones presented here are rather intriguing. In this research effort, an efficiency of degradation of more than 70% is achieved. The presented data suggest that the metal ion concentration was reduced in the synthetic water that had been treated.

3.6. Biofilm Study via SEM. The collection of different bacterial species in and around the anode electrode is what makes up the biofilm. The biofilm oversees energy generation and transmission, as well as metal degradation. During the procedure, the biofilm was produced in a natural way without any assistance from the outside. About 97% of biofilm is made up of water, 3%–6% extracellular polymeric materials (EPSs), and 2%-2% bacterial species [21, 30]. The extracellular polymeric substance is the primary component of the biofilm and is responsible for the bacterial activities that simultaneously generate electrons and breakdown metals [31]. It is the region of the biofilm that contains the most water content. The biofilm's age is determined by the EPS, and the effectiveness of the EPS depends on the availability of the organic substrate. In addition, the components of EPS compositions include 40–95% polysaccharides, 1–60% proteins, 40% lipids, and more than 5% nucleic acids [32, 33]. The organic substrate improves the functioning of the EPS, which ultimately results in a biofilm that is durable and robust. According to the CV data shown previously, the existing biofilm seemed to have a high degree of stability. On the other hand, the SEM images of the electrode exhibiting biofilm are shown in Figures 5(a) and 5(b). Images taken using a scanning electron microscope (SEM) revealed that the anode electrode containing the biofilm demonstrated the normal proliferation of bacterial species (Figure 5(a)). Therefore, bacterial behavior and stability are very useful tools in environmental remediation. In addition, scanning electron microscopy (SEM) inspection of the anode biofilm revealed a surface with a similar shape. The presence of conductive pili-type bacterial species is indicated by the presence of the rod filament structure in the anode electrode biofilm. According to the findings of a number of studies, the rod-shaped and filamentous appendage structures are characteristic of bacterial species that are of the conductive pili type [21, 24, 27]. The most frequently mentioned conductive pili species are *Klebsiella pneumoniae*, *Acinetobacter*, *Escherichia*, *Bacillus*, and *Lysinibacillus* [24, 34].

4. Mechanism of the Present Study

MFC rely on bacterial activity for metal ion production, transport, and reduction. In the literature, numerous bacterial species, including *Bacillus*, *Klebsiella*, *Escherichia*, and *Actinobacillus*, are well-known exoelectrogens [21, 24]. In the current investigation, the waste from mango peels was used as an organic substrate for several kinds of bacteria. The mango peel waste started off as polysaccharides, but it will eventually be converted into simple glucose. This glucose will then be oxidized further by bacterial species, which will result in the production of electrons and protons. The oxidation and reduction reactions that were seen in this investigation may be expressed in the following way:

$$\begin{aligned} &\text{Oxidation: mango peel waste} \longrightarrow C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 24H^+ + 24e^-, \\ &\text{Reduction: } 24H^+ + 24e^- + 6O_2 \longrightarrow 12H_2O, \\ &\text{General reaction: } C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Electricity}. \end{aligned} \tag{6}$$

After some time has passed, the electrons and protons that have been produced are moved from an anode electrode to a cathode electrode during the oxidation process. Because there was just one chamber in MFC, the protons were able to pass through with no obstruction from the anode to the cathode chamber. Before electrons can be sent to the cathode from the anode electrode, they must first go through a series of steps that include many processes. Bacterial cells are the source of these electrons. The mechanism that has received the most attention is discussed in the following paragraphs and is also shown in Figure 6.

(a) In order to move electrons from the bacterium cell to the anode, redox-active proteins such as OmcS, OmcZ, OmcB, OmcT, and OmcE are used. These proteins are responsible for the transmission of electrons. This mechanism is being used by the families of *Geobacter* to transfer electrons.

(b) Short-range electron transfer is an additional method that may be used to successfully transfer electrons. To carry out this procedure, the bacteria made use of their own self-produced reduced shuttles as well as oxidized shuttles. Both the *Desulfuromonadaceae* and *Geobacteraceae* families can produce their own electron shuttles. Components of the electron shuttle include certain molecules such as OmcA, MtrF, MtrE, and MtrC.

(c) The ability of a bacterial population to transmit electrons over long distances relies on the bacteria's conductive pili. It has the appearance of a conducting metal, but in reality, it is a component of the bacterial body that was used to carry electrons straight from the cell of the bacterium to the anode [35].

In addition, after the biofilm research and the bacterial analyses, it was discovered that the current study followed the long-range mechanism for electron transfer. This was

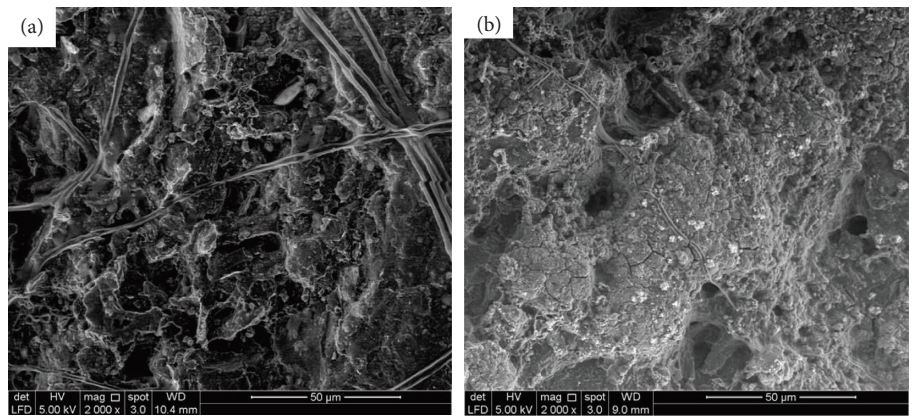


Figure 5: SEM images of (a) treated anode and (b) treated cathode.

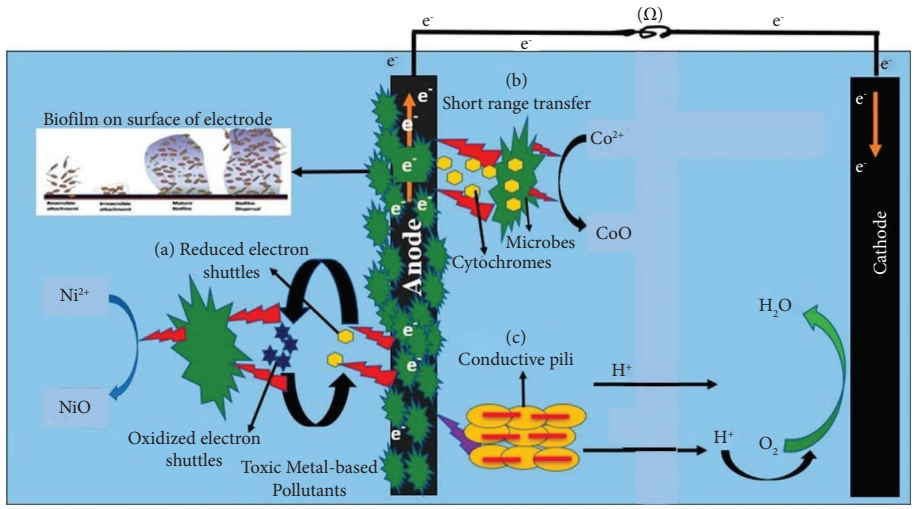


Figure 6: Mechanism of the present study (modified from reference [35], open access MDPI publisher).

discovered after analyzing the biological tests. In addition, scanning electron micrographs of the biofilm revealed the existence of a rod-shaped structure, indicating that all the detected species are of the conductive pili type of bacterial species. According to all the evidence (biological testing and earlier published research), the conductive pili of bacteria species are to blame for the electron transfer that was seen in this investigation. During a redox reaction, the soluble metal ions are effectively transformed into a state that is insoluble. The insoluble condition was discovered in the form of sludge, as was mentioned earlier in the sentence. In addition, the findings of the AAS test only indicated the amounts of metal ions that were still present in the water samples. The metal ions that were removed were changed to oxides, and a sludge-like substance was seen in the MFC because of this transformation. According to the previous literature, the metal ions that are extracted are changed into their oxide forms, and the sludge that is produced contains the metals in their oxide forms [21, 24, 36]. The following is a mechanism of the biological events that occur during metal reduction:

(i) Reduction of Cr^{3+}

$$\begin{aligned} Cr^{3+} + 3e^- &\longrightarrow Cr_{(s)}, \\ 2Cr^{3+} + 3H_2O &\longrightarrow Cr_2O_3 + 6H^+, \\ Cr_2O_3 + 6e^- + 6H^+ &\longrightarrow 2Cr_{(s)} + 3H_2O. \end{aligned} \quad (7)$$

(ii) Reduction of Ni^{2+}

$$\begin{aligned} Ni^{2+} + 2e^- &\longrightarrow Ni_{(s)}, \\ 2Ni^{2+} + 2H_2O &\longrightarrow 2NiO + 4H^+, \\ NiO + 2e^- + 2H^+ &\longrightarrow Ni_{(s)} + H_2O. \end{aligned} \quad (8)$$

(iii) Reduction of Co^{2+}

$$\begin{aligned} Co^{2+} + 2e^- &\longrightarrow Co_{(s)}, \\ 2Co^{2+} + 2H_2O &\longrightarrow 2CoO + 4H^+, \\ CoO + 2e^- + 2H^+ &\longrightarrow Co_{(s)} + H_2O. \end{aligned} \quad (9)$$

5. Concluding Remarks and Future Recommendations

The present research focused on the usage of the residual mango peel in MFC to generate energy and degrade metal ions. The voltage that could be measured to the greatest level was 102 mV. The presence of many bacterial species on the anode surface suggests that the metal degrading process has been effective. In addition, according to the data, well-known species of exoelectrogenic bacteria were found in the MFC operation that is now underway. The favorable bacterial activities that take place because of the oxidation of the organic substrate are responsible for the high efficiency with which metals are degraded. It indicates that the organic substrate underwent a vigorous oxidation process, which led to an abundant production of electrons as a byproduct. The current electrochemical measurements, on the other hand, demonstrate an energy efficiency that is much lower than that of prior studies. After conducting in-depth research and analysis, the conclusion was reached that, despite all the contributing elements, there has been a decline in energy efficiency. The investigation revealed that there was a fault with electron transportation, namely, that electrons were not delivered to the cathode in an efficient manner. It is because of the quality of the electrode that the current investigation utilized the commercial graphite electrode, which was not successful in properly transferring the electron. Electrodes that are based on derivatives of graphene should be used since they are highly conductive and modern materials. This will result in improved electron transport. For the electrode material to be effective over the long term, it must be biocompatible, chemically stable, ultraconductive, and thermally balanced. The problems that need to be overcome to get the MFC to the level of commercial viability may be tackled with the collaboration of professionals from various sectors, including microbiology, material science, and bioelectrochemistry.

Data Availability

All the data used to support the findings of the study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ghada Mohamed Aleid and Anoud Saud Alshammari equally contributed to this work.

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