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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

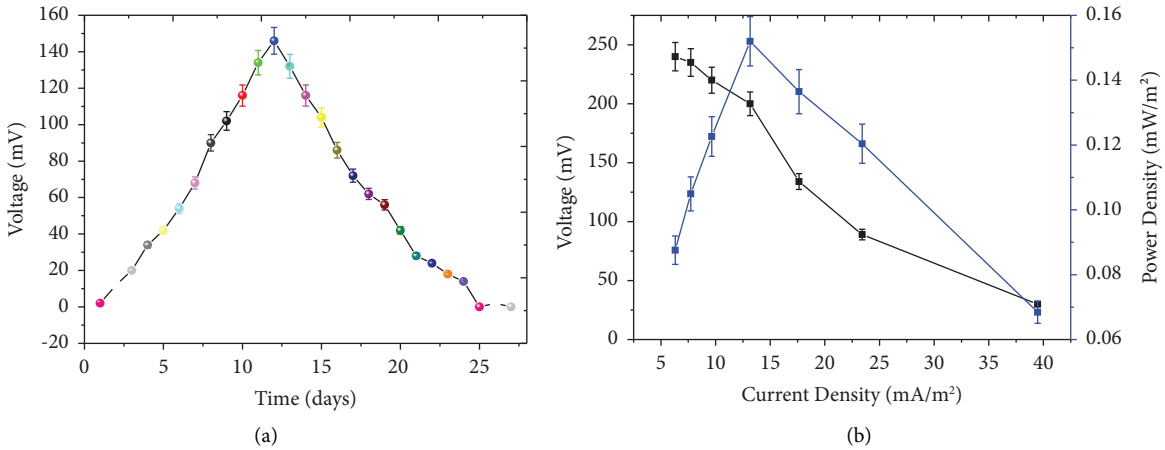


Figure 1: (a) Voltage output distribution; (b) polarization curve.

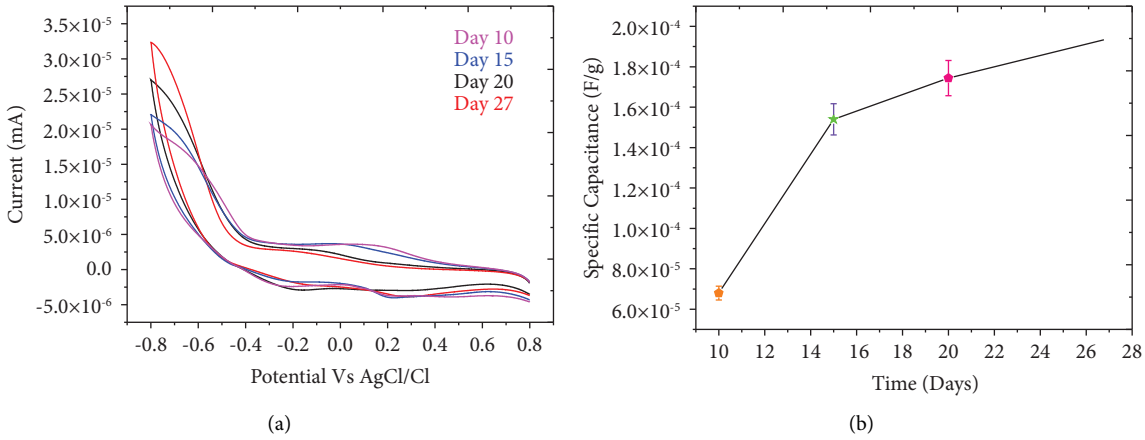
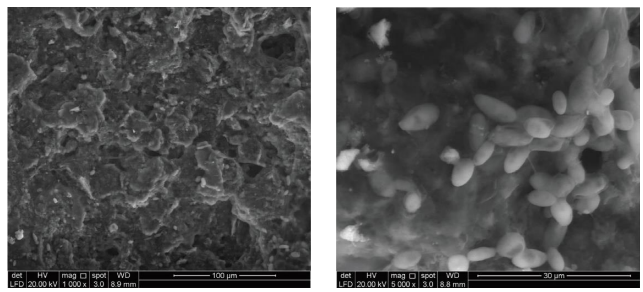


Figure 2: (a) Plot of CV at various time intervals; (b) specific capacitance pattern.

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.

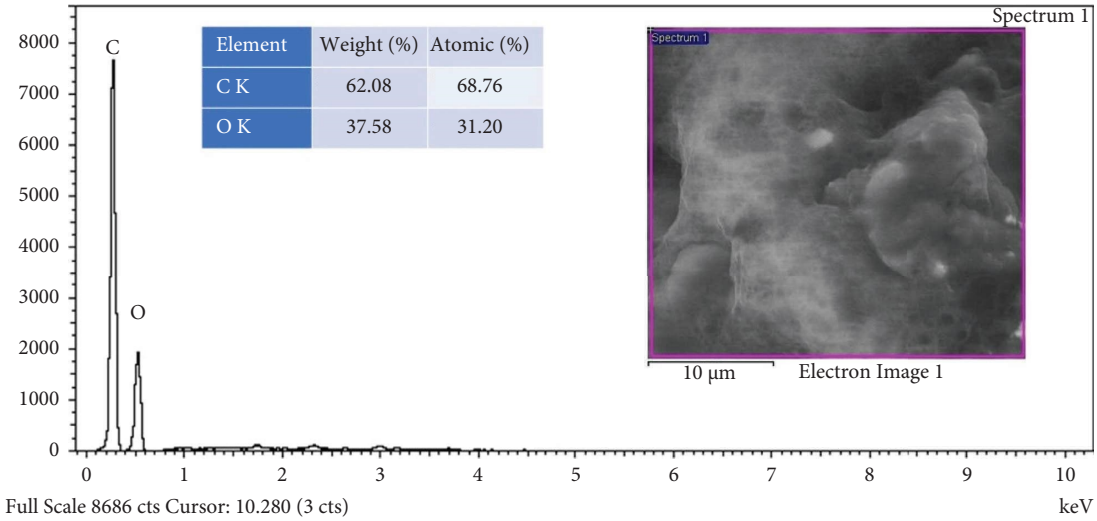


Figure 4: The EDX spectra of the anode biofilm at the completion of the MFC process.

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

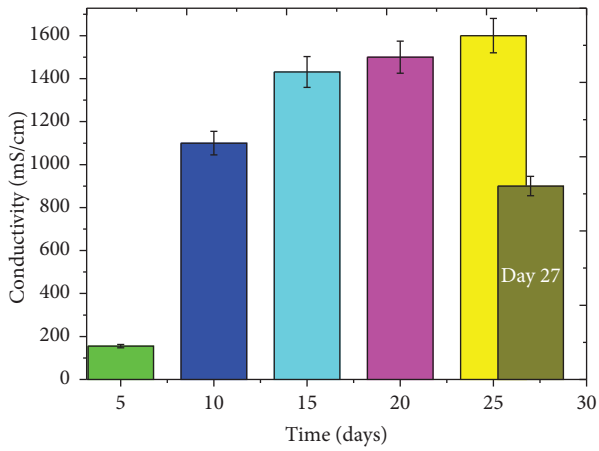


Figure 5: Conductivity value at various intervals of days.

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

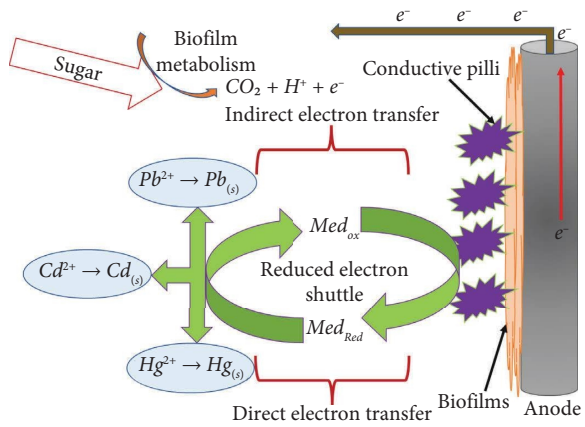


Figure 6: Proposed mechanism of the toxic metal degradation with the electron transfer mechanism.

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.

Acknowledgments

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