International Journal of Environmental Research (2025) 19:221
https://doi.org/10.1007/s41742-025-00889-2

RESEARCH PAPER

Energy and Pollution Mitigation: The Role of Coconut Sugar in Microbial Fuel Cell

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Received: 5 February 2025 / Revised: 25 May 2025 / Accepted: 24 August 2025 / Published online: 11 September 2025
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This study explores the use of microbial fuel cell (MFC) for wastewater treatment and energy production, focusing on enhancing electron generation. To encourage bacterial activity in removing metal ions from the inoculation source, coconut sugar and organic contaminants were used as dual substrates. With phenol degradation at 82.18% and removal efficiencies for Pb^{2+}, Cd^{2+}, and Cr^{3+} at 92.10%, 89.85%, and 90.60%, respectively, the study's energy efficiency was 72.7 mW/m² within 21 days. Compared to the external resistance of 1000 Ω, the internal resistance was 514.39 Ω. On day 30, CV studies revealed maximum oxidation currents of 4.0×10^{-5} mA and peak reduction currents of -1.6×10^{-5} mA, suggesting increasing metal and phenol treatment. *Lysinibacillus*, *Lysobacter*, and *Pseudoxanthomonas* were found to be the dominating bacterial species at the anode biofilm, confirming the efficacy of this strategy via electrochemical and biological testing. The potential of coconut sugar in MFC applications was highlighted by its greater voltage generation when compared to other substrates. The research highlighted pH 7 and room temperature as optimal conditions while discussing processes and parameter optimization. The challenges of electron transportation are crucial for advancing MFC to a practical stage: hence, using waste materials for electrode production is a novel approach to address this challenge. However, there has been a thorough discussion of promising key challenges and potential future perspectives.

Highlights

- Coconuts sugar is being used as an organic substrate in microbial fuel cells.
- Multiple water pollutants have been treated in the present study.
- Amazing maximum power density 72.7 mW/m² within 30 days has been achieved.
- Phenol degradation reached up to 82.18% with a high metal removal percentage (more than 90%).
- *Lysinibacillus*, *Lysobacter* and *Pseudoxanthomonas* are the most dominant species.

Keywords Cocunut sugar · Microbial fuel cell · Metals · Phenol · Energy · Wastewater

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Introduction

Modern water pollution is now a major global concern and is increasingly becoming a serious issue in world (Afilah et al. 2014). Some organic pollutants such as benzene, cresol, toluene, resorcinol, xylene, etc. and inorganic pollutants are hazardous to the environment. These pollutants may accumulate and stay in wastewater, which endangers environmental safety and public health. According to evidences, among the pollutants undoubtedly phenol has become one of the most important environmental problems in the world (Al-Zaqri et al. 2023). The presence of phenols in wastewater that needs to be removed to make the environment green. In most industries, such as petrochemicals, refining, and other highly polluting chemical sectors, phenol is a common industrial pollutant (ElMekawy et al. 2014). According to Afilah et al. (2014) due to the rapid rise of the Malaysian industry in recent decades, the problem is getting worse. Groundwater and soil quality are greatly affected by increased production or leakage of hazardous materials into the environment. Inhalation of phenol over a long period can cause respiratory problems and cancer. Phenolic wastewater treatment from industry is very important because of the possibility of harm to health and the environment (Friman et al. 2013). According to the literature to remove phenols from wastewater, various techniques such as branding filtration, adsorption, condensation, oxidation, adsorption, and others have been used. In the opinion of Montagnolli et al. (2017) oxidation is one of the most successful and efficient techniques, however, due to its high cost, it is not suitable. Water supplies across the world are now contaminated with harmful metals. Conventional approaches of addressing this problem demand a significant amount of allocation. As a result, microbial fuel cell (MFC) may be a suitable industrial-scale strategy for these underdeveloped nations to address these problems without a significant costs (Yaakop andAhmad 2021). According to the literature study, a number of research documented the high removal efficiency of using MFC to remove a single metal at a time (Low et al. 2016). According to the professionals' recommendation and the problems that have been identified in MFC one of the most important components of MFC functioning is the organic substrate. According to a number of research, another issue with metal removal using MFC is the instability of substrate to bacterial species. This research investigates the simultaneous removal of many very hazardous metals, including Pb, Cd, and Cr.

Based on evidence from previous studies, MFC is one of the appropriate approaches to lower organic/inorganic pollution, and at the same time are capable of producing bioenergy that makes them more prominent (Khatoon et al. 2020). MFC, which turn organic waste into bioelectricity, are a promising technique for producing sustainable energy. MFC produce energy by using electrochemically active microorganisms to break down organic materials in wastewater. Both environmental degradation and energy shortages are concurrently addressed by this dual function of waste treatment and energy production (Apollon et al. 2025). The efficiency and scalability of MFC technology have increased recently, giving it a competitive alternative to conventional fossil fuels. MFC provide an economical and environmentally responsible solution by using the chemical energy contained in waste, making a substantial contribution to the energy industry of the future (Elhenawy et al. 2022).

MFC use microbes as biocatalysts to convert pollutants into more ecologically friendly end products such as CO_2, biomass, and water. MFC is one of the most accurate green approaches resulting in lower environmental hazards (Asim et al. 2021). Several studies report phenol reduction through MFC, for example by Luo et al. (2009) who studied the degradation of phenol (400 ppm) by using a single phenol without a substrate in MFC and achieved a reduction efficiency of 20% while the energy produced was 9.1 mW/m^3. Then, in another effort, they used phenol-glucose as the inoculation source in MFC and achieved a degradation efficiency of 90% with an energy efficiency of 28.3 mW/m^3. Similarly, Pradhan et al. (2015) reported phenol-glucose as the main source of carbon for generating energy as well as reducing phenol but its maximum power was 27.5 mW/m^3 with 90% phenol reduction. MFC have proven to be an efficient approach to degrade phenols by energy generation because phenols function as electron donors during electrochemical oxidation (Shen et al. 2020; Wang et al. 2015). Currently, the energy efficiency generated is still not encouraging due to the use of substrates that may not be suitable. During the oxidation of organic compounds, microbial communities need fuel to survive in hazardous environments. Although aromatic compounds (phenol), also function as electron donors, their toxicity affects microbial activity (Yang et al. 2020). High carbon sourced substrates are capable of bringing great success in degradation efficiency as well as energy generation performance.

In this study, to improve the efficiency of MFC, local coconut sugar was used as the substrate while phenols acted as pollutant and also as a substrate in a single-chamber MFC. Coconut sugar is easily available in Malaysia. This Malaysian coconut sugar, according to Chong et al. (2019), contains 87.30% simple sugar, and the rest is various sweeteners. This study is the first attempt to report the use of local coconut sugar as a substrate to lower metals and phenols while producing bioenergy through a single-chamber MFC. Moreover, this work pioneered efforts to reveal the process of phenol degradation (with the formation of intermediates during oxidation) transforming into CO_2 and water.

Moreover, various materials have been used in the past as anode and cathode electrodes, but such materials are quite expensive and not durable. They also show toxic effects on microorganisms during biocatalytic activity, which can interfere with energy generation and biodegradation performance (Guerrero-Barajas 2021). In this study, graphite electrodes were chosen for their unique attributes, including durability, availability, affordability, and non-toxicity. The primary focus of the research is on the performance of the organic substrate rather than the electrode type. This innovative work utilizes organic pollutants as a substrate to boost bacterial populations and reduce the impact of metals.

Materials and Methods

Chemical Reagents and Materials

Phenol (>95%; R&M Chemicals), cadmium nitrate (R&M Chemicals), lead nitrate (R&M Chemicals), chromium nitrate (R&M Chemicals), coconut sugar (500 g/L) a gift received from Tycoon Global, Lunas, Kedah, Malaysia, distilled water received from MUPA Lab Universiti Sains Malaysia (USM), commercial graphite rods, 0.1 M buffer solution (LBS) was used as an analyte-containing KH_2PO_4 and Na_2HPO_4.

Pre-Treatment of the Inoculation Source

The wastewater of the local USM pond (2000 mL) was collected and filtered to remove impurities such as soil particles or sludge particles. Prior to addition with phenol and metals, several physicochemical properties were analyzed as shown in Table S1 while the metal and phenol information has been presented in Table 1. The presence of phenolic pollutant was not found in the wastewater of the local USM pond. An 500 ppm phenol was supplemented to 2000 mL wastewater and continued to be used as an inoculation source during MFC operation. This research focusses on a 500 ppm concentration of phenol, in accordance with a prior literature review (Shen et al. 2021). According to Ziaedini et al. (2018) the microbial community can lower up to 1000 ppm of phenol through MFC operation. The local USM pond water was thereafter supplemented with various metal ions at a concentration of 50 mg/L each. Similarly, the metal concentration of 50 mg/L was also selected on the basis of a prior literature survey (Mathuriya and Yakhmi 2014; Omenesa Idris et al. 2023). The local water supplemented with metal and phenol is referred to as M-phenolic wastewater in this research. The initial pH of inoculation has been adjusted to 7. For comparison, Table 1 shows the physicochemical properties of M-phenolic wastewater and local USM pond wastewater.

Table 1 List of metal ions and phenol supplementation with defined concentration

Physicochemical variables	Local USM pond wastewater	M-phenolic wastewater
Phenol	0 ppm	500 ppm
Pb^{2+}	0 ppm	50 ppm
Cd^{2+}	0 ppm	50 ppm
Cr^{3+}	0 ppm	50 ppm

MFC Configuration and Operating Mode

The single-chamber configuration of MFC was used to reduce water pollution from M-phenolic wastewater and simultaneously produce bioenergy in this study. A used model of MFC in the present study is presented in Fig. 1. Earlier research indicated that single-chamber MFC are more effective in facilitating efficient electron transport compared to their double-chamber counterparts (Fadzli et al. 2021; Obileke et al. 2021). In this research, the single-chamber configuration could hold a total volume of 3500 mL, with an operational volume of 3000 mL. The height of the chamber was 13 cm with a diameter of 18 cm. Double anode electrodes (two graphite rods attached together) with a length of 9.5 cm and a radius of 1 cm per anode were used. The surface area per anode was 65.94 cm^2. Whereas, the cathode electrode was a single graphite rod with a length of 9.5 cm and a radius of 1 cm. The electrodes, comprising an anode and a cathode, were linked by a copper wire with a fixed external resistance of 1000 Ω. The distance between the electrodes was 10 cm. The chamber was filled with 1000 mL of LBS and 2000 mL of M-phenolic wastewater. To ensure adequate oxygen levels within the chamber, an outdoor aquarium air pump was used (One bubble per 2 seconds is the aquarium air pump working rate). To prevent water loss during operation, 10 mL of the coconut sugar solution was added continuously until the operation was completed. An 200 g of coconut sugar was mixed with 500 mL of distilled water to prepare a solution of coconut sugar. This solution played a dual role in this study: firstly it facilitated the provision of nutrients to the microbial community to enhance biocatalytic activity, secondly helped compensate for water loss due to the evaporation process. The experiment was conducted at a temperature of 27.0 ± 3.0 °C for 30 days. The experiment was repeated twice to ensure that the findings were accurate.

Analytical Measurements

Several analysis tools were used to analyze MFC operations. Voltage trends were recorded using a UNI-T digital

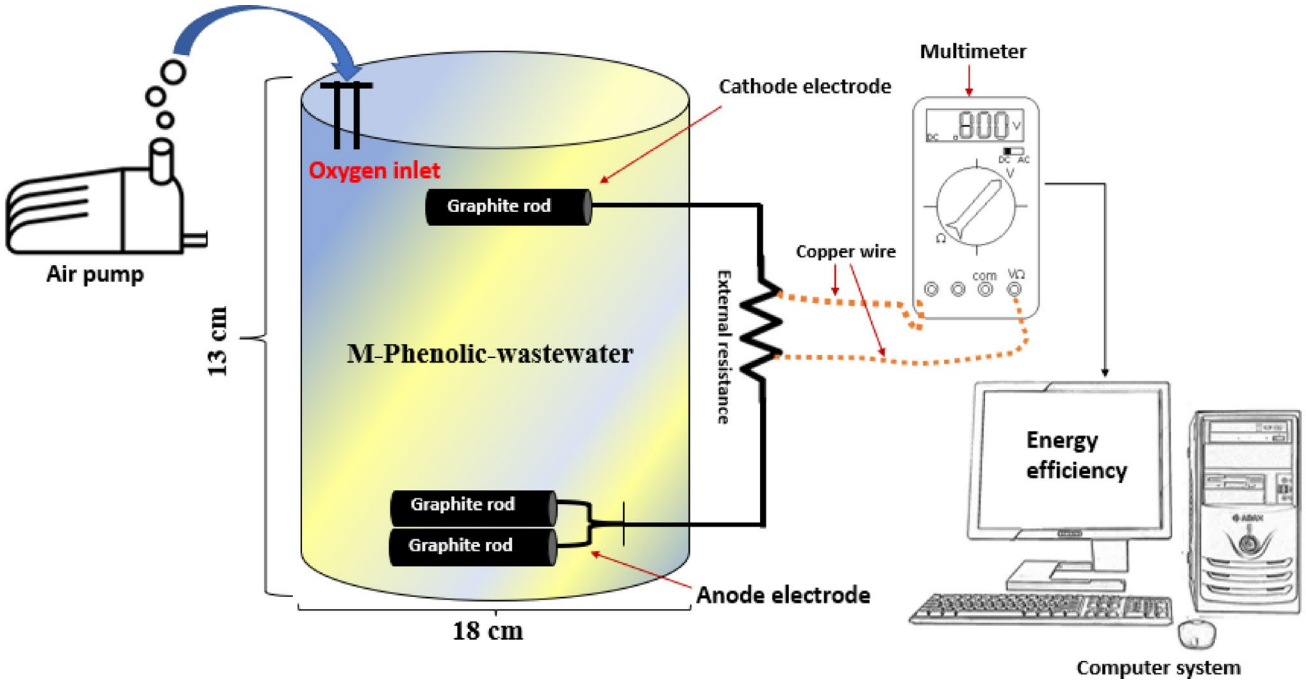


Fig. 1 A schematic diagram of MFC, developed on the concept of the present work

multimeter (China). According to Eqs. 1–4 presented in supporting file, the present study interpretation of the recorded voltage flow to the current, current density and power density (Alshammari et al. 2024). Next, polarization curve analysis was performed. The polarization curve describes the voltage output of a fuel cell for a given current density. Polarization curves are often generated using different external resistors, which draw a constant current from the fuel cell and monitor the output voltage of the fuel cell. In this study, it was measured when the voltage generation was at its peak. The MFC reactor was allowed to operate in open circuit condition for 1.5 h before polarization measurement, it was then connected non-stop to several resistance values (100 Ω to 5 kΩ) in descending flow direction (each resistor connected for 10 min) until maximum PD was reached.

Furthermore, to study the bio generation of electron pathway cyclic voltammetry (CV; BAS model Epsilon Version 1.4, Germany) was used in the present study. The anode and cathode act as working and counter electrodes respectively while the reference electrode was Ag/AgCl. The scan rate was 5 mV/s within a potential window range of – 0.8 to 0.8 V. To study the biofilm growth rate on the electrode surface, the specific capacitance (C_p) was calculated from the CV curve. Equation 4 (presented in supporting file). is used to calculate the C_p data.

Electrochemical spectroscopy (EIS) was used in the present study to characterize the MFC system. This is a valuable method in distinguishing fast or gradual electron transport flows as it analyzes single frequencies as well as large frequencies. This analysis simplifies the description of mass transfer resistance and electron transfer resistance in electrochemical reactions. It also helps calculate the internal resistance of the cell which is an important parameter in a fuel cell. Electrodes similar to those used in CV were also used for EIS analysis. The frequency spectrum range of 10 kHz – 5 mHz was used in this study.

Pollutant Treatment Efficiency Calculation

A UV-visible spectrophotometer (Shimadzu UV-Vis UV-2600i Spectroscopy) was used to study the degradation of phenol pollutant from M-phenolic wastewater. A sample of about 1 mL from the M-phenolic wastewater MFC was taken every 10 days, diluted to 50 ppm, and the amount of organic pollutant was determined. The following Eq. 5 was used to calculate the degradation efficiency (DE %) from UV results.

$$\mathrm{DE}\% = \frac{\mathrm{Ci} - \mathrm{Cf}}{\mathrm{Ci}} \times 100 \tag{5}$$

where, Ci = initial concentration of pollutant, Cf = final concentration of pollutant. A PerkinElmer AAnalyst 400 atomic absorption spectrometer (Germany) was employed to evaluate the efficiency of metal removal. The effectiveness of metal removal indicates the success of converting metals from a soluble to an insoluble form. A 1.0 mL sample

was extracted from the MFC chamber for metal analysis at specified intervals over a 15-day period. The removal efficiency of metals was calculated using Eq. 5.

Biological Characterizations of MFC Operation

In biological characterization, scanning electron microscopy (SEM)-Energy Dispersive X-ray analysis (EDX), and microbial sequencing studies were performed. The surface morphology of both electrode biofilms was observed using SEM before and after the operation. With the presence of coconut sugar solution and M-phenolic wastewater in MFC, the biofilm will grow. Upon completion of the operation, it was scraped from the electrode surface using a sterilized blade. Before SEM analysis, the biofilm was immersed in 0.1 M LBS solution at pH 7. Then it was dried and sent for SEM-EDX analysis to the USM Global Archaeological Research Center. The EDX analysis is carried to study the composition of electrode biofilm. Next, for microbial identification, the scraped electrode biofilm containing mixed microbial colonies, a 10^{-3} to 10^{-7} series dilution was performed. After the mixed cultures were placed on the nutrient agar surface, the T-streaking method was employed to isolate single colonies. Once single colonies were obtained, DNA amplification was performed using the reverse primer (1492R) and forward primer (27 F). The DNA was then amplified via polymerase chain reaction (PCR). The resulting amplified DNA was sent to the Centre for Chemical Biology (CCB) at USM for ribotyping. Upon receiving the raw data from CCB, it was analyzed using the Basic Local Alignment Search Tool (BLAST) available at https://blast.ncbi.nlm.nih.gov/Blast.cgi. Finally, the 16 S rRNA microb ial strains were deposited in GenBank (Daud et al. 2021). Lastly, the identified list of bacterial communities is plotted in the phylogenetic tree to demonstrate using the MEGA software.

Parameters Optimizations

The only purpose of the parameter optimizations examined was to investigate the trend in voltage generation. This investigation took into account the organic substrate, temperature, and pH. To begin, in order to optimize the generation of energy, a pH range of 4 to 10 was optimized. To change the pH, we utilised NaOH and H_2SO_4. The identical approach that was described before was used to conduct a series of tests. After 10 days, the voltages across each pH range were recorded. Similarly, the method described above was optimized for a temperature range of 20 °C to 35 °C. After 10 days, the voltage was also measured in each temperature range. Similarly in organic substrate, this study's inoculation source was the same as the previous one, but the organic substrates used were different. In this research, we compared the volatge performance of two organic substrates obtained from waste: food waste and bakery waste. To make the mixture, we simply mixed 100 g of waste with 200 mL of distilled water.

Result and Discussion

Electrochemical Results and Discussion

Voltage and Polarization Studies

Energy was produced by using MFC for a month. Despite the presence of toxic metals, the growth of bacteria fed by coconut sugar caused the cell's potential to rise as the phenol/metals were degraded as well. By day 21, the voltage generation had increased to a high of 799 mV. This pattern suggested that phenol functioned as an organic substrate, supplying bacterial populations with fuel and producing electrons as it degraded. The research showed that organic pollutant, such as phenol, may be useful for microbial activity and energy generation. The formation of biofilm on the anode surface significantly influenced the voltage production in the MFC. This biofilm, essential for electron transport to the anode, took several days to develop. During the initial 21 days, the voltage increased steadily from 0 to 799 mV. However, after this peak, the voltage began to decrease, as shown in Fig. 2a, and this downward trend continued for the rest of the study. The steady decrease in voltage after day 21 suggests that the organic substrate was fully oxidized, and the bacterial species could no longer produce electrons, leading to a constant reduction voltage level. The decline in voltage after day 21 in experiment could be attributed to several factors. Firstly, the complete oxidation of the organic substrate likely resulted in a lack of fuel for the bacteria, terminating electron production. Additionally, as the biofilm matured, its efficiency in electron transfer might have decreased due to structural or compositional changes. Nutrient limitations might have also played a role, as essential nutrients for bacterial growth and metabolism could have been depleted over time. Lastly, changes in environmental conditions such as pH or temperature could have negatively impacted bacterial activity, contributing to the observed decline in voltage (Hassan et al. 2019). Figure 2a shows that the voltage began to decrease as electron flow stopped from the anode to the cathode. Based on these results, it seems that the life cycle of these bacteria has expired.

Figure 2b demonstrates the relationship between stable voltage, CD, and PD in MFC. The study recorded the voltage using a variable resistor box and measured the current density using Ohm's law (Simeon et al. 2020). As shown,

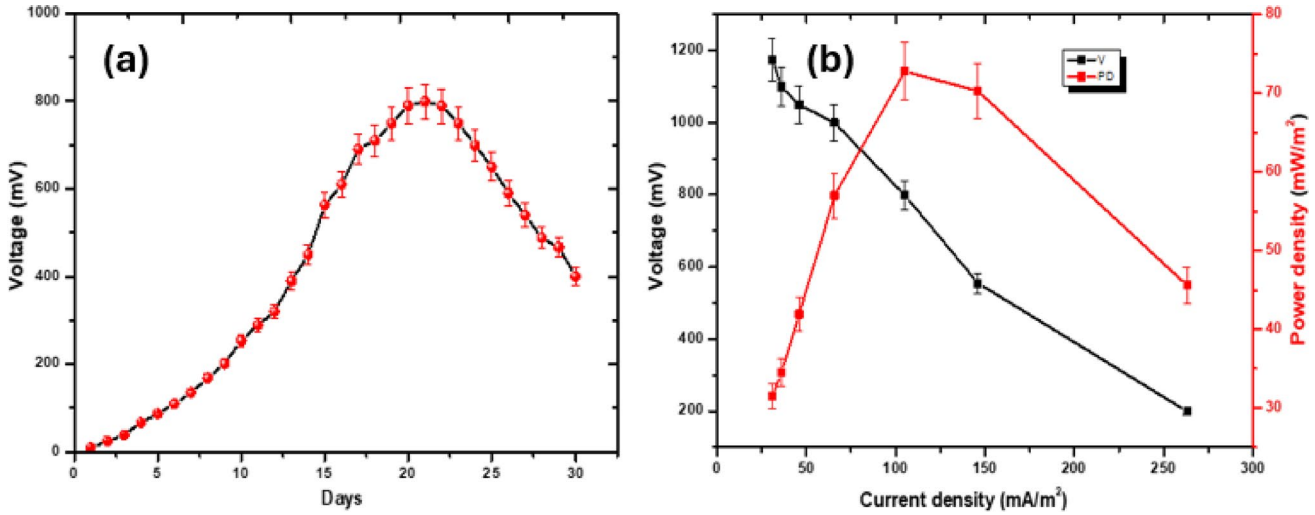


Fig. 2 Presentation of **a** voltage generation with passage of time and **b** polarization behaviour

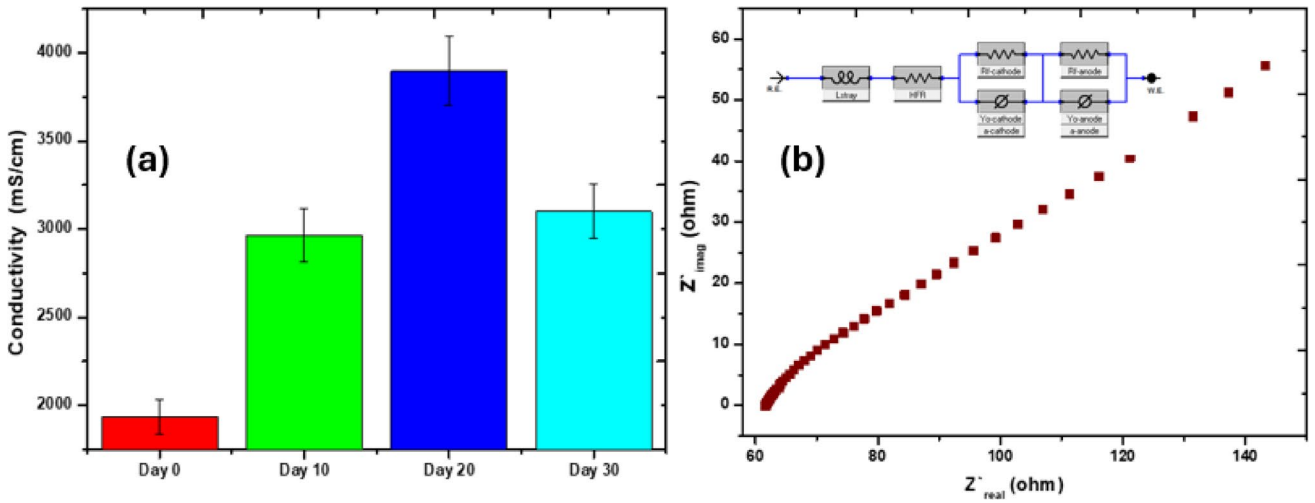


Fig. 3 **a** Condictivity test. **b** EIS curve of the study

the activation potential decreased from 1175 mV to 200 mV as the resistance increased from 5 kΩ to 100 Ω. This drop could be attributed to heat energy loss during oxidation or reduction, or to electron loss from bacterial terminal proteins to the anode. Using the external resistance from 5 kΩ to 100 Ω significantly increased the power density rate. At an external resistance of 1000 Ω, the power density was 72.77 mW/m^2, and the current density was 105.13 mA/m^2. Despite the slower stability at reduced resistance, we made progress towards a higher power density. The system minimized voltage destabilisation and maintained a relatively stable voltage even at low resistance levels, allowed electrons to move more rapidly, resulting in maximum current levels and power densities but with lower stability (Prakash et al. 2018). The electrode's electrical resistance and energy loss during proton transport via the cathode caused this phenomenon, known as ohmic overpotential (Abbas et al. 2018). High oxidative pressures at the anode led to a voltage drop as current density increased .

Conductivity and Electrochemical Spectroscopy (EIS)

Figure 3a illustrates how the cell's conductivity evolved over the 30-day experiment. Conductivity measurements were taken at various intervals, showing a range from 1935 mS/cm on day 0 to 3100 mS/cm on the day 30, with a peak of 3898 mS/cm on day 20. This high conductivity on day 20 correlates with a significant amount of power production, as indicated by the voltage patterns, which peaked on days 20 and 21. The system's performance remained largely consistent throughout the experiment, but long-term exposure to environmental factors such as pH changes, ubstrate degradation, and temperature fluctuations gradually reduced its efficiency. These findings align with a study by

Rojas-Flores et al. (2021), which also explored the impact of conductivity on MFC performance. The data suggests that maintaining high conductivity is essential for maximizing power output in MFC. However, the gradual decline in efficiency highlights the challenges of sustaining optimal conditions over extended periods. Addressing these environmental pressures through improved system design and management could enhance long-term performance. This study underscores the importance of continuous monitoring and adjustment to maintain high efficiency in MFC, making them more viable for practical applications in renewable energy generation. According to the present results, the author concluded to address "How might researchers practically enhance conductivity in MFC systems?" Researchers can enhance conductivity in MFC systems by improving electrode materials, such as using highly conductive carbon-based materials or metal alloys, which facilitate better electron transfer. Optimizing biofilm formation on the anode with bacterial strains that produce conductive biofilms and maintaining optimal environmental conditions like pH, temperature, and nutrient levels can also boost conductivity. Additionally, selecting easily degradable substrates and ensuring their continuous supply can sustain high metabolic activity. Using electrolytes with high ionic conductivity and integrating nanomaterials into the electrode structure can further enhance performance. Finally, innovative system designs that optimize flow patterns and minimize distances between electrodes can reduce resistance and improve overall conductivity, making MFC more efficient for renewable energy applications (Al-Zaqri et al. 2023).

In order to effectively illustrate the bio-electrochemical processes that occurred in the MFC, the Nyquist plot was developed using the EIS approach and is shown in Fig. 3b. As shown within Fig. 3b, the fuel cell circuit was used. Typically, the Nyquist plot is either a semicircle or a straight line. The diffusion-regulated reaction is shown by the straight line in this figure, and the resistance to charge transfer via EIS is shown by the semicircle's diameter (Yaakop andRafatullah 2022). This plot is shown in Fig. 3b, as previously described by other studies in a similar manner (Anwer et al. 2019; Bakar et al. 2022; Serrà et al. 2022). On the 30th day, after the biofilm had fully developed, the fuel cell was tested using an EIS-potentiostat. In terms of electrical accessibility, semi-circles or semi-bent lines indicate high mobility, while straight lines with a high Z'img (Ohm) suggest limited mobility. Evidence from day 30 showed that the biofilm was fully established and stable, as indicated by enhanced electronic mobility and semi-bent curves (He andMansfeld 2009). The internal resistance of the MFC was measured at 514.39 Ω. Low charge resistivity and internal resistance facilitate easier electron movement (Hung et al. 2019). However, after peaking on day 30, the voltage output began to decline. Internal resistance reduces electron mobility, and factors such as the electrolytes and the efficiency of the organic substrate also impact internal resistance (Idris andNoh 2023). Enhanced electronic mobility is crucial for the performance of MFC because it directly impacts the efficiency of electron transfer within the system. When electronic mobility is high, electrons can move more freely from the bacteria in the biofilm to the anode, which improves the overall electrical conductivity of the cell. This efficient electron transfer leads to higher voltage outputs and better power generation. Additionally, low internal resistance, which is associated with high electronic mobility, means that less energy is lost as heat, making the system more efficient. This also allows for a more stable and sustained power output over time. Enhanced electronic mobility can also reduce the impact of factors like electrolyte resistance and substrate efficiency, further optimizing the MFC performance. In summary, improving electronic mobility helps maximize the energy conversion efficiency of MFC, making them more effective for practical applications in renewable energy generation.

Cyclic Voltammetry Studies

Electrochemical techniques are commonly used to analyze the electrochemical behavior of MFC, particularly focusing on electron transfer interactions at the biofilm-anode interface. On the 30th day of the experiment, a forward peak at 0.8 V and a reverse peak at – 0.5 V were observed in the anodic chamber, indicating strong oxidizing and reducing properties, respectively. This suggests a well-established redox loop, essential for efficient electron transfer by the microbial communities (Fricke et al. 2008; López Zavala et al. 2019). The CV on day 30 showed maximum reversible peaks due to reduction electron-transfer, highlighting the role of redox potential in controlled electron migration to the anode as shown in Fig. 4a. Oxygen played a significant role in the oxidation and reduction processes, while the organic substrate was the main driver of the oxidation rate (Wen et al. 2021). Over time, the exoelectrogenic density on the electrode increased, enhancing potential values and affecting the electrical solution's flexibility and conductivity (Torres et al. 2010). Peak reduction currents were -1.6×10^{-5} mA on day 30, whereas the maximum oxidation currents were 4.0×10^{-5} mA. The decrease peaks seen between −0.5 and −0.6 V on day 30 most likely show the reduction of Pb^{2+} along with other metals such as Cd^{2+} and Cr^{3+} (Guerrero–Barajas andYaakop 2022). The electrochemical behaviour of phenol degradation over time is also shown by CV experiments. Current (mA) vs. voltage (V) profiles during various operating days (Day 1, Day 10, Day 20, and Day 30) are shown on the graph. Progressive phenol

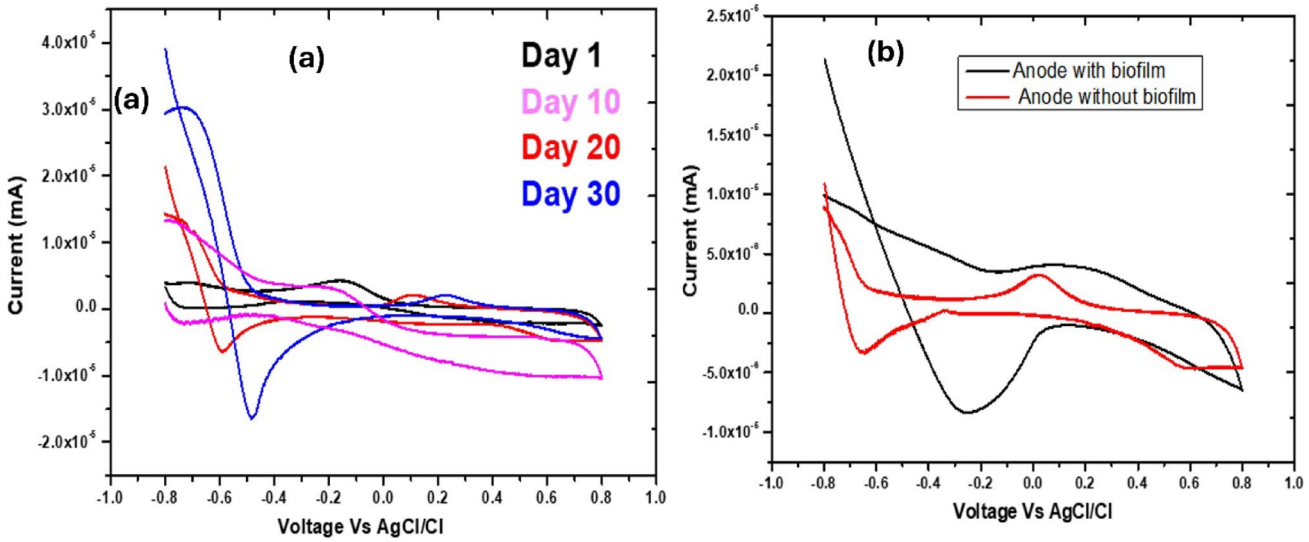


Fig. 4 **a** CV pattern on different days of operation. **b** CV pattern of anode with and without biofilm development

Table 2 The Cp values at various points in time

Day	Capacitance (F/g)
1	0.00007
10	0.00010
20	0.00019
30	0.00015

degradation is suggested by the rising current peaks over time, which show increased electrochemical activity. This is due to the fact that phenol oxidation generates intermediates that undergo further degradation, resulting in enhanced current responses. The successful breakdown of phenol is confirmed by the CV data, which shows larger current values at later stages. This is consistent with the MFC's increased microbial activity and substrate utilization. The study also examined the electrocatalytic activity of the inoculum on different days, with the 30th day showing the highest peak, indicating superior stability and specific capacitance (see Table 2). Specific capacitance quantifies the capacity to store charge per unit mass of the electrode material. Table 2 indicates that the specific capacitance rises from 0.00007 F/g on day 1 to a maximum of 0.00019 F/g on day 20, reflecting the development of the biofilm and improved charge storage capacity. By day 30, the specific capacitance decreases to 0.00015 F/g. This reduction may be ascribed to many sources, including the deterioration of the biofilm and the electrode material (Gallay 2016). Over time, the stability of biofilm may be undermined by environmental variations or nutrient depletion, resulting in diminished microbial activity and electron transfer efficiency (Lv et al. 2020). Additionally, the electrode material itself may undergo wear and tear, affecting its conductivity and overall performance Two. These effects combined result in the noted reduction in specific capacitance on day 30. The overall anode potential increased as the biofilm developed, likely due to the redox enzymes in the bacterial cell membrane and the charge storage capabilities in the bacterial cytoplasm (Al-Zaqri et al. 2023). These findings underscore the importance of biofilm development and electron transfer efficiency in optimizing MFC performance.

The redox activities of the bioanode electrode system, both with and without biofilm (on different days), were examined using CV as shown in Fig. 4b. Figure 4b illustrates that CV, within a potential range from −0.8 to 0.8 V, revealed redox activity in both the presence and absence of biofilm. Phenol oxidation exhibited a distinct peak in both conditions. The CV results with biofilm showed a higher oxidation current of 2.2×10^{-6} mA compared to 1.12×10^{-5} mA without biofilm. Therefore, the results with biofilm confirm redox activity. However, redox activity alone does not fully account for power generation, as it also relies on extracellular electron transport (Hassan et al. 2018). The possible electron transfer route in phenol degradation, which involves periplasm-membrane embedded electron respiration (Yu et al. 2018), was similar to the redox peak observed in CV. The electrode with biofilm demonstrated higher oxidation and reduction peaks (Umar et al. 2021a).

Pollutant Treatment Results and Discussion

Phenol Degradation Mechanism and Present Study Efficiency

The incorporation of coconut sugar as an organic substrate significantly enhanced the degradation rate by boosting bacterial metabolism. Organic substrates are essential for phenol degradation in MFC since they augment microbial activity

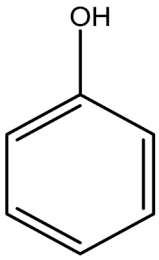
and facilitate electron transfer mechanisms. Although phenol may be directly degradable by microbes, the addition of an extra organic substrate can markedly enhance the overall effectiveness of the degradation process. Organic substrates, such coconut sugar or carbohydrates based material, provide an extra carbon source that facilitates the proliferation and metabolic function of electroactive bacteria (Al-Zaqri et al. 2023; Wei et al. 2018). The enhanced microbial activity results in the development of a more resilient and conductive biofilm on the anode, hence improving electron transport and energy production (Al-Zaqri et al. 2023). Furthermore, the co-metabolism of phenol and the organic substrate might result in synergistic effects, optimising the degradation routes of both molecules. The organic substrate may enhance the synthesis of redox-active chemicals and enzymes that promote the degradation of phenol. This dual-substrate methodology enhances phenol degradation efficiency and increases the total power output of the MFC, making it a more effective and sustainable technology for wastewater treatment and energy generation (Wei et al. 2018). This study demonstrated an impressive 82.18% efficiency in phenol degradation using MFC. The process involved two types of microbes: bio-degradative and electroactive. The bio-degradative bacteria precisely targeted phenol, resulting in complete ring cleavage, while the electroactive bacteria aided in the decomposition of intermediates. Phenolic acid, an intermediate, was produced through a comprehensive carboxylation process facilitated by the enzyme carboxylase phenol. The subsequent breakdown of phenol by electroactive bacteria yielded carbon dioxide (CO_2) and electrons, as illustrated in the electrochemical processes. Equations visually demonstrate the degradation of phenol and phenolic acid into their final products, with absorbance peaks of phenol at distinct time intervals clearly depicted. This study highlights the synergistic role of bio-degradative and electroactive bacteria in phenol degradation, emphasizing the potential of MFC in environmental bioremediation. The use of coconut sugar not only improved bacterial activity but also demonstrated a sustainable approach to enhancing degradation processes. Figure 5a and b displays the estimated degradation percentage based on UV curves. The absorbance peak of phenol at $\lambda max = 275$ nm, which is induced by the transition $\pi \rightarrow \pi^*$ of aromatic (C=C), appears on curve day 10 before the reactor operates (Dearden andForbes 1959). The phenol peak of the curve has shifted to a maximum wavelength of 245 nm after the reactor cycle is completed. This is where the degradation of phenol into phenolic acid starts (Kowalski and-Kowalska 2005). Phenolic acid is produced from phenol. The intermediate exhibits a greater degree of conjugation than phenol due to the delocalisation of electrons (Craft et al. 2012). As can be observed from the curve that appeared between days 20 and 30, which exhibited a band at 245 nm, the breakdown of the intermediate phenolic acid was largely degraded. An further indication of a slow deterioration trend is provided by the computation of degradation efficiency. In light of these discoveries, the electrochemical equation that is shown below provides a description of the anodic and cathodic processes that occur inside the MFC.

Metal Removal Efficiency and its Mechanism

Table 3 showcases the research findings on metal ion removal. Utilizing a bioelectrochemical system for metal removal is emerging as one of the most promising and innovative techniques, especially with MFC. The present study reveals new insights into the removal of metal ions in M-phenolic-wastewater. As the process nears completion, there is a gradual increase in the efficiency of metal removal. The results provided here are notably noteworthy in comparison to past study. This study achieves a removal efficiency over 90%. The findings indicate a reduction in metal ion concentration in the M-phenolic-wastewater.

During a redox reaction, soluble metal ions undergo a transformation into an insoluble state, resulting in the formation of sludge. This phenomenon was previously mentioned and is crucial for understanding the process. The AAS test results provided insights into the concentrations of metal ions that remained in the water samples post-reaction. These tests are essential for quantifying the efficiency of the redox process in removing metal ions from the solution. The metal ions that were successfully removed from the solution were converted into their oxide forms thus precipitating out of the solution as sludge. This sludge-like substance was observed in the MFC, indicating the successful conversion and removal of metal ions. Extensive research supports these findings, demonstrating that the extracted metal ions consistently convert into their oxide forms. This conversion is a critical aspect of the redox reaction, as it ensures the metal ions are no longer soluble and can be effectively separated from the water. The resulting sludge contains these metals in their oxide states, which is a key indicator of the reaction's success. Furthermore, the formation of metal oxides and their subsequent precipitation as sludge is not only a marker of the reaction's efficiency but also has practical implications for waste management and environmental protection. By converting soluble metal ions into insoluble oxides, the redox reaction helps in reducing the mobility and bioavailability of potentially harmful metals, thereby mitigating their environmental impact (Abbas et al. 2018; Umar et al. 2021a; Yaqoob et al. 2020). The following is a mechanism of the biological events that occur during metal reduction (also shown in figure "Basic Mechanism of Coconut Sugar Oxidation and Electron Transportation"):

Anode reaction:

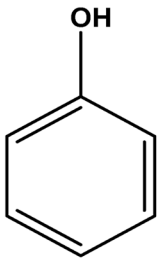


$$+ \quad 11H_2O \longrightarrow 6CO_2 + 14H^+ + 14e^-$$

Cathode reaction:

$$3.5O_2 + 14H^+ + 14e^- \longrightarrow 7H_2O$$

Overall electrochemical reaction:



$$+ \quad 7O_2 \longrightarrow 6CO_2 + 3H_2O$$

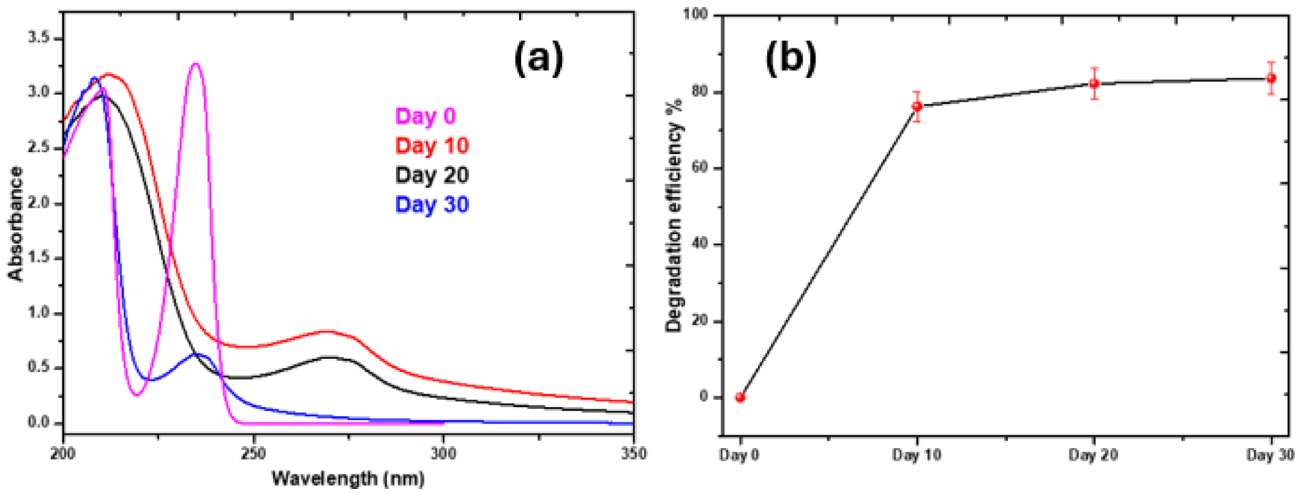


Fig. 5 **a** UV spectra at different time intervals. **b** Degardation percentage in 30 days

Table 3 Removal efficiency trend of metal in the presence of M-phenolic-wastewater in MFC

Organic matter	Initial concentration (ppm)	Experimentation days	Removal % of Pb^{2+}	Removal % of Cd^{2+}	Removal % of Cr^{3+}
Coconut sugar	50	0	0.00	0.00	0.00
		10	34.29±0.5	35.27±0.5	34.00±0.5
		20	77.56±0.5	69.45±0.5	70.17±0.5
		30	92.10±0.5	89.85±0.5	90.60±0.5

- Reduction of Pb^{2+}.

$$Pb^{2+} + 2e^- \rightarrow Pb_{(s)}$$
$$2Pb^{2+} + 2H_2O \rightarrow 2PbO + 4H^+$$
$$PbO + 2e^- + 2H^+ \rightarrow Pb_{(s)} + H_2O$$

- Reduction of Cr^{3+}.

$$Cr^{3+} + 3e^- \rightarrow Cr_{(s)}$$
$$2Cr^{3+} + 3H_2O \rightarrow Cr_2O_3 + 6H^+$$
$$Cr_2O_3 + 6e^- + 6H^+ \rightarrow 2Cr_{(s)} + 3H_2O$$

- Reduction of Co^{2+}.

$$Cd^{2+} + 2e^- \rightarrow Cd_{(s)}$$
$$2Cd^{2+} + 2H_2O \rightarrow 2CdO + 4H^+$$
$$CdO + 2e^- + 2H^+ \rightarrow Cd_{(s)} + H_2O$$

Biological Results and Discussion

Electrode Biofilm Studies

To gain deeper insights into the bacterial morphology on electrode surfaces, SEM analysis was performed on both the anode and cathode before and after the operation. The SEM images highlighted significant differences among untreated graphite, treated anode, and treated cathode, as depicted in Fig. 6a–c. Figure 6a illustrates the untreated graphite electrode, characterized by a smooth, planar surface typical of unused commercial graphite. Conversely, the anode electrode displayed a dense, filamentous structure on its surface, indicative of bacterial colonies (Fig. 6b). These colonies are likely composed of biodegradative and electroactive microorganisms. The bioanode substantially influences the efficacy of MFC by improving electron transport and energy storage capacities. The thick, filamentous structure on the anode surface signifies the existence of biodegradative and electroactive bacteria, essential for effective electron transfer (Khater et al. 2022). As the biofilm develops, the specific capacitance rises to a peak of 0.00019 F/g on day 20, indicating enhanced charge storage inside the bacterial cytoplasm. This maturation results in an elevated anode potential, facilitated by redox enzymes inside the bacterial cell membrane. Thus, the power density and current density reach their maxima at 72.77 mW/m² and 105.13 mA/m², respectively, with an external resistance of 1000 Ω. The anode electrode's stability over 30 days reinforces constant power output and voltage patterns, underscoring the need of bioanode advancement for optimum MFC performance. The cathode also exhibited few bacterial clusters, although these were less dense and less developed compared to those on the anode (Fig. 6c). A mature biofilm was observed on the anode electrode, which was subsequently scraped to identify the bacterial species present. The SEM images of the anode revealed a predominance of tube or rod-shaped morphologies, suggesting the presence of bacteria with conductive pili. These pili are filamentous structures that resemble rod-shaped wires, which bacteria use to transfer electrons from the cell to the anode electrode. This analysis highlights the significant role of bacterial morphology and biofilm development in the electrochemical performance of the electrodes, particularly the anode, where the presence of conductive pili facilitates electron transfer (Serrà et al. 2021). The interaction between bacterial cells and the anode surface is both significant and direct. Numerous experiments have demonstrated that conductive pili play a crucial role in this process. These pili effectively transmit electrons from the bacterial cells to the anode surface, facilitating efficient electron transfer and enhancing the overall electrochemical performance of the system (Guang et al. 2020; Lovley 2017; Reimers et al. 2017). Based on this research, it can be concluded that the dense biofilm development signifies the anode's high biocompatibility with living organisms. The high sugar content of the provided organic substrate results

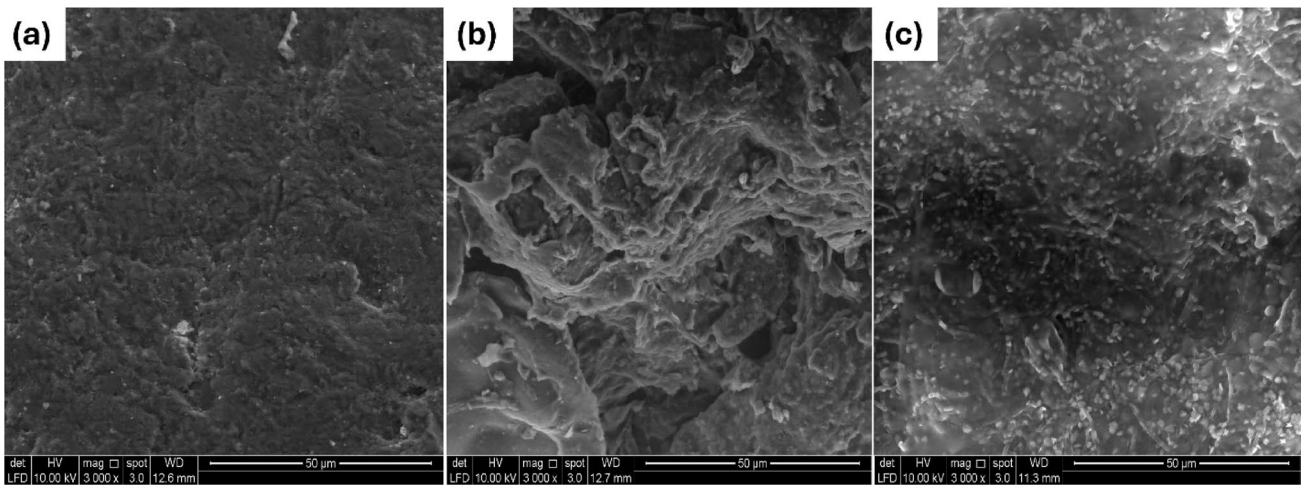


Fig. 6 SEM images of **a** Original graphite rod. **b** Anode electrode after operation. **c** Cathode electrode after operation

in a limited variety of bacterial species, which are densely concentrated on the biofilm's surface (Aleid et al. 2023). An EDX analysis was conducted on the biofilm to identify the components present on the anode's surface. As shown in Fig. 7, the analysis revealed that toxic metals does not adversely affect bacterial populations. This inference is corroborated by the finding that the anodic biofilm comprised solely carbon and oxygen upon conclusion of the operation. In essence, the study demonstrated that the inorganic/organic pollutants present during the process did not manifest any toxic effects on the bacterial community.

Bacterial Identification from Anode Biofilm

As shown in Fig. 8, the clone library realization technique was used to evaluate the anode microbial composition. The results show that the biofilm on the anode is quite similar in shape. In this investigation, the most common types of bacteria were *Lysinibacillus*, *Lysobacter* and *Pseudoxanthomonas*. Moreover, numerous scholarly publications have delineated these exoelectrogens and biodegradative microbial species as pivotal agents in pollutant remediation and energy production (Fang and Achal 2019; Hosseini et al. 2023; Wang et al. 2023; Yang et al. 2021b). For example, recently, Hosseini et al. (2023) stated that the degradation of phenol is caused by Lysinibacillus. It indicates that the electrons in the final product are also being produced. In their investigation of *Pseudomonas* species, Zhang et al. (2020) demonstrated the degradation of xylene and toluene. The toxicity of organic pollutant was reduced by the *Pseudomonas* species biofilm on the MFC electrode surface. In order to lessen the adverse environmental effects of resorcinol, *Pseudomonas* species were used in the present investigation to degrade phenol. However, in this research, *Pseudomonas* and *Bacillus* species were unique biocatalysts that degraded the pollutant by turning it into non-toxic CO_2 while also producing energy. The identified bacterial species are classified as conductive pili-typed bacterial species based on the literature (Li et al. 2017; Seymour et al. 2023; Yang et al. 2021a). In conclusion, we can say that the degradation of phenol and the removal of metal ions are caused by the bacterial species *Lysinibacillus*, *Lysobacter* and *Pseudoxanthomonas*, respectively.

Basic Mechanism of Coconut Sugar Oxidation and Electron Transportation

MFC depend on the activity of bacteria for the production, transport, and reduction of metal ions. Various bacterial species are recognized in the literature as effective exoelectrogens, which are microorganisms capable of transferring electrons to external electrodes. Notable examples include *Bacillus*, *Klebsiella*, *Escherichia*, and *Actinobacillus*. These bacteria play a crucial role in the electrochemical processes within MFC, facilitating the conversion of chemical energy into electrical energy through their metabolic activities (Umar et al. 2021a; Yaqoob et al. 2020). In the current investigation, coconut sugar was utilized as an organic substrate

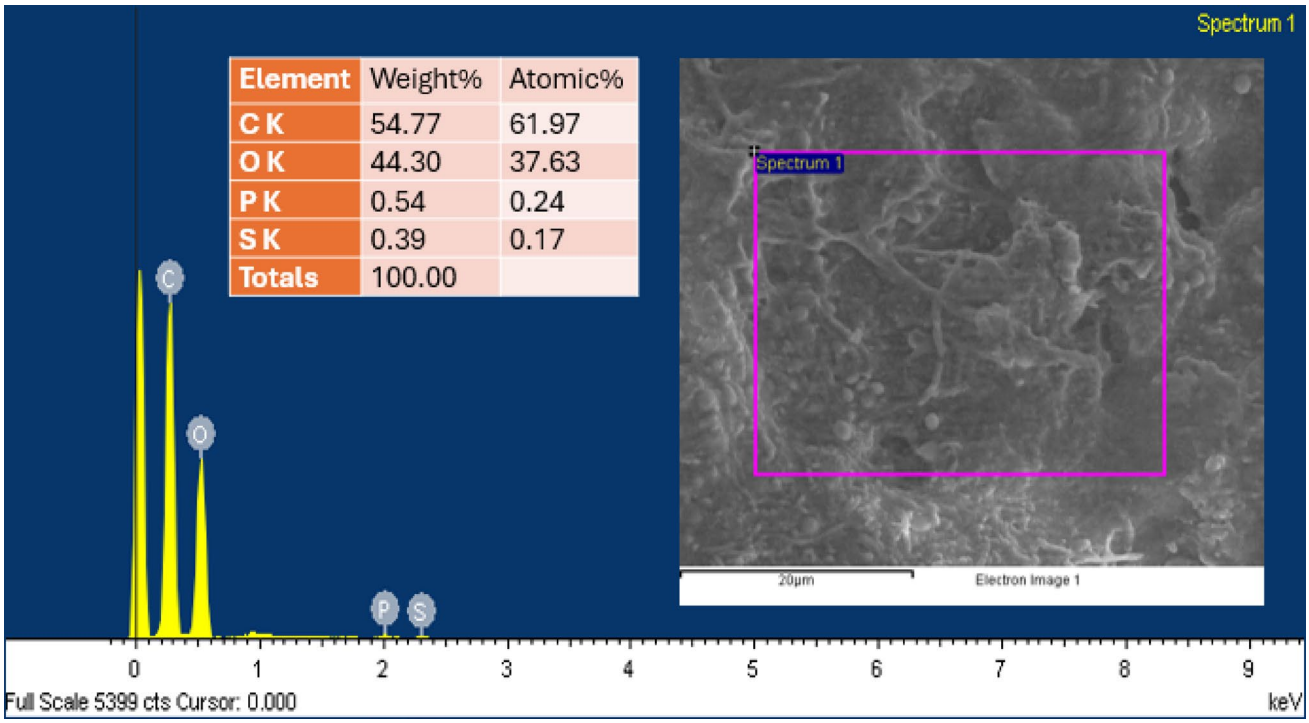


Fig. 7 EDX spectra of anode electrode after 30 days operation of MFC

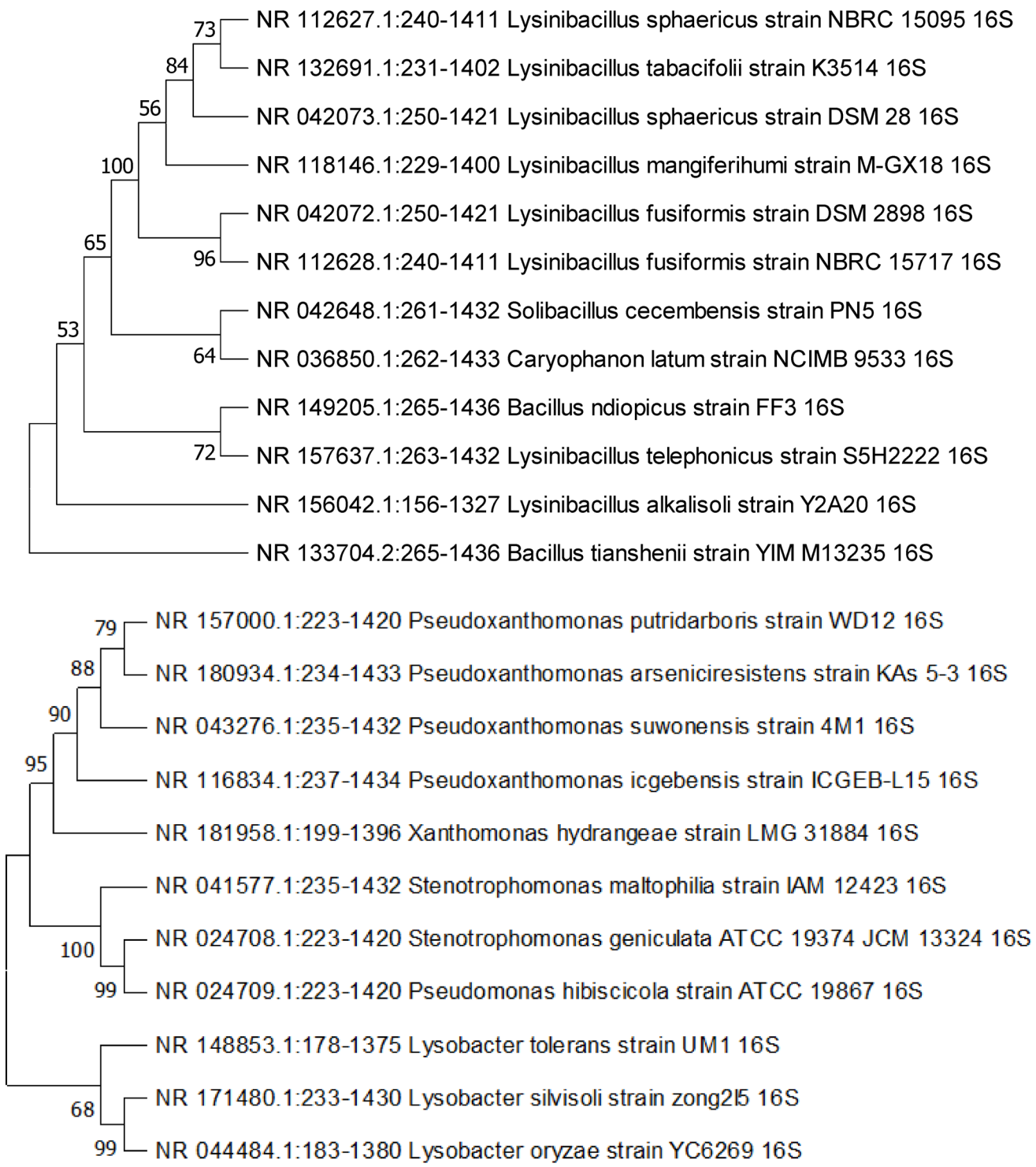


Fig. 8 Phylogenetic review of the MFC anode biofilm, displaying the key isolates' phylogenetic positions

for various bacterial species. Initially, the coconut sugar, which is a disaccharide, undergoes conversion into simple glucose. This glucose is subsequently oxidized by the bacterial species, leading to the production of electrons and protons. The observed oxidation and reduction reactions in this study can be represented as follows: According to equations and Fig. 9 (a detailed discussion on Fig. 9 has been reported by Umar et al. (2021b) the emission of CO_2 at the anode surface is a consequence of anaerobic metabolic activities. In anaerobic situations, glucose is metabolized by bacteria via fermentation and methanogenesis processes. Glucose is first transformed into intermediary molecules, including

acetate and hydrogen. The intermediates undergo further processing by various microbial populations, resulting in the creation of CO_2 and CH_4. The CO_2 detected at the anode surface is a consequence of metabolic processes, indicating the continuous anaerobic breakdown of organic substrates.

$$\text{Oxidation}: \text{Coconut sugar} \rightarrow C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$
$$\text{Reduction}: 24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O$$
$$\text{General reaction}: C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Electricity}$$

After a period of time, the electrons and protons produced during the oxidation process are transferred from the anode electrode to the cathode electrode. In the single-chamber MFC, protons can move free from the anode to the cathode chamber, while electrons travel through the external circuit. Previously reaching the cathode, electrons must undergo several intermediary steps involving multiple processes. These electrons originate from bacterial cells. The most widely studied mechanism is detailed in the following paragraphs and illustrated in Fig. 10. The Fig. 10 also showed the metal conversion from soluble to insoluble form.

To facilitate the transfer of electrons from bacterial cells to the anode, redox-active proteins such as OmcS, OmcZ, OmcB, OmcT, and OmcE are used. These proteins play a crucial role in electron transmission, a mechanism predominantly utilized by the *Geobacter* family for electron transfer.

(a) Short-range electron transfer is another effective method for electron transport. In this process, bacteria utilize self-produced reduced and oxidized shuttles. Both the *Desulfuromonadaceae* and *Geobacteraceae* families are capable of producing their own electron shuttles. Key components of these electron shuttles include molecules such as OmcA, MtrF, MtrE, and MtrC.

(b) The ability of bacterial populations to transmit electrons over long distances is dependent on the presence of conductive pili. These pili, which resemble conductive metals, are integral parts of the bacterial structure and are used to directly transfer electrons from the bacterial cell to the anod. Furthermore, it was found that the present study used a long-range method for electron transfer after the biofilm research and the bacterial investigations. After examining the biological testing, this was found. The electron transfer mechanisms in MFC are crucial for several reasons:

1. Efficient energy conversion: The ability of bacteria to transfer electrons efficiently from their cells to the anode is fundamental to the operation of MFC. This process converts chemical energy from organic substrates into electrical energy, making MFC a promising technology for sustainable energy production.
2. Role of redox-active proteins: Proteins are essential for facilitating electron transfer. These proteins act as conduits, enabling the movement of electrons from the bacterial cells to the anode. This mechanism is particularly significant in the Geobacter family, which is known for its high efficiency in electron transfer.
3. Short-range electron transfer: This method involves the use of self-produced electron shuttles by bacteria.

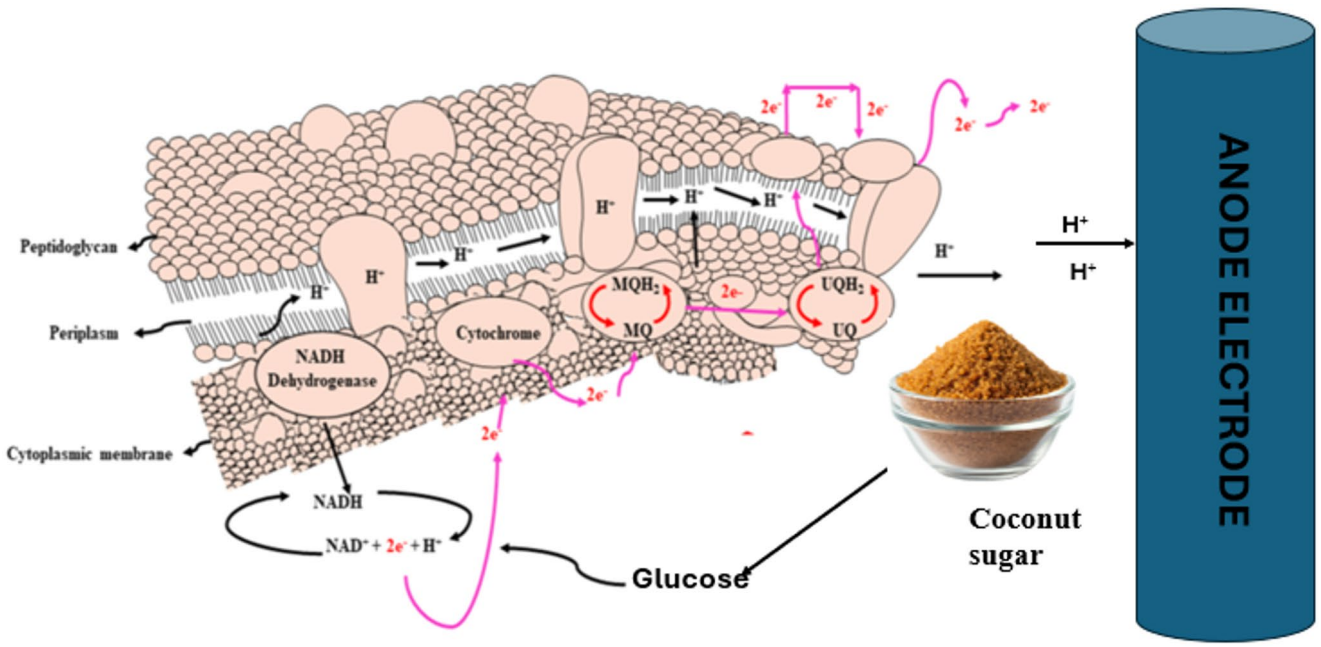


Fig. 9 Demostrataion of coconut sugar oxidation- leading to electron generation (Figure modified from reference (Umar et al. 2021b) with Elsevier permission)

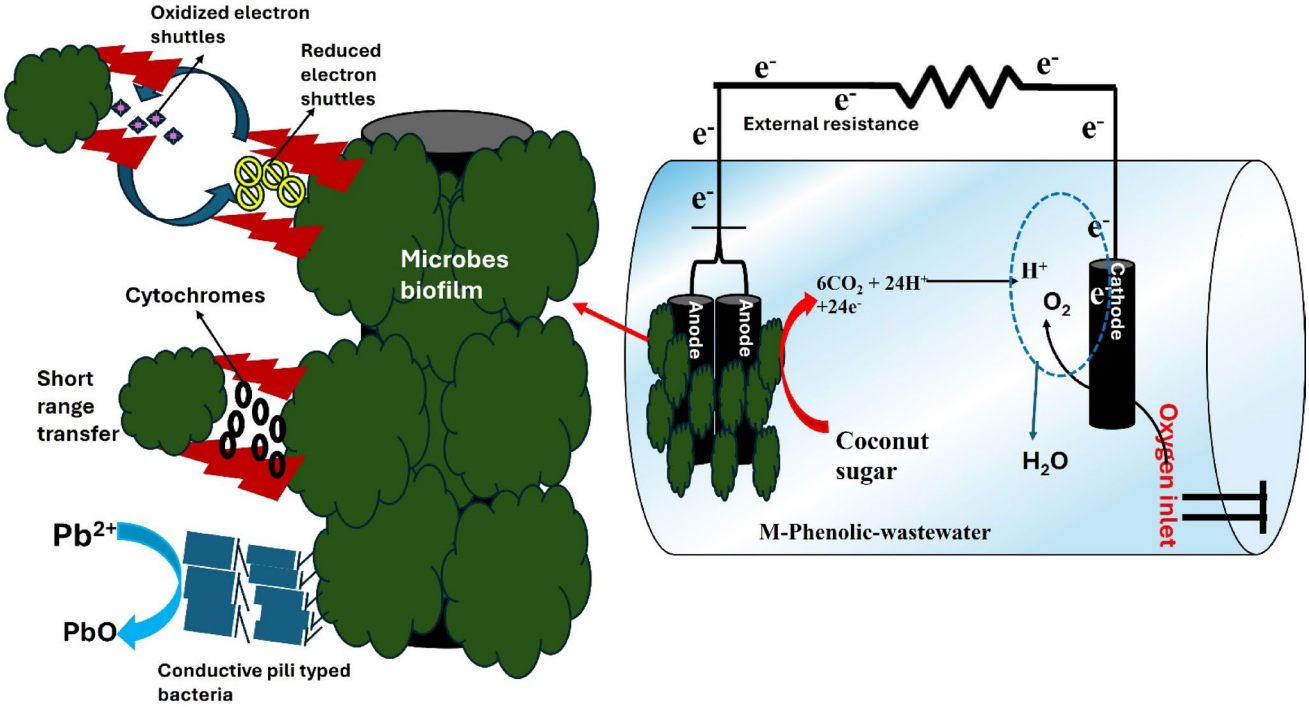


Fig. 10 Demostration of electron generation and electron transfer mechanism from bacteria to anode in MFC

These shuttles, which include molecules like OmcA, MtrF, MtrE, and MtrC, allow for the transfer of electrons over short distances. This process is vital for maintaining the flow of electrons within the MFC, ensuring continuous energy production.

4. Long-range electron transfer: Conductive pili, which resemble metallic conductors, enable bacteria to transfer electrons over longer distances. This capability is crucial for connecting bacterial cells directly to the anode, enhancing the overall efficiency of the electron transfer process. The conductive pili ensure that electrons can be transported effectively, even in densely packed bacterial communities.
5. Environmental impact: These electron transfer mechanisms also play a significant role in bioremediation. By facilitating the reduction of metal ions and other pollutants, MFC can help in cleaning up contaminated environments. The bacteria involved in these processes can convert harmful substances into less toxic forms, contributing to environmental sustainability.
6. Technological advancements: Understanding and optimizing these electron transfer mechanisms can lead to significant advancements in MFC technology. Improved efficiency and scalability of MFC could make them a viable alternative for renewable energy sources, wastewater treatment, and other applications.

In summary, the electron transfer mechanisms in MFC are integral to their function and efficiency. They not only enable the conversion of organic matter into electrical energy but also have broader implications for environmental protection and technological innovation.

Parameter Optimization

pH and Temperature

Figure 11a illustrates how pH affects the slope of voltage generation when coconut sugar is present. Observations indicate that voltage generation steadily increases between pH 4 and 7, but declines beyond pH 7. This suggests that highly alkaline or highly acidic conditions are not ideal for maintaining stable operations. During continuous operation of the MFC, the highest voltage recorded was 250 mV at pH 7. In contrast, pH 10 resulted in only 65 mV, and pH 4 produced 199 mV. The low voltage generation in alkaline or acidic conditions is primarily due to the disruption in bacterial growth, which directly impacts biofilm production. A weak and unstable biofilm leads to poorer performance. However, the study only examines a narrow pH range (4 to 10), and it would be beneficial to explore a broader spectrum to fully understand the pH influence on voltage generation. The explanation attributes low voltage generation to biofilm instability but does not delve into the specific mechanisms or potential solutions to enhance biofilm resilience.

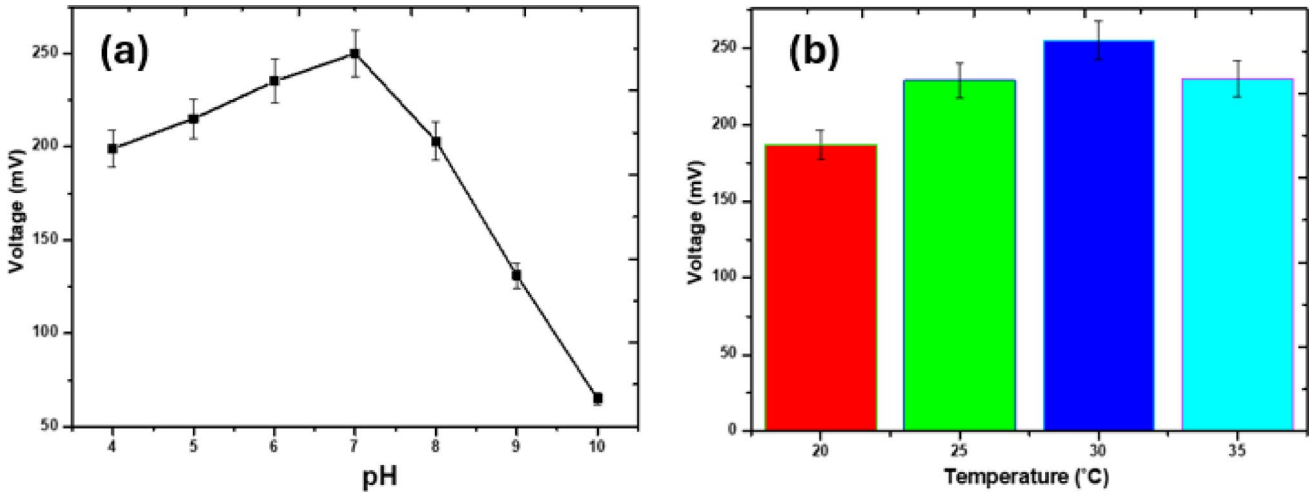


Fig. 11 **a** pH optimization and, **b** range of temperature optimization in term of voltage generation trend

Additionally, the study assumes continuous operation of MFC, but it does not address how intermittent or variable conditions might affect voltage generation and biofilm stability. While coconut sugar is used in this study, it would be useful to compare its effects with other types of sugars or organic substrates to determine if the observed trends are unique to coconut sugar or more generalizable. These comments highlight areas where further research and more detailed analysis could strengthen the findings and provide a more comprehensive understanding of the factors influencing voltage generation in MFC. In the previous sources, Huang et al. (2012) extensively investigated optimization parameters, with a particular focus on pH optimization. Their study found that the highest voltage output occurred at a neutral pH, while a pH of 5.2 led to the formation of acidic sludge, significantly disrupting voltage output. The findings suggest that a near-neutral pH provides the most optimal conditions for voltage generation.

To maximize the impact of temperature on energy production, the MFC setups were run at the temperature range of 20 ˚C to 35 ˚C, which is above or below room temperature. The results showed that between 25 ˚C and 30 ˚C was the ideal temperature range for MFC operation, obtaining maximum energy production (Fig. 11b). Because of the unfavourable circumstances for bacterial development, the voltage potential was much lower at 20 ˚C, which is below room temperature. For good development, exoelectrogenic bacteria need ideal circumstances, such as a favourable temperature. The research only looks at a small range of temperatures, however, and a wider range would provide more thorough information on how temperature affects MFC performance. Furthermore, it would be helpful to look at how temperature fluctuations affect MFC performance and bacterial growth, since the research makes the assumption that temperature conditions are constant. Since various strains of exoelectrogenic bacteria may have different ideal temperature ranges, which might affect the findings, the paper does not say if several strains were examined. Additionally, knowing how temperature impacts long-term operation may be essential for practical applications, since the research does not address the stability of MFC performance over the long term at various temperatures. These remarks point out areas that need further investigation and provide ideas for improving the results' reliability and relevance.

Organic Substrate

During the operation of MFC, the organic substrate provides the energy needed for bacterial growth and respiration. Further, which in turn generates electrons and protons while simultaneously removing pollutants. One of the most crucial components of bioelectrochemical cells is the organic substrate, as respiration fuel is essential for bacteria during oxidation (Barbosa et al. 2017). Research into MFC has made use of a wide variety of organic substrates, including glucose, carbohydrates, amino acids, sucrose, fructose, cellulose, vegetable scraps, and biomass (Gezginci andUysal 2016; Meshram andJadhav 2017; Pant et al. 2010). If the system is used on a big scale, these natural wastes may be used as an organic substrate to reduce running expenses. According to present research, coconut sugar is superior, but its value is limited to areas where it is readily available and inexpensive as shown in Table 4. Waste products are often the best substitute since they are used as organic substrate. Coconut sugar is an better organic substrate for MFC due to its rich nutrient profile, including amino acids, vitamins, minerals, polyphenols, and antioxidants, which support robust microbial growth and activity. Its low glycemic index ensures a steady energy release, sustaining microbial activity over extended periods (Asghar et al. 2020; Saraiva et al.

Table 4 Comparing the voltage generation profiles of different organic substrates

Organic substrate	Inoculation source	MFC operational days	Voltage
Food waste	M-phenolic wastewater	10	116
Bakery waste	M-phenolic wastewater	10	204
Cocunut sugar	M-phenolic wastewater	10	254

Table 5 Comparison of metal ion removal efficiencies from previous studies

Organic substrate	Metal	Initial concentration (ppm)	Metal removal efficiency (%)	References
Sodium acetate	Cd^{2+}	–	31.00	Habibul et al. (2016)
Sodium acetate	Pb^{2+}	50	44.11	Habibul et al. (2016)
Acetate	Cd^{2+}	50	60.00	Gai et al. (2018)
Oil palm trunk sap	Cd^{2+}	50	70.10	Yaakop and Ahmad (2021)
Oil palm trunk sap	Pb^{2+}	50	75.00	(Yaakop and Ahmad 2021)
Sapodilla waste	Cd^{2+}	50	92.88	Fadzli and Yaakop (2023)
Sapodilla waste	Pb^{2+}	50	95.40	Fadzli and Yaakop (2023)
Sapodilla waste	Cr^{3+}	50	89.70	Fadzli and Yaakop (2023)
Potato waste	Cr^{3+}	100	84.12	Al-Zaqri et al. (2022)
Rotten rice	Cd^{2+}	–	82.2	Daud et al. (2021)
Coconut sugar	Cr^{3+}	50	90.60	Present study
Coconut sugar	Cd^{2+}	50	89.85	Present study
Coconut sugar	Pb^{2+}	50	92.10	Present study

2023). Compared to food waste and bakery waste, coconut sugar generates higher voltage, indicating superior bioelectricity production. Additionally, its sustainable production methods and non-toxic nature make it an environmentally friendly choice. These scientific advantages make coconut sugar a highly effective and sustainable substrate for MFC.

Comparative Analysis/Highlighting the Contribution of the Present Study

This study demonstrated the efficiency of coconut sugar as an electron source in MFC for energy generation and pollutant treatment. The literature review highlights the unique aspects of phenol degradation and metal removal. Table 3 presents the removal efficiency, while Fig. 2 illustrates the voltage generation trend. For instance, Ibrahim et al. (2021) investigated the removal of Pb^{2+} using sweet potato as an organic substrate, achieving a total energy efficiency of 1.05 mW/m² and a 60.33% removal of Pb^{2+} over 40 days. In comparison, Wang et al. (2020) reported a voltage of 451 ± 19.1 mV using MFC, whereas the current study achieved 2900 mV. Table 5 compares the metal ion removal efficiencies of various organic substrates used in different studies. Coconut sugar, as highlighted in the present study, shows remarkable removal efficiencies for Cr^{3+} (90.60%), Cd^{2+} (89.85%), and Pb^{2+} (92.10%) at an initial concentration of 50 ppm. These results are among the highest compared to other substrates such as sapodilla waste, which also demonstrated high removal efficiencies for Cd^{2+} (92.88%), Pb^{2+} (95.40%), and Cr^{3+} (89.70%) (Fadzli andYaakop 2023). Other substrates like oil palm trunk sap and acetate showed lower removal efficiencies, ranging from 60.00 to 75.00% (Yaakop andAhmad 2021). The superior performance of coconut sugar underscores its potential as an effective substrate for metal ion removal in MFC, contributing positively to the field of environmental remediation and sustainable energy production. This study provides valuable insights into optimizing substrate selection for enhanced MFC performance. To compare the energy efficiency of different organic substrates used in MFC, a Table S2 is provided. Coconut sugar outperformed other substrates, including wastewater from the chocolate industry, macroalgae, and food waste, with the maximum current density 105.13 mA/cm² and power density 72.77 mW/m². This work brings attention to the possibility of using coconut sugar as a substrate for MFC applications, which might have a favourable impact on sustainable energy generation. Coconut sugar improves MFC efficiency by increasing electron production and energy storage, according to the results. This work demonstrates the superior efficiency of coconut sugar, paving the way for more effective and ecologically sustainable energy solutions, and providing crucial insights for the design and operation of future MFC. The study's thorough methodology, including electrochemical and biological assessments, validates the effectiveness of coconut sugar in MFC applications. The discovery of predominant bacterial species, including *Lysinibacillus*, *Lysobacter*, and *Pseudoxanthomonas*, substantiates the technique, demonstrating the interplay between microbial activity and substrate efficacy. This study enhances our understanding of MFC technology and creates new opportunities for using organic waste in energy generation, therefore promoting environmental sustainability and resource efficiency. The higher removal efficiency and voltage generation in this study could be attributed to the use of a high-carbohydrate organic substrate like coconut sugar, which is also an environmental waste product.While the results are promising, several factors warrant further investigation. The significantly higher voltage generation in this study compared to previous research suggests that substrate composition plays

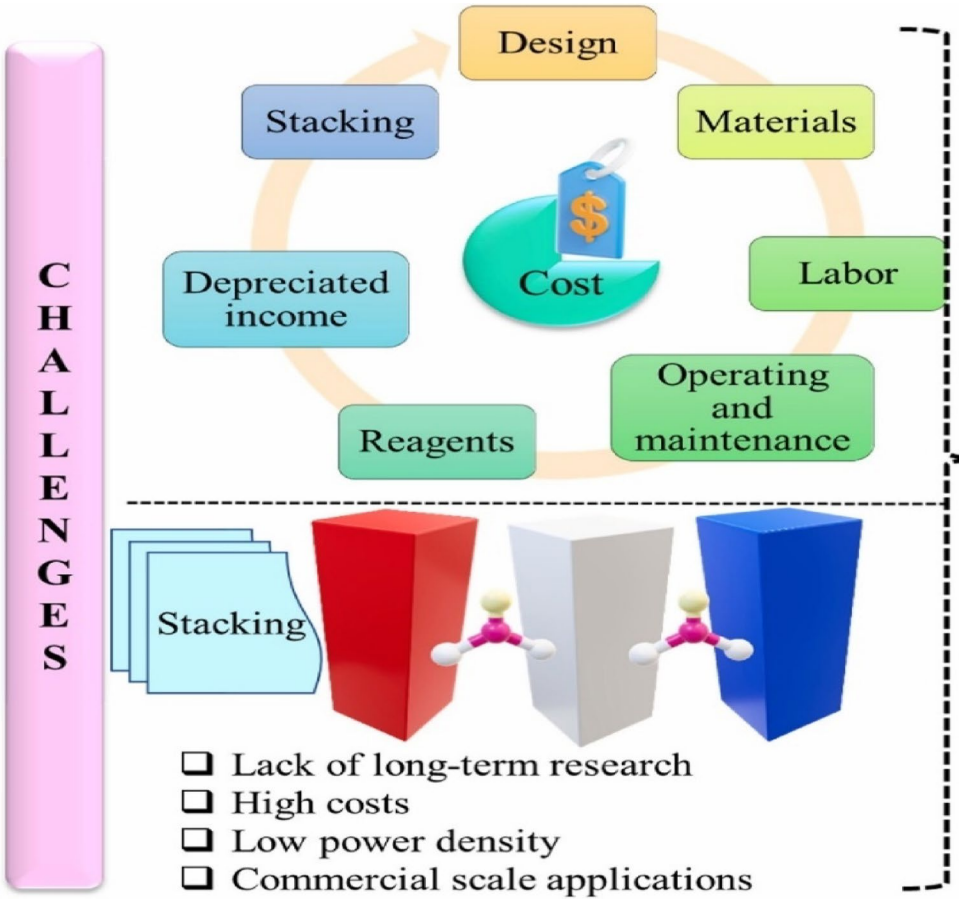


Fig. 12 List of challenges of MFC (Figure adapted from reference (Hassan et al. 2023) with permission of Elsevier)

a crucial role. However, the long-term stability and scalability of using coconut sugar in MFC need to be evaluated. Additionally, the environmental impact of sourcing and processing coconut sugar should be considered, despite its classification as waste. Future research should also explore the comparative efficiency of other organic substrates and their potential environmental benefits. Addressing these aspects will provide a more comprehensive understanding of the viability and sustainability of using coconut sugar in MFC for wastewater treatment and energy generation. To highlight a broad picture of MFC performance, a comparative tables (Table 5; table source is reference (Guerrero–Barajas andYaakop 2022) are given here.

Challenges and Future Directions

MFC technology encounters several problems, such as instability of electrode materials, instability of organic substrates, and long-term bacterial instability. These problems are well elucidated in several review papers. The identified issues in this study are the most troubling about the potential commercialisation of this technology. A brief and comprehensive analysis about the modern barriers are presented by Hassan et al. (2023) and showed in Fig. 12.

- Low power output is a significant challenge for MFC, limiting their practical applications. This issue arises from inefficient electron transfer between microbes and the anode, high internal resistance within MFC components, and unstable or insufficiently conductive biofilms. Additionally, the type and concentration of substrates can affect microbial metabolic activity, influencing power generation. To address these challenges, enhancing the conductivity of anode materials and optimizing the microbial-electrode interface can improve electron transfer efficiency (Rafatullah et al. 2020). Using advanced materials with lower resistance and optimizing the design can reduce internal losses. Engineering more robust and conductive biofilms through genetic modification or selecting more efficient microbial strains can enhance performance. Finally, optimizing substrate types and concentrations to match microbial metabolic needs can improve energy output, making MFC more viable for broader applications (Do et al. 2018).
- MFC have potential for sustainable energy generation; nevertheless, their performance is often limited by the

stability of anode electrodes. Bioanodes have difficulties related to biofilm development and upkeep. The stability of the biofilm is essential for reliable electron transport; yet, environmental changes might disturb it, resulting in diminished efficiency (Naaz et al. 2023). Furthermore, bioanodes may experience biofouling, whereby undesirable microbial proliferation impedes their performance. Biofouling may result in heightened internal resistance and decreased power production (Kolajo et al. 2022). Conversely, normal anodes in MFC, often composed of materials such as carbon fibre or metal oxides, may undergo deterioration over time. This deterioration impacts their conductivity and electron transfer efficacy. Furthermore, conventional anodes may exhibit restricted biocompatibility, affecting the proliferation and functionality of electroactive bacteria. Both anode types need regular maintenance and optimization to guarantee long-term stability and efficient performance in integrated MFC systems. Moreover, the expenses related to materials and the intricacies of producing conventional anodes may provide further obstacles to the widespread use of MFC (Guerrero-Barajas 2021). Mitigating these restrictions is essential for the progression of MFC technology and the attainment of dependable, sustainable energy generation.

- The MFC must be scaled up in order to handle massive volumes of water and produce high current. It is known that as MFC size and dimensions grow, the power density decreases because of an increase in volumetric ohmic resistance and reserve container volume, which leads to subpar energy production performance (Clauwaert et al. 2008). Scaling up MFC systems necessitates merging many MFC systems to create a large stack for energy generation rather than only enlarging each reactor (Ieropoulos et al. 2008). Unfortunately, a number of studies revealed that, in comparison to the traditional anaerobic MFC system, power density was still inadequate. As an example, Dekker et al. (2009) used synthetic wastewater to build a 20-liter stacked MFC, however the outcomes was very low.
- Internal resistance and biofilm issues: The internal resistance in MFC arises from the components like electrodes and membranes, reducing overall efficiency (Wu et al. 2016). Biofilm limitations occur when the microbial biofilm on the anode is unstable or poorly conductive, hindering electron transfer. To address internal resistance, using advanced materials with lower resistance and optimizing the design can help. For biofilm limitations, engineering more robust and conductive biofilms through genetic modification or selecting efficient microbial strains can enhance performance. These solutions aim to improve the efficiency and viability of MFC for broader applications.

According to Do et al. (2018) they presented an ideal future perspective debate for researchers. MFC are emerging as a promising technology in wastewater treatment, and future research should address several key areas:

- **Metabolic mechanisms**: Understanding the metabolic mechanisms of electrochemically active microorganisms is crucial. This involves selecting microorganisms with high electrochemical activity, constructing conductive thick biofilms, and optimizing operating conditions.
- **Reactor design and architecture**: The design and architecture of MFC reactors are critical for their effective application in wastewater treatment. Innovative designs can enhance performance and scalability.
- **MFC stacks**: Addressing issues such as voltage reversal and ionic short circuits in MFC stacks is essential. These challenges, caused by biocatalyzed electrode reactions, are significant barriers to practical application.
- **Power collection and utilization**: Developing efficient power collection and utilization systems is necessary to accelerate the commercial application of MFC. Detailed investigations into these systems will enhance their viability.
- **Eelctron transportation**: The production of high-quality, waste-derived anode electrodes is the most effective and most economical method of addressing electron transit. Researchers have recently begun to fabricate electrodes from waste (Goglio et al. 2025; Senthilkumar andNaveenkumar 2023).
- **Synergy with other technologies**: Exploring the potential synergy between MFC and other wastewater treatment technologies could improve their effectiveness and accelerate their application (Do et al. 2018; He et al. 2017).

Focusing on these areas will help advance the development and implementation of MFC in wastewater treatment and energy production.

Conclusion

In this study, coconut sugar was used as an organic substrate in MFC to enhance electron generation, addressing one of the primary challenges of MFC technology. The research also incorporated both organic and inorganic pollutants within the same chamber to demonstrate that organic pollutants can serve as substrates for microbial activities when combined with coconut sugar. This dual support for bacterial fuel significantly increased energy production. Over a period of 21 days, the MFC achieved a maximum voltage output, and the removal efficiency for metal ions exceeded

90%. The bacterial communities identified in the study provided strong evidence that the observed phenol degradation and metal removal were facilitated by microbial activities within the MFC. These findings underscore the potential of using coconut sugar not only to boost electron generation but also to enhance the overall efficiency of pollutant removal in MFC. The study's results highlight the importance of selecting appropriate organic substrates to optimize the performance of MFC in treating wastewater and generating energy. Additionally, this research offers several key insights that could be beneficial for novice researchers in the field. It emphasizes the significance of understanding the metabolic mechanisms of electrochemically active microorganisms and the role of substrate composition in influencing MFC performance. The study also points to the need for further exploration of different organic substrates and their potential environmental benefits, as well as the long-term stability and scalability of using coconut sugar in MFC. In conclusion, the use of coconut sugar as an organic substrate in MFC presents a promising approach to overcoming the challenges of electron generation and pollutant removal. The dual support of organic and inorganic pollutants enhances microbial activity, leading to increased energy production and efficient metal ion removal. This study not only contributes to the advancement of MFC technology but also provides valuable guidance for future research, paving the way for more sustainable and effective wastewater treatment solutions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s41742-025-00889-2.

Acknowledgements The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number "NBU-FFR-2025-1101-01.

Author Contributions Mohamad Nasir Mohamad Ibrahim, Anoud Saud Alshammari, Asim Ali Yaqoob: Experimentation, Conceptualization, Methodology, writing-original draft preparation, visualization, investigation. Amira Suriaty Yaakop: Biological tests and analysis. Akil Ahmad, Anoud Saud Alshammari, Mohamad Nasir Mohamad Ibrahim: Writing reviewing and language editing, supervision, funding acquisition. This article has been read and approved by all listed authors.

Funding Open access funding provided by The Ministry of Higher Education Malaysia and Universiti Sains Malaysia

Data Availability The authors confirm that all data underlying the findings are fully available without restriction. Data can be obtained after submitting a request to the corresponding/first author.

Declarations

Competing Interests The authors declare no competing interests.

Ethical Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

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