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Research Article

Bioelectricity Generation from Palm Oil Mill Effluent Using Microbial Fuel Cells: Electroactive Bacterial Identification and Bioelectrochemical Performance Evaluation

Zulkarnaini Zulkarnaini, Shinta Silvia, and Fujiati Fujiati
Department of Environment Engineering, Faculty of Engineering, Universitas Andalas, Padang, Indonesia

Micko Tomas* and Melda Latif
Department of Electrical Engineering, Faculty of Engineering, Universitas Andalas, Padang, Indonesia

Akmal Jamaan
Department of Pharmacy, Faculty of Pharmacy, Universitas Andalas, Padang, Indonesia

Diana Rahayuning Wulan
Research Center for Environmental and Clean Technology, National Research and Innovation Agency (BRIN), Indonesia

Shahrul Ismail
Faculty of Ocean Engineering Technology, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia

*Corresponding author: micko.tomas@eng.unand.ac.id DOI: 10.14416/j.asep.2026.11.006
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Abstract
Palm oil mill effluent (POME) is a high-strength agro-industrial wastewater with a high organic content, offering considerable potential as a renewable substrate for bioelectricity generation through microbial fuel cell (MFC) technology. However, the bioelectrochemical performance of MFCs treating POME remains strongly influenced by wastewater characteristics and the activity of electroactive microorganisms. This study evaluated the bioelectrochemical performance of a dual-chamber MFC using POME as the substrate by investigating wastewater characteristics, electricity generation, and electroactive bacterial communities. The MFC consisted of graphite electrodes separated by a proton-exchange membrane and was operated under continuous monitoring using an Arduino Uno-based data-acquisition system. The POME sludge exhibited total suspended solids, volatile suspended solids, and chemical oxygen demand of 103,070, 21,340, and 72,365 mg/L, respectively. The reactor achieved maximum electrical performance on day 8, producing a current density of 481,79 mA/m² and a power density of 765,32 mW/m². Microbiological analysis identified *Alcaligenes* as the dominant electroactive bacterium colonizing the anode, indicating its potential role in extracellular electron transfer and bioelectricity generation. These results demonstrate that POME is a promising feedstock for the simultaneous valorization of wastewater and the production of renewable bioelectricity. Furthermore, integrating physicochemical characterization, bioelectrochemical performance evaluation, and electroactive bacterial identification provides a comprehensive understanding of the factors governing MFC performance. It offers valuable insights for the development of bioelectrochemical systems for palm oil wastewater treatment.

Keywords: Anode-associated bacteria, Bioelectricity generation, Bioelectrochemical performance, Microbial fuel cell, Palm oil mill effluent, Wastewater treatment

1 Introduction

The increasing global demand for energy, coupled with the depletion of fossil fuel reserves and growing environmental concerns, has intensified the search for sustainable and renewable energy technologies. Indonesia, as one of the world's largest palm oil producers, has experienced a continuous increase in

Applied Science and Engineering Progress, Vol. 20, No. 4, 2027, 8384

electricity demand driven by population growth, industrial expansion, and economic development. According to PT PLN, the national electricity demand has shown a sustained upward trend, with the connected electrical load projected to increase by approximately 6.77% annually through 2021 [1],[2]. Microbial activity at the anode plays an important role in the oxidation of organic substrates and the transfer of electrons to the electrode, thereby influencing the electrical performance of Microbial fuel cell (MFC) systems [3].

MFCs have emerged as a promising bioelectrochemical technology for converting the chemical energy stored in organic substrates directly into electrical energy through the metabolic activity of electroactive microorganisms [4]-[7]. In an MFC, microorganisms oxidize biodegradable organic compounds at the anode, releasing electrons and protons. The electrons are transferred through an external electrical circuit to the cathode, while protons migrate through a proton exchange membrane, thereby generating an electrical current. Unlike conventional biological wastewater treatment processes, which primarily consume energy, MFCs offer the additional advantage of generating renewable electricity while reducing organic pollutant load, making them an attractive technology for sustainable wastewater management [8]-[11]. The broader potential of waste-derived materials for electrochemical energy recovery has also been demonstrated in bio-battery systems, where the composition and electrochemical characteristics of biomass-derived electrolytes strongly influence electrical performance [12].

Among various organic waste streams, palm oil mill effluent (POME) is considered one of the most suitable substrates for MFC applications because of its exceptionally high biodegradable organic content. More broadly, biological conversion of organic biomass and wastewater into renewable energy has also been extensively investigated through anaerobic digestion, where substrate characteristics, inoculum selection, pretreatment, and operating conditions strongly influence energy-recovery performance [13].

Conversely, its rich organic composition provides an abundant source of biodegradable carbon that can serve as an electron donor for electroactive microorganisms, making POME a promising feedstock for simultaneous wastewater treatment and bioelectricity generation [14]-[16]. Extensive research has demonstrated the feasibility of employing POME in MFC systems. Previous studies integrating MFC units with biological wastewater-treatment processes demonstrated that POME could effectively serve as an electron donor for electricity generation while simultaneously supporting wastewater treatment. The dual-chamber MFCs inoculated with a pure culture of *Pseudomonas aeruginosa* ZH1 produced approximately five times higher power density than reactors inoculated with anaerobic sludge obtained from POME treatment ponds, highlighting the significant influence of microbial communities on electrical performance [17], [18]. Similarly, another study demonstrated that controlled inocula composed of selected electroactive microorganisms generated approximately twice the electrical power of conventional anaerobic sludge inocula [19]-[21]. These studies collectively indicate that the composition of microbial communities is a critical factor governing extracellular electron transfer and overall MFC performance.

Despite these advancements, several knowledge gaps remain. Previous studies have primarily focused on reactor configurations, electrode materials, operating conditions, and inoculation strategies. Relatively few studies have simultaneously evaluated the physicochemical characteristics of POME, time-resolved bioelectrochemical performance, pollutant reduction, and anode-associated cultivable bacteria in a single POME-fed MFC system. Such integrated characterization can provide valuable insights into reactor performance and the biological environment surrounding the anode. However, culture-based bacterial characterization alone cannot determine microbial dominance, electroactivity, or specific extracellular electron transfer mechanisms; these aspects require molecular identification and specific electrochemical validation of the isolates [22], [23].

Inoculum selection is a crucial factor in MFC performance, as microbial composition and initial adaptation to wastewater conditions can influence organic matter degradation, biofilm formation, and electricity output. Previous studies indicate that sludge origin and treatment history can affect MFC performance, as microbial communities may differ across stages of wastewater treatment [24].

This study aims to evaluate the bioelectrochemical performance of a dual-chamber MFC using POME as the substrate by characterizing its physicochemical properties and culturable bacterial isolates associated with the anode. Unlike studies that focus primarily on electrical output, this research integrates wastewater characterization, temporal bioelectrochemical evaluation, pollutant reduction, and culture-based bacterial characterization within the same POME-fed MFC system [25], [26].

2 Methods

2.1 *Sampling site and sample collection*

Raw POME and sludge were collected from the wastewater treatment facility of PT AMP Plantation, Agam Regency, West Sumatra, Indonesia. The sampling locations are presented in Figure 1. These locations were selected to obtain fresh POME with a high organic content as the substrate and biologically active sludge as the microbial inoculum for the MFC system. Previous studies have shown that wastewater-treatment sludge contains microbial communities adapted to wastewater conditions and can therefore serve as a suitable inoculum for MFC applications [27].

Approximately 10 L of raw POME and 10 L of sludge were collected in sterile polyethylene terephthalate (PET) containers. The samples were immediately transported to the laboratory in insulated containers and stored at 4°C before reactor preparation to minimize changes in physicochemical characteristics and microbial activity. Raw POME was allowed to cool before being introduced into the MFC reactor.

Figure 1 illustrates the geographical location of PT AMP Plantation and the two sampling points used in this study. Point 1 represents the aerobic pond inlet channel, where sludge was collected as the microbial inoculum. In contrast, Point 2 represents the raw POME discharge channel, where untreated palm oil mill effluent was collected as the substrate for bioelectricity generation experiments.



Figure 1: Location of POME sludge sampling points.

2.2 *Proton exchange membrane preparation*

Prior to assembly, the Nafion® 117 proton exchange membrane (PEM) (DuPont Co., USA) was chemically pretreated to improve proton conductivity and remove residual contaminants following an established procedure [28]. The membrane was boiled in deionized water for 1 h, followed by 3% (v/v) H_2O_2 for 1 h. After rinsing with deionized water, it was boiled in 1 N H_2SO_4 for 1 h, rinsed three times, and stored in deionized water until reactor assembly. This pretreatment enhanced membrane hydration and supported proton transport between the anode and cathode chambers during MFC operation.

2.3 *Electrode preparation*

Graphite rods were used as both anode and cathode electrodes because of their high electrical conductivity, chemical stability, corrosion resistance, and biocompatibility, which support biofilm attachment during MFC operation [29]. Each electrode was 10 cm long and 1 cm in diameter, and the exposed geometric surface area of the graphite anode was used to normalize current and power densities.

Before reactor assembly, the electrodes were pretreated by immersion in 1 N HCl for 24 h, rinsed with distilled water, immersed in 1 N NaOH for 24 h, and rinsed again before being stored in deionized water until use. The graphite electrodes were connected with insulated copper wires through a fixed external resistance of 1000 Ω. Continuous aeration was supplied to the cathode chamber using an aquarium air pump to provide dissolved oxygen as the terminal electron acceptor. The recorded voltage was subsequently used to calculate current, power, current density, and power density according to conventional MFC performance evaluation methods [30].

2.4 *Dual-Chamber MFC assembly*

A single independent laboratory-scale dual-chamber MFC reactor (n = 1) was used in this study. The system consisted of two identical cylindrical glass chambers serving as the anode and cathode compartments, interconnected by a 10 cm-long PVC tube (½-inch internal diameter) containing a Nafion® 117 PEM. The membrane facilitated proton transport while preventing direct mixing of the anodic and cathodic electrolytes.

The anode chamber was filled to approximately 75% of its working volume with a mixture of raw

Applied Science and Engineering Progress, Vol. 20, No. 4, 2027, 8384

POME and aerobic sludge, serving as the organic substrate and microbial inoculum, respectively. The anode was maintained under anaerobic conditions, while the cathode chamber contained distilled water and was continuously aerated using an aquarium air pump to provide dissolved oxygen as the terminal electron acceptor. Graphite rod electrodes (10 cm × 1 cm) were positioned vertically at the center of each chamber and connected through insulated copper wires and a fixed 1000 Ω external resistor. The voltage across the resistor was monitored using an Arduino Uno-based data-acquisition system and recorded on a computer for subsequent analysis.

As illustrated in Figure 2, electrons generated during microbial oxidation at the anode flowed through the external circuit to the cathode, while protons migrated through the Nafion® 117 membrane. This configuration separated the anodic oxidation and cathodic reduction processes, limited oxygen diffusion into the anode chamber, and enabled continuous electrical operation of the MFC.

2.5 *MFC operation and electrical measurements*

MFC was operated in batch mode under ambient tropical laboratory conditions, with a temperature range of 27 to 30°C and no external temperature regulation, for a total operational period of 28 days. During operation, microorganisms in the anode chamber metabolized biodegradable organic compounds present in POME, while the resulting bioelectrochemical reactions generated measurable voltage across the external circuit.

The electrical performance of the MFC was continuously monitored using an Arduino Uno-based data acquisition system connected across a fixed external resistor of 1000 Ω. The cell potential (V) was automatically recorded at 10 min intervals throughout the experimental period and stored for subsequent analysis. The recorded voltage data were used to determine the electrical current, power output, current density, and power density of the MFC using conventional bioelectrochemical performance evaluation methods reported for microbial fuel cells.

The electrical current I was calculated according to Ohm's law, as expressed in Equation (1):

$$I = \frac{V}{R} \tag{1}$$

where I is the electrical current (A), V is the measured cell voltage (V), and R is the external resistance $(1000\,\Omega)$.

The electrical power generated by the MFC was calculated as described in Equation (2):

$$P = VI = \frac{V_2}{R} \tag{2}$$

where P is the electrical power output (W).

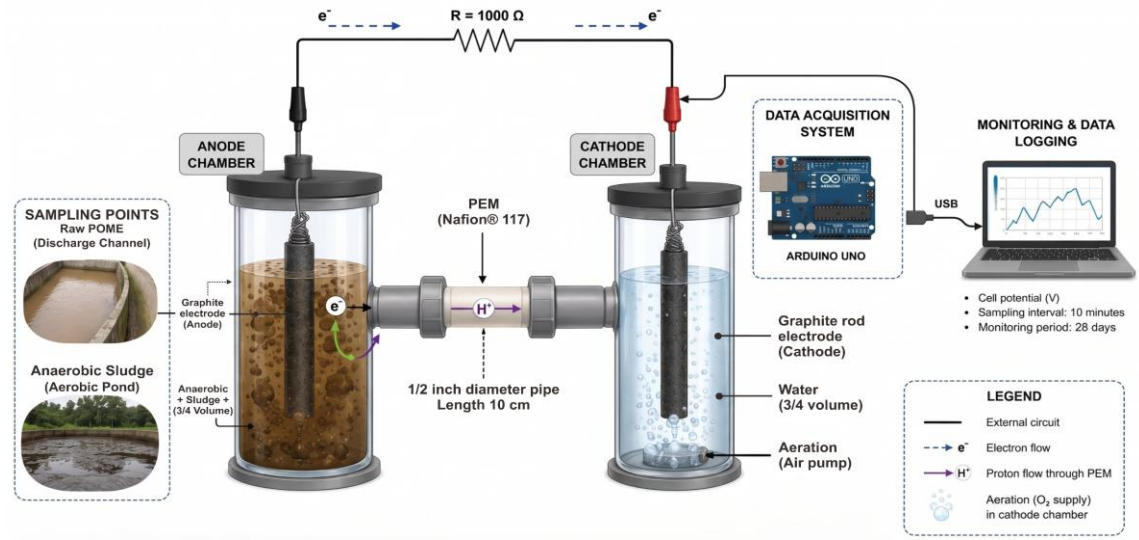


Figure 2: Schematic diagram of dual-chamber MFC used in this study. The system consists of an anaerobic anode chamber containing raw POME and aerobic sludge, an aerated cathode chamber, graphite rod electrodes connected via a 1000 Ω external resistor, a Nafion® 117 PEM, and an Arduino Uno-based data-acquisition system.

The current density was calculated by normalizing the electrical current to the exposed geometric surface area of the anode, as defined in Equation (3):

$$J = \frac{I}{A} \tag{3}$$

where J is the current density $(A\,m^{-2})$ and A is the projected surface area of the anode electrode (m^2).

The power density was determined by normalizing the electrical power output to the exposed geometric surface area of the anode, as expressed in Equation (4):

$$P_D = \frac{P}{A} \tag{4}$$

where P_D is the power density $(W\,m^{-2})$.

For the cylindrical graphite electrode, the exposed geometric surface area was calculated by considering both the cylindrical lateral surface and the two circular end surfaces exposed to the electrolyte, as described in Equation (5):

$$A = \pi dL + \frac{\pi d^2}{2} \tag{5}$$

where d is the electrode diameter (m) and L is the immersed electrode length (m). The equation includes the cylindrical lateral surface and both circular end surfaces exposed to the electrolyte.

Voltage was recorded at 10-min intervals throughout the 28-day operation. For graphical presentation, measurements recorded within each day were aggregated to obtain daily mean values of voltage and the corresponding electrical parameters. Repeated 10-min measurements represent temporal observations from the same reactor and should not be interpreted as independent biological replicates. Because only one independent MFC reactor was operated $(n = 1)$, no between-reactor statistical inference was performed.

2.6 *Physicochemical analysis*

The physicochemical characteristics of POME were determined before MFC operation to characterize the wastewater matrix and assess its suitability for bioelectricity generation. Total suspended solids (TSS), volatile suspended solids (VSS), and chemical oxygen demand (COD) were measured according to the Standard Methods for the Examination of Water and Wastewater [31]. TSS represented the suspended particulate fraction, VSS indicated the volatile organic fraction, and COD reflected the overall organic load available for microbial conversion.

These measurements provided baseline information on substrate composition and biodegradability for interpreting MFC performance. Because COD, TSS, and VSS were measured only at the available initial and final conditions rather than continuously throughout operation, no temporal correlation between pollutant concentrations and daily electrical output was inferred.

2.7 *Isolation and phenotypic characterization of anode-associated bacteria*

At the end of the 28-day operating period, biofilm samples were aseptically collected from the graphite anode and processed for culture-based microbiological characterization. The analysis was performed to obtain preliminary phenotypic information on culturable bacteria associated with the anode surface, rather than to establish microbial community dominance or direct extracellular electron transfer activity.

The collected biofilm was serially diluted using sterile physiological saline solution and aseptically streaked onto nutrient agar (NA) plates. The inoculated plates were incubated aerobically at 37 ± 1°C for 24–48 h until well-isolated colonies were obtained. Distinct colonies exhibiting different morphological characteristics were purified by repeated streaking to obtain pure bacterial isolates for subsequent identification. Bacterial identification was performed according to the taxonomic procedures described in Bergey's Manual of Determinative Bacteriology and the Manual for the Identification of Medical Bacteria [32].

The characterization process combined colony morphology, Gram staining, and biochemical tests to obtain a presumptive phenotypic assignment of the purified bacterial isolate. Morphological characterization included observations of colony diameter, pigmentation, shape, margin, elevation, surface texture, opacity, and consistency. Gram staining was performed according to the standard four-step procedure, using crystal violet as the primary stain, Gram's iodine as the mordant, 95% ethanol as the decolorizing agent, and safranin as the counterstain. Stained preparations were examined under an oil immersion objective (1000× magnification) to determine the Gram reaction and cellular morphology of each isolate.

Biochemical characterization was subsequently performed to evaluate the physiological and metabolic properties of the purified isolates. The culture-based analysis provides preliminary information on a culturable bacterial isolate associated with the anode environment. Because molecular identification, microbial community profiling, and isolate-specific electrochemical characterization were not performed, the resulting phenotypic assignment was not considered definitive evidence of microbial dominance, electroactivity, or a specific extracellular electron transfer mechanism.

3 Results and Discussion

3.1 *Physicochemical properties of POME*

The initial physicochemical characteristics of the POME sludge (inoculum) and raw POME wastewater (substrate) used in the MFC are summarized in Table 1. The POME sludge contained 103,070 mg/L TSS and 21,340 mg/L VSS. The resulting VSS/TSS ratio of approximately 0.21 indicates that volatile solids represented about one-fifth of the measured suspended solids, whereas the larger fraction consisted of fixed or nonvolatile solids. Therefore, VSS should not be interpreted directly as active microbial biomass. The relatively low volatile fraction may also limit the proportion of suspended material readily available for biological conversion during MFC operation.

The raw POME exhibited a COD concentration of 72,365 mg/L, confirming its classification as high-strength organic wastewater. This high organic content served as an abundant carbon source for electrogenic microorganisms, enabling continuous oxidation of organic compounds and electricity generation. The measured pH values of 7.0 (sludge) and 6.1 (raw POME) were within the favorable range for microbial activity.

The sludge temperature of 30°C was within the optimum mesophilic range for electrogenic bacteria, supporting efficient microbial metabolism. Although raw POME had a temperature of 62°C at the time of sampling, it was cooled before reactor operation. Consequently, the measured sampling temperature should not be interpreted as the actual operating temperature experienced by the anode microbial community. The DO concentration of 0.1 mg/L indicated a highly anaerobic condition, which is beneficial for the anode chamber because oxygen competes with the anode as the terminal electron acceptor. The low DO level therefore favored electron transfer to the anode and improved the bioelectrochemical performance of the MFC.

Table 1: Initial physicochemical characteristics of POME sludge and raw POME wastewater.

Sample	Parameter	Unit	Value
POME sludge (Inoculum)	TSS	mg/L	103,070
	VSS	mg/L	21,340
	Temperature	°C	30
	pH	–	7.0
Raw POME (Substrate)	COD	mg/L	72,365
	Temperature	°C	62
	pH	–	6.1
	DO	mg/L	0.1

3.2 *Bioelectricity generation performance*

The bioelectrochemical performance of MFC was evaluated by monitoring the current density and power density over a 28-day batch operation. The daily average current density and power density are presented in Figure 3 and Figure 4, respectively.

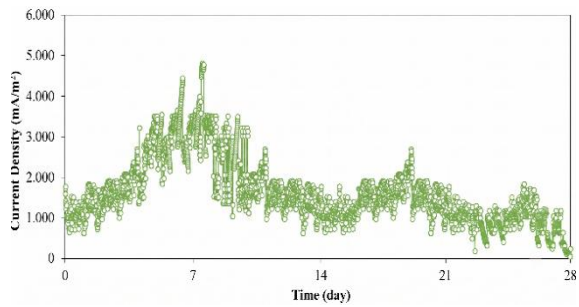


Figure 3: Daily current density profile during the 28-day MFC operation.

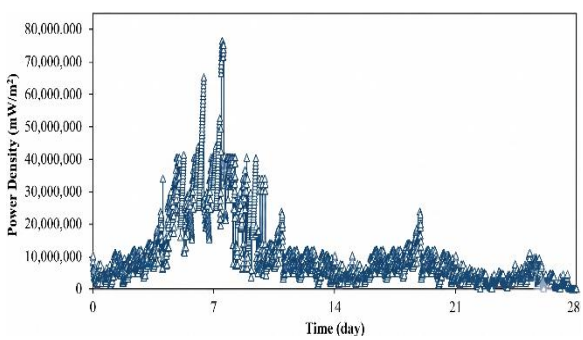


Figure 4: Daily power density profile during the 28-day MFC operation.

As shown in Figure 3, the current density increased during the initial operating period and reached a maximum of 481.79 mA/m² on day 8, followed by a gradual decline toward the end of the experiment. The initial increase may reflect progressive microbial adaptation and biofilm development on the graphite anode, together with

increased utilization of readily biodegradable organic compounds in POME.

The decline after day 8 may be associated with progressive depletion of readily biodegradable substrates and changes in microbial activity during batch operation. Because COD was measured only at the available endpoint conditions rather than throughout the 28-day period, a direct temporal relationship between substrate depletion and the daily electrical profile cannot be established from the present data.

As shown in Figure 4, the power density increased during the initial operating period and reached a maximum of 765.32 mW/m² on day 8, followed by a subsequent decline during the remaining operating period. The increasing electrical output during the initial operating period may be associated with microbial adaptation to the anode environment and progressive establishment of the anode biofilm. These processes can improve substrate oxidation and electron transfer as reactor operation proceeds.

After reaching a peak on day 8, power density decreased as readily biodegradable organic substrates were depleted. In addition, part of the metabolic energy was used for biofilm formation and maintenance rather than for electricity generation, reducing the number of electrons available for transfer to the anode. The observed decline may also have been influenced by changes in ionic transport, electrode conditions, or other internal losses during prolonged batch operation. However, internal resistance was not directly measured in the present study. The use of distilled water as the catholyte should also be considered when interpreting the electrical performance. Because distilled water has relatively low ionic conductivity, its use may increase ohmic losses and constrain ionic transport between the anode and cathode compartments. Proton and ion transport through the Nafion® 117 membrane, together with catholyte conductivity, may therefore have limited the observed cell performance. However, electrochemical impedance spectroscopy and direct internal-resistance measurements were not conducted; consequently, the magnitude of these losses cannot be quantified from the present experiment.

3.3 *Pollutant removal performance*

Changes in TSS, VSS, and COD were evaluated using measurements obtained at the initial and final conditions of the 28-day MFC operation. These measurements represent endpoint changes and therefore do not describe pollutant-removal kinetics during reactor operation. The results are summarized in Table 2.

Table 2: Pollutant removal performance of the microbial fuel cell.

Parameter	Unit	Initial	Final	Removal Efficiency (%)
TSS	mg/L	103,070	35,180	65.87
VSS	mg/L	21,340	3,580	83.22
COD	mg/L	72,365	37,318	48.43

As shown in Table 2, the MFC effectively reduced suspended solids and organic pollutants during the 28-day operation. The TSS concentration decreased from 103,070 to 35,180 mg/L, corresponding to a removal efficiency of 65.87%, indicating the effective removal of suspended particles through sedimentation, microbial degradation, and biofilm attachment to the anode surface. In contrast, VSS exhibited the highest removal efficiency (83.22%), decreasing from 21,340 to 3,580 mg/L. The reduction in VSS indicates a decrease in the volatile fraction of suspended solids during the operating period. This change may reflect biological degradation, biomass transformation, sedimentation, and retention of solids within the reactor; therefore, it should not be interpreted solely as evidence of electroactive microbial metabolism.

The moderate COD reduction observed in the present study should also be interpreted in relation to wastewater characteristics and operating conditions. Biological treatment performance is strongly dependent on substrate concentration, pH, microbial consortium, and reactor conditions. Previous work has also investigated a POME-fed dual-chamber MFC supplemented with Lactobacillus bulgaricus [33]. Recent work on microbial treatment of high-strength industrial wastewater similarly demonstrated that COD-removal performance can vary substantially with the operating environment and substrate loading [34]. The COD concentration decreased from 72,365 to 37,318 mg/L, corresponding to a 48.43% reduction, provided that the initial and final measurements reflect the same reactor matrix. The moderate COD reduction relative to the observed electrical output indicates that electrical generation and total COD removal are not necessarily proportional. Readily biodegradable soluble organic compounds may preferentially support electrical generation, whereas total COD also includes particulate, slowly biodegradable, and refractory organic fractions.

3.4 *Isolation and phenotypic characterization of anode-associated bacteria*

A culturable bacterial isolate recovered from the anode biofilm after 28 days of MFC operation was characterized using colony morphology, Gram staining, and biochemical tests. The colony and microscopic characteristics are presented in Figures 5 and 6, while the phenotypic and biochemical characteristics are summarized in Tables 3 and 4.

As shown in Figure 5, bacterial colonies isolated from the MFC anode exhibited a uniform morphology, characterized by circular, creamy-white colonies with smooth margins and convex elevation. The relatively uniform colony morphology indicates successful purification of the selected culturable isolate for subsequent phenotypic characterization. After three days of incubation, the bacterial population on the MFC anode reached 9.0×10^5 CFU/mL.

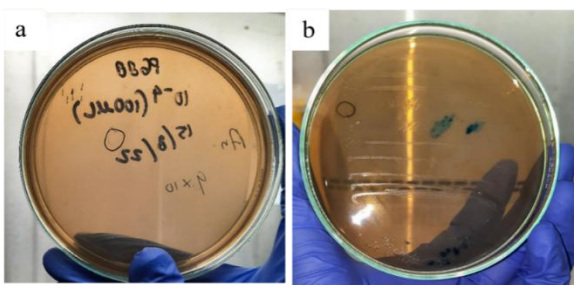


Figure 5: Colony morphology of bacterial isolates grown on APW medium: (a) purified colonies obtained from the 10^{-4} dilution and (b) pure bacterial isolate.

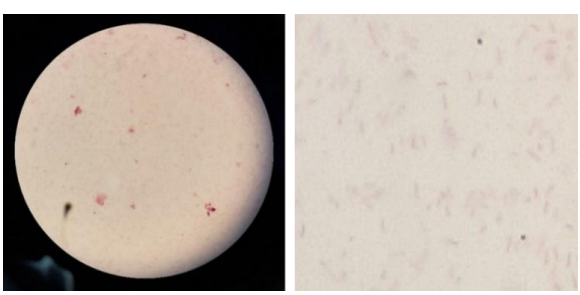


Figure 6: Gram staining of the anode-associated bacterial isolate showing Gram-negative rod-shaped cells under light microscopy at 1000× magnification.

Figure 6 shows that microscopic observation revealed Gram-negative rod-shaped cells, indicating that the isolate belongs to the Gram-negative bacterial group. This morphological characteristic, together with the colony morphology and biochemical test results, supports a presumptive phenotypic assignment of the isolate to the genus *Alcaligenes*. The recovery of a Gram-negative rod-shaped isolate from the anode demonstrates the presence of a culturable bacterium associated with the anode environment; however, culture-based isolation alone does not establish its abundance within the biofilm or its direct contribution to extracellular electron transfer.

Table 3 summarizes the morphological characteristics of the dominant bacterial isolate recovered from the MFC anode. The isolate formed round colonies with smooth margins, convex elevation, and punctiform size, indicating a homogeneous colony morphology. Microscopic observation revealed Gram-negative rod-shaped cells lacking endospores, suggesting that the isolate belongs to a non-spore-forming Gram-negative bacterium. As shown in Table 4, the isolate was catalase-positive, oxidase-positive, motile, and KCN-tolerant, while most biochemical and carbohydrate fermentation tests were negative. Combined with the Gram-negative rod-shaped morphology observed in Figure 6, these characteristics are consistent with a presumptive phenotypic assignment to the genus *Alcaligenes*.

The phenotypic and biochemical characteristics of the isolate were consistent with a presumptive *Alcaligenes* sp. assignment. Microbial attachment and biofilm development are important factors in biological energy-conversion systems because interactions among microorganisms and conductive surfaces can influence substrate degradation and electron-transfer processes. For example, enhanced biofilm development on granular activated carbon has been associated with improved organic degradation and interspecies electron transfer during anaerobic waste-to-energy conversion [35]. Nevertheless, such observations cannot be directly extrapolated to the present isolate because isolate-specific electrochemical activity was not evaluated. However, recovery of this isolate from the anode does not demonstrate that it was dominant within the biofilm or directly responsible for the observed electrical output. Recent microbial-reactor studies have demonstrated the value of community-level characterization for resolving microbial composition and ecological structure within biological treatment systems [36]. Accordingly, confirmation of the present isolate and its relative abundance would require molecular approaches, such as 16S rRNA sequencing or microbial-community profiling, together with isolate-specific electrochemical characterization.

Table 3: Characteristics of the dominant bacterial isolate recovered from the MFC anode.

Characteristic	Observation
Colony morphology	
Colony shape	Round
Colony margin	Smooth
Colony elevation	Convex
Colony color	Creamy white
Colony size	Punctiform
Cell morphology	
Cell shape	Rod
Gram reaction	Negative
Endospore	Absent

Table 4: Biochemical characteristics of the dominant bacterial isolate recovered from the MFC anode.

Biochemical Test	Result	Biochemical Test	Result
TSIA	M/M	MR	–
Gas production	–	VP	–
H_2S production	–	Oxidation/Fermentation (O/F)	–
Catalase	+	KCN tolerance	+
Oxidase	+	Arginine hydrolysis	–
Motility	+	Lysine decarboxylase	–
Indole	–	Ornithine decarboxylase	–
Urease	±	Phenylalanine deaminase	–
Citrate utilization	–	Esculin hydrolysis	–
Lactose fermentation	–	Arabinose fermentation	–
Glucose fermentation	–	Raffinose fermentation	–
Sucrose fermentation	–	Sorbitol fermentation	–
Mannitol fermentation	–	Trehalose fermentation	–
Xylose fermentation	–	Dulcitol fermentation	–
Gelatin hydrolysis	–		

3.5 *Comparison with previous MFC studies*

To evaluate the performance of the developed MFC, the maximum electrical output (MEO) obtained in the present study was compared with those reported in previous studies employing different inoculum sources and operating conditions. Because MFC performance is strongly influenced by reactor architecture, electrode material and surface area, substrate composition, inoculum, external resistance, membrane, catholyte, and normalization basis, the values in Table 5 are presented only as contextual benchmarks rather than as direct evidence of performance superiority. As shown in Table 5, the MEO obtained from microbial fuel cells varies considerably depending on the inoculum source and operating conditions. MFCs inoculated with pure *Geobacter sulfurreducens* achieved an MEO of 0.60 mW/m², whereas wastewater- and soil-seeded MFCs produced lower outputs of 0.47 and 0.10 mW/m², respectively.

Direct quantitative ranking among these studies should be interpreted cautiously because the reported electrical outputs were obtained using different reactor configurations and operating conditions. In contrast, a

Table 5: Comparison of MEO achieved by microbial fuel cells using different inoculum sources and operating conditions.

Inoculum	MEO (mW/m²)	Dominant Bacteria	Reference
G. sulfurreducens-seeded MFC	0.60	*Geobacter sulfurreducens*	[22]
Wastewater-seeded MFC	0.47	Nonelectrogenic bacteria	[22]
Soil-seeded MFC	0.10	Nonelectrogenic bacteria	[22]
Controlled inoculum	107.35	*Pseudomonas aeruginosa*, *Azospira oryzae*, *Acetobacter peroxydans*, and *Solimonas variicoloris*	[6]
Ferricyanide catholyte	222.59	–	[23]
Aerated catholyte	190.28	–	[23]
Lactobacillus bulgaricus in POME	248.04	*Lactobacillus bulgaricus*	[33]
Aerobic-pond sludge/POME MFC	765.32	Presumptive *Alcaligenes* sp.	This study

controlled inoculum containing *Pseudomonas aeruginosa*, *Azospira oryzae*, *Acetobacter peroxydans*, and *Solimonas variicoloris* increased the MEO to 107.35 mW/m², demonstrating that microbial composition plays a critical role in facilitating extracellular electron transfer. The cathodic electron acceptor also significantly affected electrical performance. The ferricyanide catholyte generated a higher MEO (222.59 mW/m²) than the aerated catholyte (190.28 mW/m²), reflecting the superior electrochemical properties of ferricyanide for oxygen reduction.

4 Conclusions

This study demonstrated the feasibility of utilizing palm oil mill effluent (POME) as a substrate for bioelectricity generation in a dual-chamber microbial fuel cell (MFC). The POME sludge obtained from PT AMP Plantation exhibited high organic strength, with total suspended solids (TSS), volatile suspended solids (VSS), and chemical oxygen demand (COD) of 103,070 mg/L, 21,340 mg/L, and 72,365 mg/L, respectively, indicating its suitability as a carbon-rich substrate for microbial metabolism. The MFC achieved its maximum bioelectrochemical performance on the eighth day of operation, producing a maximum current density of 481.79 mA/m² and a maximum power density of 765.32 mW/m². These results demonstrate the capability of anaerobic POME sludge to support efficient electron generation and transfer during MFC operation. Morphological, Gram-staining, and biochemical characterization of a culturable anode-associated isolate indicated a presumptive assignment to *Alcaligenes* sp. Because molecular identification and isolate-specific electrochemical testing were not performed, the isolate cannot be conclusively considered dominant or directly responsible for extracellular electron transfer. The results, demonstrate laboratory-scale POME-fed MFC performance while highlighting the need for replicated reactor experiments, and additional electrochemical validation in future studies.

Author Contributions

Z.Z.: conceptualization, methodology, investigation, formal analysis; S.S.: investigation, laboratory experiments, data curation; F.F.: investigation, validation, laboratory analysis; M.T.: conceptualization, project administration, writing review and editing; M.L.: software, data acquisition, visualization, validation; A.J.: microbiological analysis, bacterial identification, validation; D.R.W.: formal analysis, validation; S.I.: supervision, conceptualization, methodology. All authors have read and agreed to the published of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors utilized ChatGPT solely to improve the language and readability of the manuscript. All scientific content, analysis and interpretation of results were developed and verified by the authors.

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