Pollutant Removal Efficacy and Bioelectricity Generation of Microbial Fuel Cells from Polluted Water using Different Electrodes

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Abstract

The discharge of untreated wastewater into water bodies creates serious ecological and health problems, highlighting the necessity for a sustainable, energy-efficient treatment solution. Microbial fuel cells (MFCs) have emerged as a promising bioelectrochemical technology that can treat wastewater while generating electricity from organic matter. This study evaluated double-chambered MFCs for concurrent electricity generation and wastewater treatment from dairy farm effluent and Turag River water over a 25-day operation. Three anode-cathode configurations: Graphite-Cu (G-Cu), Carbon fiber-Stainless steel (Cf-SS), and Carbon rod-Zn (CR-Zn), were compared for their electrochemical performance and pollutant removal efficiency. In dairy farm effluent, G-Cu produced the highest peak voltage (706.67 mV), current (0.2667 mA), and power (0.1884 mW) with 98.53% COD removal. In Turag River water, Cf-SS produced the highest voltage (676.67 mV) with COD removal (70.59%), while CR-Zn produced the highest current (0.2667 mA) and power (0.1751 mW). COD, TDS, TSS, and turbidity were reduced by the MFCs,

although the removal efficiency varied among electrode configurations and wastewater types. Changes in pH were also observed after treatment. In addition, bacterial isolates obtained from the layer formed on the anode electrodes after 25 days of operation were identified using MALDI-TOF MS (Bruker MALDI Biotyper). The identified bacteria included species previously reported to have electroactive potential, suggesting their possible association with electricity generation in MFCs. *Escherichia coli* was also detected in both wastewater types, which may indicate fecal or domestic contamination. Overall, the study demonstrated the feasibility of using different electrode configurations for treating real wastewater while generating electricity under laboratory conditions. Among the tested configurations, G-Cu showed comparatively consistent electrical and treatment performance, although the highest performance for individual parameters varied among the electrode configurations and wastewater types.

Keywords: *Bioelectricity generation; Electrogenic bacteria; Biofilm formation; Dairy farm effluent; Electrode material; Microbial fuel cell (MFC); Renewable energy; River water*.

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1. Introduction

The treatment of wastewater has become a global challenge due to the increasing discharge of industrial, domestic, and agricultural effluents into water systems. According to the United Nations World Water Development Report (1), more than 80% of the world's wastewater is discharged untreated into the environment. This problem is especially evident in Bangladesh, where nearly 70% of industrial effluents in the Dhaka region are released directly into rivers such as the Buriganga, Shitalakhya, and Turag without adequate treatment, which significantly increases turbidity, depletes oxygen, elevates BOD and COD levels, and creates a significant risk to local biodiversity. (2,3). Besides industrial effluents, agricultural and livestock activities add considerable organic load to water bodies. Nearly 270 million tons of manure are generated each year globally, adding nutrient-rich waste into water (4).

Conventional wastewater treatment methods, like aerobic treatment of low-strength wastewater (domestic sewage, dairy effluents), involve high capital costs along with considerable operational and energy requirements. Aeration alone consumes about 0.5 kWh per cubic meter of

wastewater, which corresponds to roughly 30 kWh of energy use per person each year (5). Moreover, it requires high operational costs, and these processes generate a large volume of excess sludge that must be further treated and disposed of. These shortcomings have spurred the search for alternative approaches that can simultaneously reduce wastewater and recover resources. One such promising emerging technology is the microbial fuel cell (MFC), a type of bioelectrochemical system, which is considered a cost-effective and sustainable technology (6). MFCs generate sustainable energy by utilizing microbial metabolic activity to transform organic waste into electricity, while simultaneously offering the additional benefit of wastewater treatment (7,8). An MFC typically consists of an anode and a cathode, sometimes separated by a membrane. MFCs can be single or double-chambered, with the anode maintained under anaerobic conditions and the cathode usually aerobic (8,9). Various electrogenic bacteria naturally present in wastewater act as biocatalysts at the anode, oxidizing organic and inorganic compounds to generate electrons (8). These electrons are transferred through an external circuit to the cathode, where reduction reactions occur. This dual function offers a distinct advantage over conventional treatment methods.

Beyond energy recovery, MFCs provide several operational advantages: they generate less sludge than conventional treatment processes, eliminate the need for aeration, and are relatively simple to operate. Their effective reduction of chemical oxygen demand (COD) also enables safer discharge of treated effluent with reduced environmental impact. Moreover, recent studies reveal that the wastewater contains approximately 5-10 times more energy than the energy needed for its treatment process. (6). Despite these advantages, the widespread application of MFCs remains constrained by several challenges, including low power density, high system cost, and difficulties in scaling up to industrial levels. (10). Among the various factors influencing these limitations, the choice of electrode materials is especially critical, as the electrode directly governs the efficiency and electron transfer. The anode acts as the site of biofilm attachment and electron transfer, while the cathode supports the reactions necessary for electricity generation. That's why electrode properties such as composition, conductivity, surface area, and porosity strongly affect the overall performance of MFC. (11). Carbon-based electrodes, like graphite, carbon cloth, and carbon fiber, have been widely used because of their stability, high surface area, and compatibility with microbial growth. (12,13). In contrast, metal electrodes such as stainless steel, copper, and zinc offer higher electrical conductivity and lower cost.

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Therefore, optimizing electrode material and configuration remains one of the key strategies to enhance MFC performance and make the technology applicable to wastewater treatment applications. This study seeks to improve the efficiency of MFCs by employing three different electrode configurations: Graphite-Cu (G-Cu), Carbon fiber-Stainless steel (Cf-SS), and Carbon rod-Zn (CR-Zn) in double-chambered MFCs. Two real wastewater substrates, dairy effluent and contaminated water from the Turag River, are tested to evaluate substrate suitability. In addition, microbial identification is conducted to identify bacterial species associated with the anode surface after MFC operation and to examine their reported electrogenic potential. The main aim of this study is to compare the efficiency of the three electrode combinations in power generation and wastewater treatment, evaluate the performance of MFCs using Turag River water and Dairy farm effluent, and identify the microbial species associated with the anode surface after treatment. This integrated approach makes the present research distinct, as it provides comparative insights into electrode efficiency, substrate type, and bacterial identification. MFC applications for wastewater treatment and electricity generation have been investigated in Bangladesh, including studies of municipal wastewater and different electrode combinations (37,38). To the best of our knowledge, no previous study has compared these three electrode configurations using both dairy farm and Turag River water under the same experimental conditions.

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2. Materials and methods

2.1 Study area

This study was conducted using surface water collected from the Turag River and wastewater effluent from the Central Cattle Breeding & Dairy Farm, Savar, both locations situated near Dhaka, Bangladesh. The Turag River is widely recognized as one of the most contaminated rivers in Dhaka, where untreated municipal sewage and industrial effluents are discharged. (14). The River water samples were collected from five locations along the Turag River, representing bazar, industrial, and residential areas. These locations were selected to represent different sources and levels of pollution along the river. These sites were chosen to capture the spatial variation in pollution levels. The Dairy wastewater was collected from the main discharge point of the Central Cattle Breeding & Dairy Farm, Savar. This effluent contained high organic matter, suspended solids, and nutrient concentrations, which is a good source of environmental pollution and a

potential substrate for MFC. The sample sites are presented in Figure 1, which marks the five points along the Turag River, and in Figure 2, the dairy farm effluent outlet at Savar. The geographic coordinates of the sampling sites were recorded using a Garmin GPSMAP 64s handheld GPS navigator (Garmin International, Inc., USA).

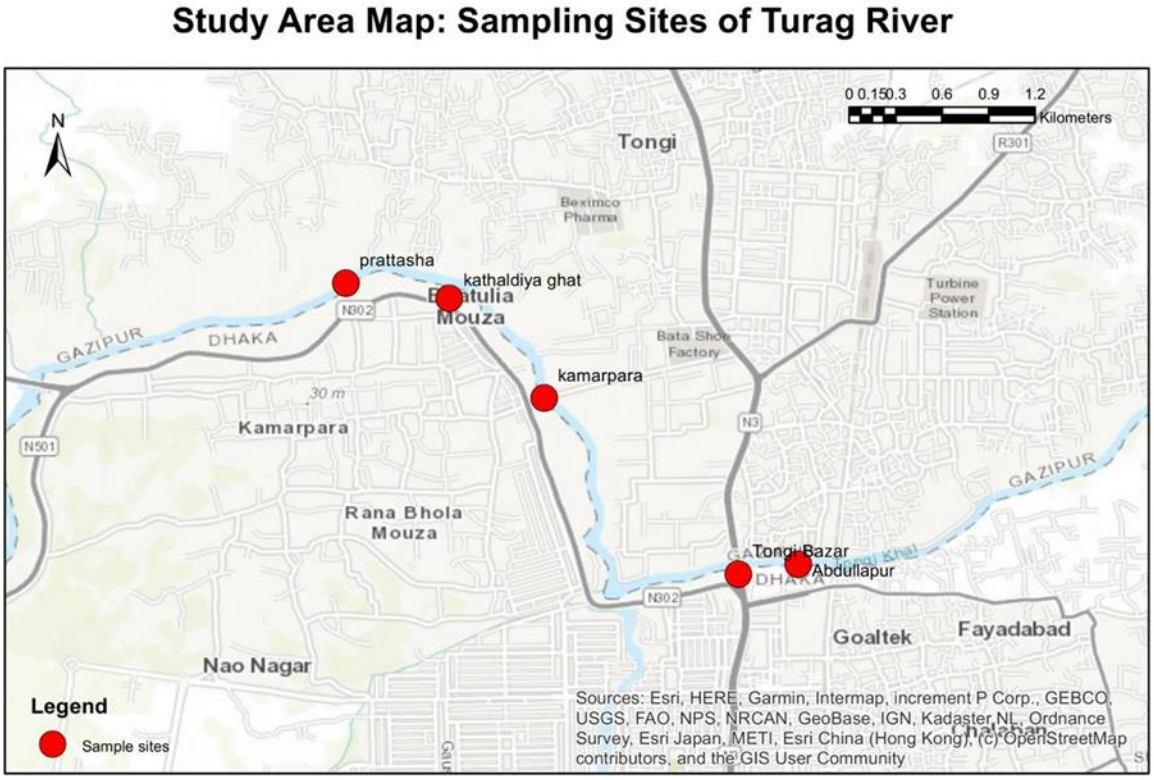


Figure 1. Sample (Dairy Effluent) collection site

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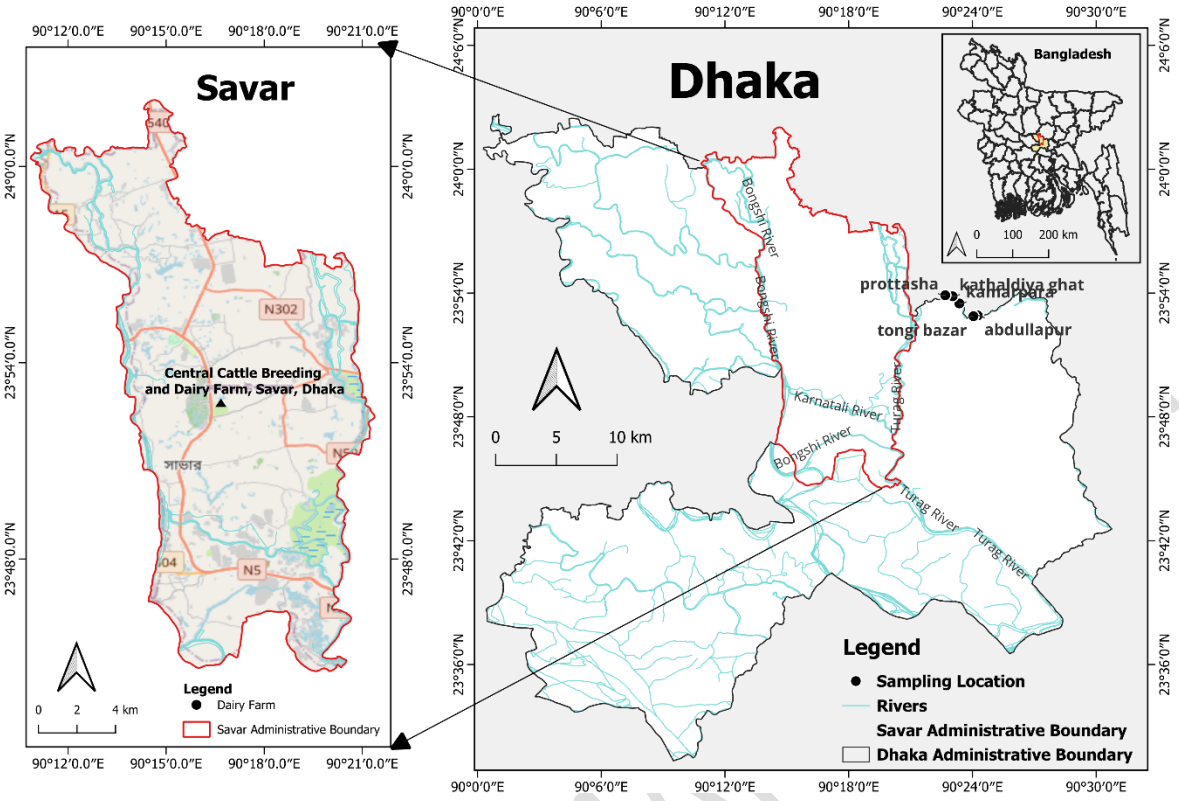


Figure 2. Sampling sites of the Turag River water sample

2.2. Design of MFC

A microbial fuel cell is a bio-electrochemical device that converts the chemical energy stored in organic matter into electrical energy through bacterial metabolism. The design idea of the MFC was taken from the literature. (12;13;15) MFC was developed based on those ideas. Figure 3 shows the MFC design used in this research. Structurally, an MFC is composed of two primary compartments: an anode chamber and a cathode chamber that are separated by an agar KCl salt bridge. The experimental study of a double-chamber microbial fuel cell (MFC) consisted of two compartments (anode and cathode) separated by an agar salt bridge. The anode chamber is maintained under anaerobic conditions to support microbial growth and activity, while the cathode chamber is kept aerobic to facilitate the oxygen reduction reaction. (16;17;9). In the anode chamber, exoelectrogenic bacteria oxidize the organic substrate, releasing electrons and protons. The electrons are absorbed by the anode and transferred through an external circuit to the cathode,

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while protons pass through the PEM or salt bridge into the cathode chamber. At the cathode chamber, the electrons and protons combine with oxygen, which acts as the electron acceptor, and produce water as the final product. (9).

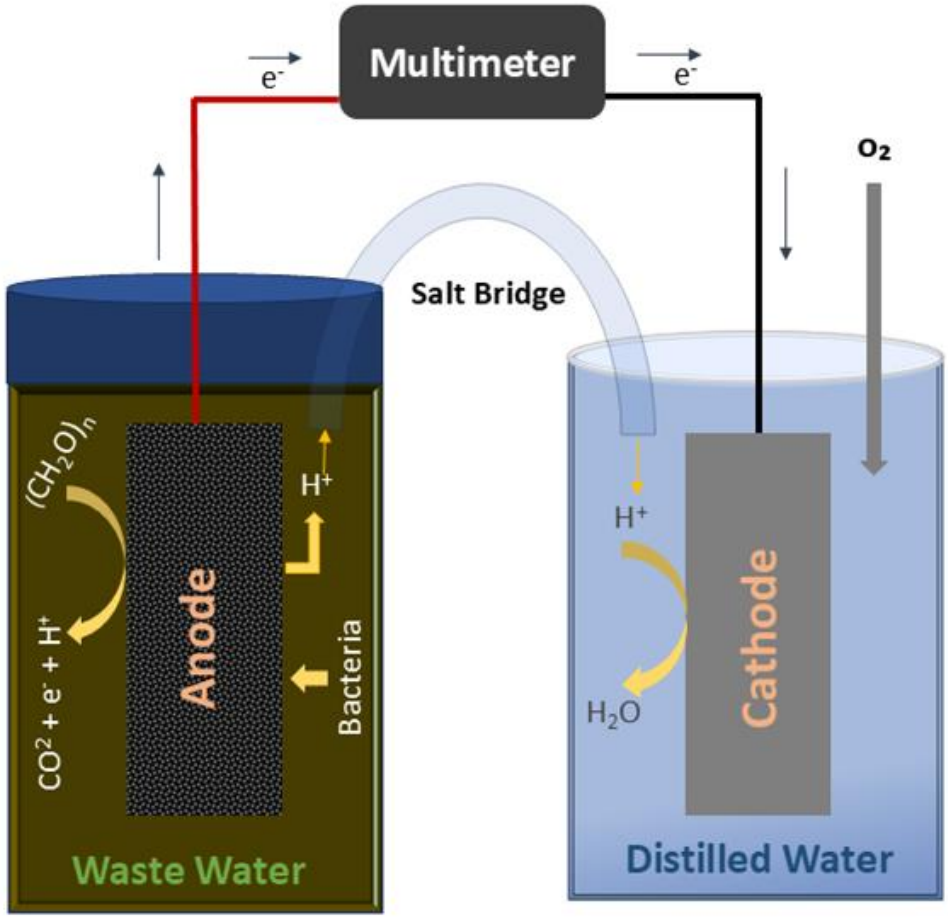


Figure 3. Design of MFC

2.2.1. Experimental setup of MFC for this study

A double-chamber microbial fuel cell was constructed using two plastic containers (1.5L capacity each, 1.2 L working volume) serving as anode and cathode chambers (Figure 4). The chambers were connected by a U-shaped agar KCL salt bridge (length 12 cm and diameter 1.6 cm), which provides ionic conductivity between the chambers. For this study, a total of six double-chambered MFCs were constructed, with three operated using Dairy effluent and three using Turag River water as substrates. For each type of wastewater, three MFCs were built using different

electrode combinations: Cf-SS, G-Cu, and CR-Zn. The anode chamber contained the wastewater sample, while the cathode chamber contained distilled water. The anode chamber was sealed tightly to ensure anaerobic conditions. The cathode chambers were left open for natural aeration, allowing oxygen to serve as an electron acceptor.

The exposed surface area of each anode electrode was measured before use and was used for calculating current density and power density. The corresponding anode surface areas were: 66 cm^2 for carbon fiber, 37 cm^2 for graphite rod, and 35 cm^2 for carbon rod and a rectangular 55.68 cm^2 electrode was used for the cathode. All the electrodes were rinsed with distilled water and immersed for 24 hours before installation. Electrodes were connected using copper wires to complete the circuit. The MFCs were operated under ambient room temperature conditions for 25 days, with natural day-night temperature variation. Voltage and current were measured using a digital multimeter three times daily (morning, midday, evening). These readings were used to calculate power, power density, and current density. Before and after treatment of wastewater water the water quality parameters like COD, TDS, TSS, EC, and turbidity were measured to evaluate the efficiency of MFCs in treating the samples.

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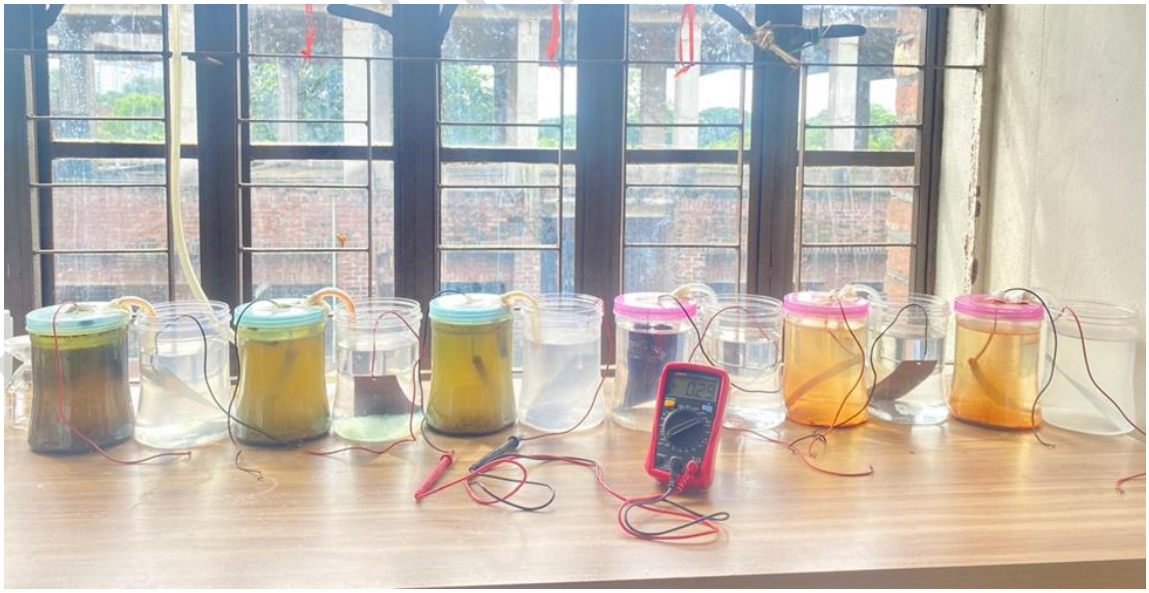


Figure 4. Experimental setup of MFC for this study

2.3. Sample collection and physicochemical analysis of wastewater

The two types of wastewater samples were employed in this study: dairy farm effluent and surface-contaminated river water. The dairy farm effluent was collected from the discharge outlet, while Turag River water samples were obtained from five selected sites. The samples were collected in a sterilized sample bottle to avoid contamination. After collection, the samples from five points were combined and thoroughly mixed to prepare a composite river water sample. The homogeneous composite sample was stored at 4°C in the laboratory for further use. The samples were brought to room temperature before use in the MFCs to maintain microbial activity.

A set of physicochemical parameters, including pH and COD. TDS and EC. TSS and turbidity were analyzed immediately after collecting the sample to establish a baseline water quality characteristic. The pH of the samples was measured using a Hanna pHep HI98107 pH meter (APHA Methods 4500-H^+ B). Electrical conductivity and total dissolved solids were measured using Hanna Dist 4 HI98304 and Hanna DiST 2 HI98302 portable meters, respectively (APHA Methods 2510 B and 2540 C, respectively). These meters were calibrated according to the manufacturer's instructions before measurement, and the probs were rinsed with distilled water before and after each measurement. The probs were immersed in the samples, and measurements were recorded after stable readings were obtained.

COD was determined using a Hanna HI83399 Multiparameter Photometer with commercial Hach HR COD digestion reagent. For TSS determination, 100 mL of well-mixed wastewater sample was filtered through a pre-weighed Whatman filter paper, and after filtration, the retained suspended solids on the filter paper were then weighed using a digital weighing balance after drying. Then, the TSS concentration was calculated from the increase in filter-paper mass relative to the volume of sample filtered (APHA Methods 2540 D). Turbidity was measured using a portable turbidity meter (APHA Methods 2130 B). All physicochemical analyses were conducted following standard laboratory procedures and, where applicable, relevant methods described in *Standard Methods for the Examination of Water and Wastewater* (APHA, 2017).

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2.4. Salt bridge preparation

To reduce internal resistance and improve ionic conductivity between anode and cathode chambers, a KCl-agar solution was prepared based on previously reported salt bridge formulations for double-chamber MFCs (39,40). In this study, 3% (w/v) agar and 22.4g KCl were used to prepare a 3 M KCl-agar solution. The components were dissolved in 100 mL of distilled water and heated over a burner for approximately 2 min until a homogeneous solution was obtained. The hot liquid solution was then poured into a 12 cm long U-shaped glass tube and allowed to cool and solidify at room temperature. After solidification, the KCl-agar gel was used as the salt bridge to connect the anode and cathode chambers of the MFC, providing proton conductivity and preventing the liquid from mixing.

2.5. Data collection sources

In order to achieve the objective of this research, data were obtained from both experimental investigation and literature-based sources. The experimental data were collected through direct measurements of wastewater samples and monitoring MFCs' performance regarding electrical properties using a multimeter. The literature data were collected to provide theoretical support, methodological guidance, and comparison with previous studies.

2.5.1. Primary data

Primary data, or first-hand measurements, were generated directly from laboratory experiments and field-collected wastewater samples, which serve as the main foundation of this study. Operations of the MFCs. These data represent the physicochemical characteristics of wastewater and the electrochemical performance of MFCs under experimental conditions. The following data were primarily collected for this study.

Electrical output

Voltage and current were monitored three times a day (morning, midday, and evening) for 25 days for each MFC using a digital multimeter. The three daily measurements represent repeated observations from each MFC system and not independent experimental replicates. For each day, the arithmetic mean and standard deviation (SD) were calculated from the three readings to describe the daily electrical output and its variability.. Power was determined from

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corresponding voltage and current measurements, while current density and power density were calculated using the exposed anode surface area. Current and voltage were initially recorded in microamperes (μA) and microvolts (μV), respectively, because of the relatively low output of the MFCs. For data analysis and presentation, the recorded current and voltage values were converted to milliamperes (mA) and millivolts (mV), respectively, using the conversion factors 1mA=1000 μA and 1mV=1000 μV.

Physicochemical parameters

The water physicochemical parameters, such as pH, TDS, TSS, and Turbidity, were measured before setting up the MFC cells and after 25 days of MFC treatment. The physicochemical parameters were measured by following standard laboratory procedures described in *Standard Methods for the Examination of Water and Wastewater* (APHA, 2017).

2.5.2. Secondary data source

Secondary data were gathered from published literature, books, and online databases (e.g., Google Scholar, PubMed, ResearchGate). These sources were used to support experimental findings, provide theoretical background, and compare results with previous studies in the field of microbial fuel cells and wastewater treatment.

2.6 Data evaluation formulas

The performance of the MFCs and the treatment efficiency of wastewater were evaluated using standard equations. Power, current density, and power density were determined from the measured voltage and current data using equations (1) - (5). Similarly, the removal efficiency of COD, TDS, EC, TSS, and turbidity was calculated using equations (6) - (10).

$$\text{Power, } P = V \times I \quad (1)$$

$$\text{Surface area of carbon fiber, } A = L \times W \text{ (rectangular)} \quad (2)$$

$$\text{Surface area of graphite and carbon rod, } A = 2\pi \times r \times L + 2\pi \times r^2 \text{ (cylinder shape)} \quad (3)$$

$$\text{Power density (PD)} = \frac{P}{A} \quad (4)$$

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$$\text{Current density (CD)} = \frac{I}{A} \quad (5)$$

$$\text{The percentage of COD removal} = \frac{Ci-Co}{Ci} \times 100 \quad (6)$$

$$\text{TDS(mg/l)} = \frac{Wf-Wi}{V} \times 1000 \quad (7)$$

$$\text{The percentage of TDS removal} = \frac{Ti-To}{Ti} \times 100 \quad (8)$$

$$\text{The percentage of EC removal} = \frac{Ei-Eo}{Ei} \times 100 \quad (9)$$

$$\text{The percentage of turbidity removal} = \frac{TRi-TRo}{TRi} \times 100 \quad (10)$$

Where, P represents power (mW), V represents voltage (mV), I represents current (mA), and A represents the exposed anode surface area (mm^2). PD and CD refer to power density (mW/mm^2) and current density (mA/mm^2), respectively. L, W, and r indicate the electrode length, width, and radius of the electrodes. In the pollution removal equations, C_i and C_0 represent initial and final COD concentrations, while T_i and T_0 refer to initial and final TDS concentrations, respectively; E_i and E_0 represent initial and final electrical conductivity, respectively; TR_i and TR_0 denote the initial and final turbidity values. W_i and W_f represent initial and final weight, while V in equation (7) represents the sample volume.

2.7. Statistical analysis

Experimental data were processed using OriginPro software for tabulation, advanced data visualization, and linear regression analysis. Linear regression was used to evaluate the temporal trends in voltage and current generation during 25-day operation, and the coefficient of determination (R^2) was used to indicate the goodness of fit of the regression lines. Microsoft Excel was used to analyze the pollution removal efficiency.

For the regression analysis, the day of operation was considered the independent variable, while the corresponding voltage and current measurements were considered as dependent variables.

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2.8. Bacterial identification

Microbial activity plays a central role in the performance of MFCs. After 25 days of MFC operation, bacterial samples were collected from the layer formed on the surface of the anode electrodes in the anode chamber. In this study, the bacterial population was identified using a combination of Gram staining and Matrix-Assisted Laser Desorption/ Ionization Time-of-flight Mass Spectrometer (MALDI-TOF MS). Initially, pure bacterial colonies were examined by Gram staining to determine their Gram reaction and cellular morphology. The isolates were subsequently analyzed using Bruker MALDI Biotyper for species-level identification. A small amount of each pure colony was transferred onto a MALDI target plate, overlaid with the MALDI matrix solution, and allowed to dry before analysis. The resulting protein mass spectrum was compared with reference spectra in the Bruker database for bacterial identification. MALDI-TOF MS is an established and reliable method for species-level identification of cultured bacterial isolates (41). The identified bacterial species were subsequently compared with published literature to determine their reported electrogenic potential.

3. Results

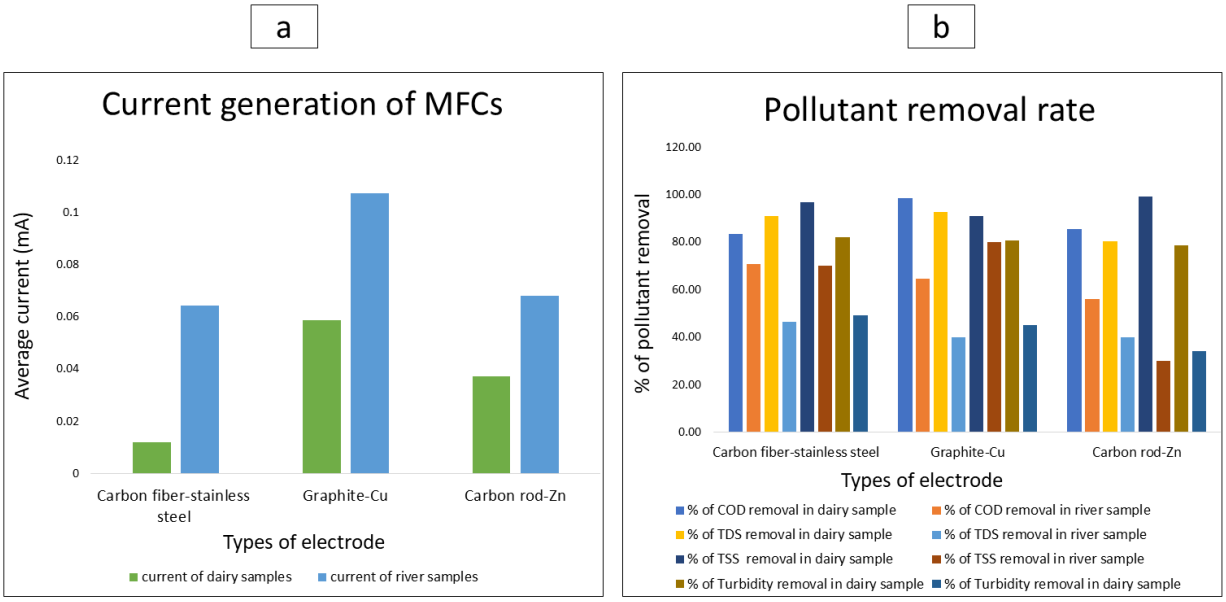
The performance of MFCs was examined using three different electrode combinations, CF-SS, G-Cu, and CR-Zn, by evaluating their electricity generation and pollutant removal efficiency in both dairy wastewater and river water. Among the tested configurations, the G-Cu electrode pair showed the highest average current output in both substrates; in particular, it generated approximately 0.106 mA in river water and 0.058 mA in dairy wastewater. In contrast, the CR-Zn electrode produced an average current of around 0.067 mA and 0.037 mA, respectively, while the Cf-SS pair demonstrated the lowest performance, generating only 0.012 mA in dairy and 0.064 mA in river samples (Figure 5).

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Figure 5. Average current generation rate (a) and pollutant removal rate (b) of the three electrodes from MFCs.

In terms of pollutant removal, the G-Cu electrode generally exhibited comparatively high performance in measured pollutant removal parameters. For dairy wastewater, this electrode achieved 98.53% COD, 91.3% TDS, 91.3% TSS, and 80.4% turbidity removal. It also maintained high performance in river water, with 64.8% COD, 39.7% TDS, 80.4% TSS, and 45.6% turbidity removal (Figure 5). Although the CR-Zn pair showed nearly complete TSS removal in dairy (99.1%), its COD and turbidity reductions in river water were comparatively low (57.1% and 33.4%, respectively). The Cf-SS configuration achieved moderate COD and TDS removal, but its turbidity and TSS performance were consistently lower than that of G-Cu.

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3.1. pH and visual variation

The variation in pH and the visual appearance of the wastewater sample before and after MFC treatment were analyzed to evaluate the influence of microbial and electrochemical processes

on the overall water quality. The measured pH values and color changes for both dairy farm and river wastewater are summarized in Table 1.

The initial pH of dairy wastewater was 7.8, which slightly decreased to 7.1-7.5 after treatment, while the river wastewater pH dropped from 8.6 to 7.2-8.0. The lowest pH of 7.1 occurred in the Graphite-Cu system, representing the greatest pH decrease among the tested configurations. Since electricity generation in the MFC is associated with electrochemical reactions in the anode chamber, proton generation and transfer through the salt bridge can occur during MFC operation. Therefore, the pH decrease observed in the G-Cu system may be related to higher proton generation and transfer during operation.

Color changes also confirmed visible improvement in the anode chamber. The dairy farm wastewater shifted from greenish yellow to light yellow, and the Turag River water shifted from light yellow to whitish, indicating effective removal of organic and suspended impurities.

In the cathode chamber, the CR-Zn system distilled water was initially clear and colourless, but gradually it became milky in appearance during the 25-day experimental period. After the end of experiment, the zinc electrode was found to have become noticeably thinner, weaker, and more brittle, and mechanically weaker with visible changes on its surface. . These observations suggest zinc corrosion and dissolution during the treatment period, and the electrode in the cathode chamber may have undergone material loss during the treatment period, potentially resulting in the release of zinc ions into the cathode. However, as zinc concentration was not measured, its release could not be directly confirmed. The G-Cu system also showed a light-golden coloration in the cathode chamber, whereas no significant visible change was observed in the CR-SS system.

Table 1: pH variation of Dairy farm and Turag River water before and after 25 days of MFC treatment

Sample type	Electrode combination	pH	
		Before treatment	After treatment
	Cf-SS	7.8	7.3

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Dairy farm effluent	G-Cu	7.8	7.1
	CR-Zn	7.8	7.5
Turag River water	Cf-SS	8.6	7.2
	G-Cu	8.6	7.8
	CR-Zn	8.6	8.0

3.2 Voltage and current generation from MFCs

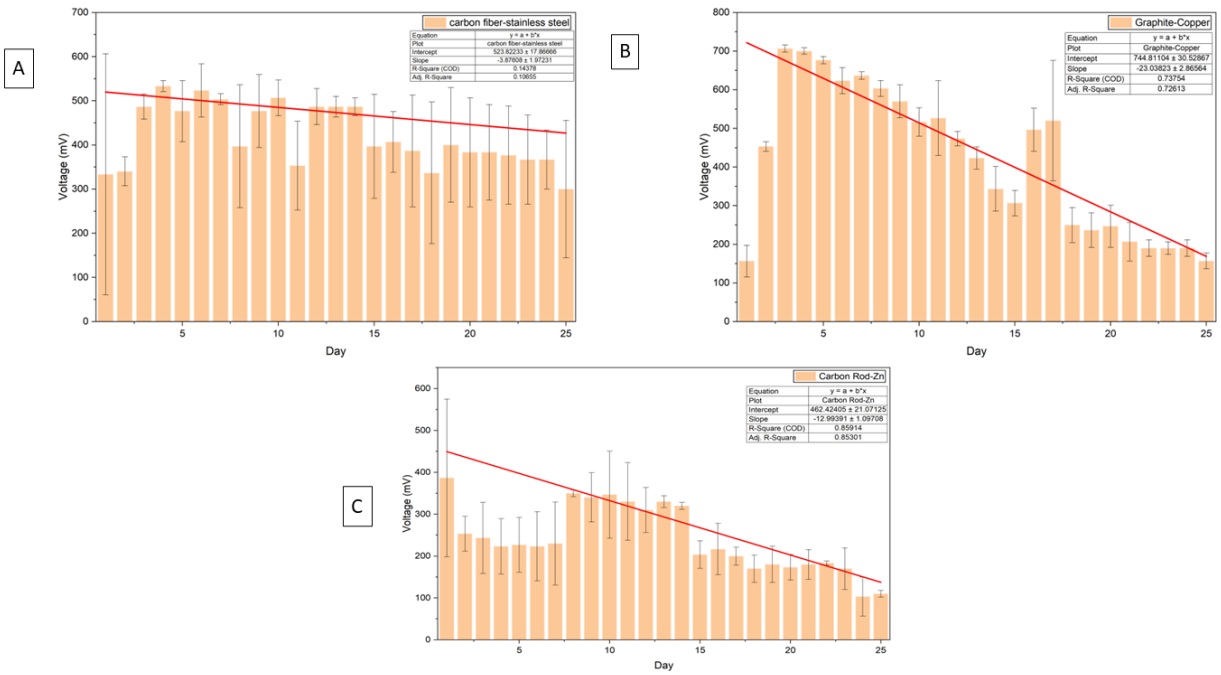
All double-chamber MFCs successfully produce electricity during wastewater treatment. The generated voltage and current were measured regularly with a digital multimeter for 25 days to monitor the performance and consistency of electricity production. The variation of voltage output in MFCs operated with the dairy wastewater system is shown in Figure 6 (a-c), and river wastewater is shown in Figure 7 (d-f). Voltage gradually increases during the first week of operation and reaches its peak on day 3 for the Graphite-Cu (G-Cu) combination, recording a maximum of 706.67 mV. The Carbon fiber- Stainless steel (Cf-SS) and Carbon Rod-Zn (CR-Zn) produce maximum voltages of 533.33 mV on day 4 and 386.67 mV on day 1, respectively. After day 10, all MFCs exhibited a gradual decline in voltage as available organics became depleted.

For the Turag River water system Figure 7 (d-f), the voltage showed a similar trend to the dairy samples but reached slightly higher peak values and became stable earlier during the operation period. The Cf-SS electrode exhibited the greatest performance, reaching a maximum of 676.67 mV on day 7, while the G-Cu pair produced approximately 456.67 mV on day 12, and the CR-Zn electrode reached about 656.67 mV on day 2, followed by a substantial decrease and subsequent fluctuation. The voltage peak occurred at different stages depending on the electrode configuration, and the outputs generally declined after their respective peak period. The higher

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initial voltages in river samples suggest more efficient electron transport, possibly aided by the slightly alkaline pH that enhanced ionic conductivity in the electrolyte bridge.

Figure 6. Graphs of voltage generation by Carbon fiber-stainless steel (a), Graphite-Cu (b), and Carbon rod-Zn (c) electrode combinations from Dairy Wastewater sample. Dashed lines indicate linear regression trends R^2. Error bars show the standard deviation (SD) of three repeated measurements (n=3)



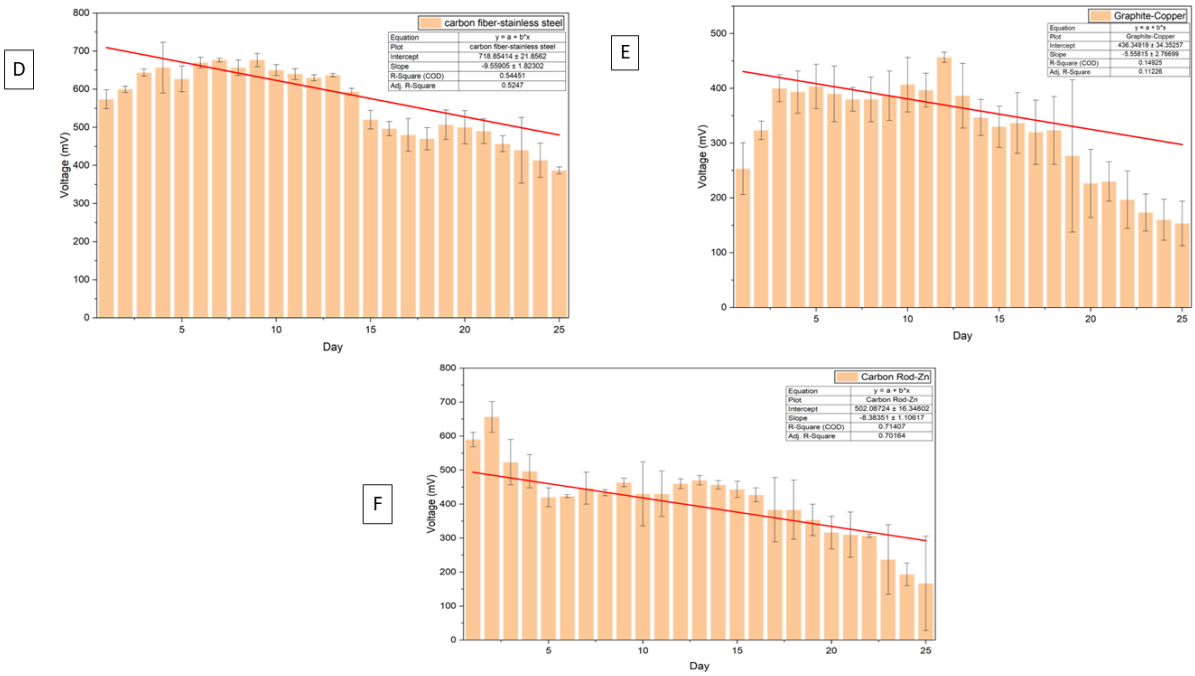


Figure 7. Graphs of voltage generation by Carbon fiber-stainless steel (d), Graphite-Cu (e), and Carbon rod-Zn (f) electrode combinations from the Turag River water sample. Dashed lines indicate linear regression trends R^2. Error bars show the standard deviation (SD) of three repeated measurements (n=3)

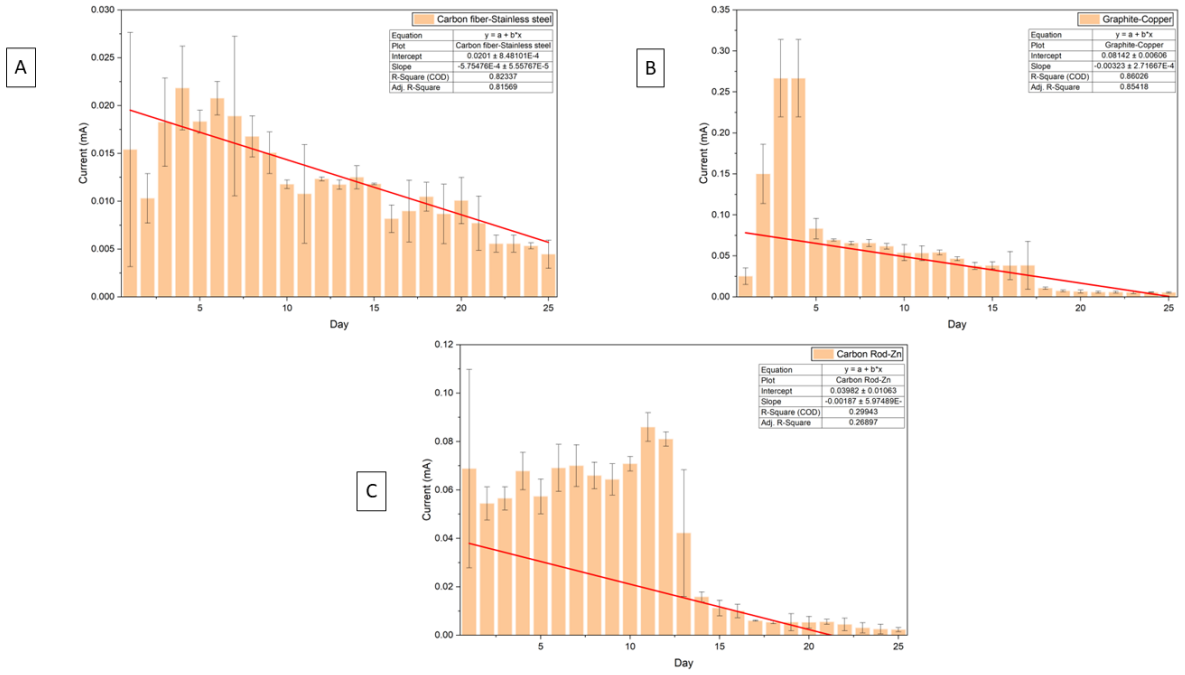
The current generation followed a similar trend to the voltage throughout the experiment. As illustrated in Figure 8. (a-c), which shows the current variation for dairy wastewater, the G-Cu electrode combination exhibited the highest current production, reaching a maximum of 0.2667 mA on day 3, corresponding to the peak voltage phase. The Cf-SS and CR-Zn electrodes generated comparatively lower peak currents of 0.0218 mA on day 4 and about 0.0859 mA on day 11, respectively. As the experiment progressed, the current values gradually declined, reaching a minimum range of 0.004-0.006 mA between days 22 and 25, which may indicate reduced microbial activity and substrate availability.

For the river water system, the current generation also showed different temporal patterns among the electrode combinations Figure 9 (d-f). The CR-Zn electrode pair produced the highest current output of 0.2667 mA on day 2, followed by G-Cu with 0.1951 mA on day 4 and Cf-SS with 0.1592 mA on day 6. For the Cf-SS and G-Cu systems, the current maxima occurred earlier than the voltage maxima, whereas the voltage and current maxima of the CR-Zn system occurred on the same day (day 2). Overall, these results demonstrate that voltage and current did not necessarily follow identical trends throughout the experiment, and their temporal variations depended on the electrode configuration and wastewater type.

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Among the three electrode configurations, G-Cu showed comparatively stable electrical performance throughout the 25-day operation period. Although CR-Zn produced a relatively high initial current, its output declined sharply after approximately two days and remained comparatively low thereafter. In contrast, Cf-SS exhibited comparatively lower voltage and current throughout the operation period. Therefore, considering both the magnitude and stability of electrical output over time, G-Cu demonstrated the most consistent performance under the tested conditions.

Figure 8. Graphs of current generation by Carbon fiber-stainless steel (a), Graphite-Cu (b), and Carbon rod-Zn (c) electrode combinations from Dairy Wastewater sample. Dashed



lines indicate linear regression trends R^2. Error bars show the standard deviation (SD) of three repeated measurements (n=3)

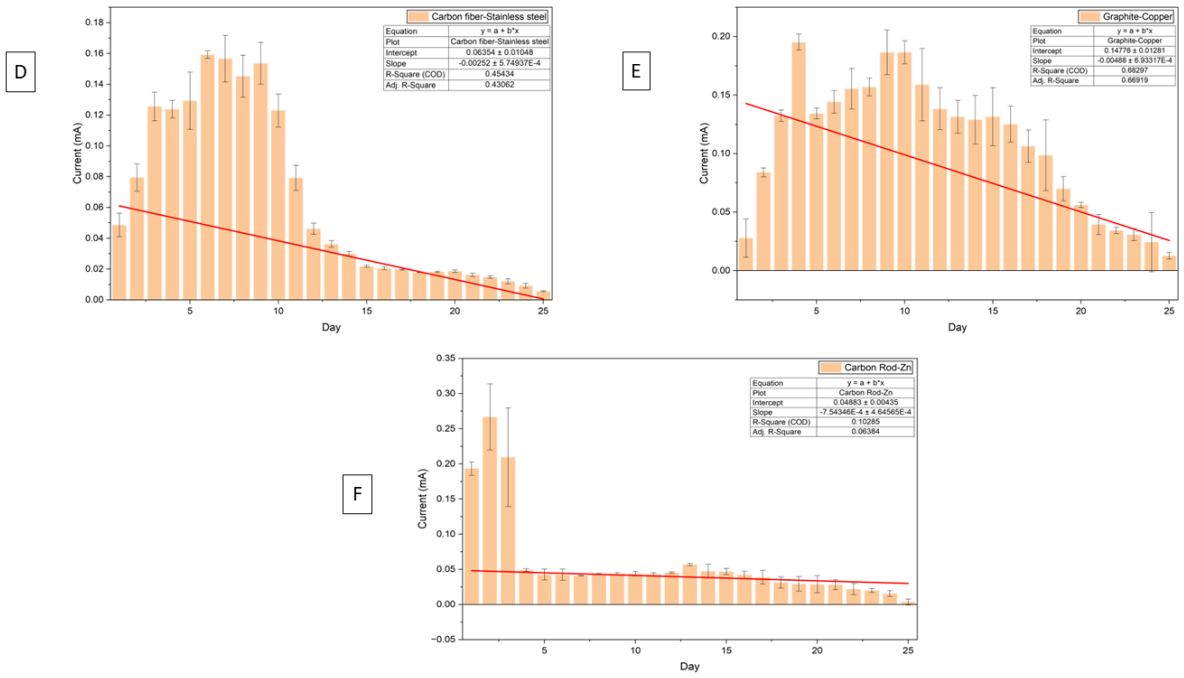


Figure 9. Graphs of current generation by Carbon fiber-stainless steel (d), Graphite-Cu (e), and Carbon rod-Zn (f) electrode combinations from the Turag River water sample. Dashed lines indicate linear regression trends R^2. Error bars show the standard deviation (SD) of three repeated measurements (n=3)

Table 2: Maximum electrical outputs of microbial fuel cells using different electrode combinations in dairy farm effluent and Turag river water

Electrical parameter	Dairy farm effluent	Turag river water
Peak voltage (mV)	706.67(G-Cu)	676.67 (Cf-SS)
Peak current (mA)	0.2667(G-Cu)	0.2667(CR-Zn)
Peak power (mW)	0.1884 (G-Cu)	0.1751(CR-Zn)

As shown in **Table 2**, the highest electrode outputs varied between the two types of wastewater. G-Cu showed the highest voltage, current, and power generation in the dairy farm effluent, while different electrode combinations performed best for different electrode parameters in Turag River water. This suggests that the performance of the electrode combinations depends on the types of wastewater and the electrical parameters considered.

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3.3. Power, Power density, and Current density analysis

The power output and density profiles reflect the combined effect of voltage and current generation, shown in Figures 10 and 11. In Dairy wastewater, the G-Cu MFC achieved the maximum power of 0.1884 mW on day 3, corresponding to a peak power density of about 5.093×10^{-5} mW/mm^2. The CR-Zn and Cf-SS electrodes followed with maximum powers of about 0.0284 mW on day 11 and 0.0116 mW on day 4, corresponding to power densities of 8.105×10^{-6} mW/mm^2 and 1.764×10^{-6} mW/mm^2, respectively. The power output generally decreased after the respective peak periods, although some fluctuations were observed throughout the operation period.

For River wastewater, the CR-Zn electrode produced the highest maximum power of 0.1751 mW on day 2. The Cf-SS electrode produced the maximum power of 0.1067 mW on day 6, while the G-Cu electrode produced 0.0767 mW on day 4, with corresponding power density were approximately 5.003×10^{-5} mW/mm^2 for CR-Zn, 1.616×10^{-5} mW/mm^2 for Cf-SS, and 2.047×10^{-5} mW/mm^2 for G-Cu, respectively. The power outputs subsequently declined with fluctuations throughout the 25-day experimental period. The corresponding power density variations have been presented in Figure 11. The overall trends for both wastewater types were consistent with their power outputs, showing distinct peaks during the early operation phase, and with time, the values declined gradually.

Similarly, Figure 12 demonstrates the current density performance for Dairy and River wastewater MFCs. In the Dairy wastewater system, the G-Cu electrode reached the highest current density of 7.207×10^{-5} mA/mm^2 on day 3, while CR-Zn and Cf-SS attained maximum current densities of 2.456×10^{-5} mA/mm^2 on day 11 and 3.308×10^{-6} mA/mm^2 on day 4, respectively. In the river water system, the CR-Zn electrode produced the highest current density of 7.69×10^{-5} mA/mm^2 on day 2, while G-Cu and Cf-SS produced 5.274×10^{-5} mA/mm^2 and 2.412×10^{-5} mA/mm^2, respectively. The density profile generally corresponds to the current and the power outputs.

Overall, the G-Cu electrode showed good power and current in dairy wastewater, while CR-Zn showed the highest power and current in the river wastewater during the early stage of operation. However, the electrical performance may vary with wastewater and electrode combination. Therefore, the performance of the electrode should not be judged only based on the

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highest power or current value; the comparative stability of electric output should also be considered.

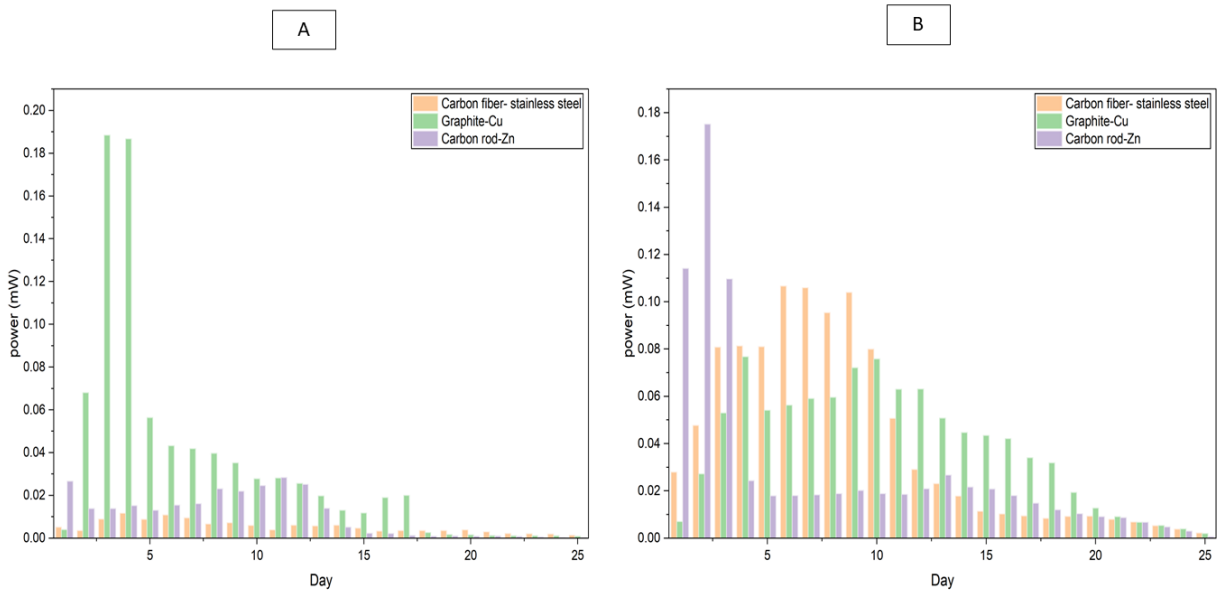


Figure 10. Power output of Dairy(a) and Turag River (b) wastewater MFCs with different electrode combinations during 25 days of operation.

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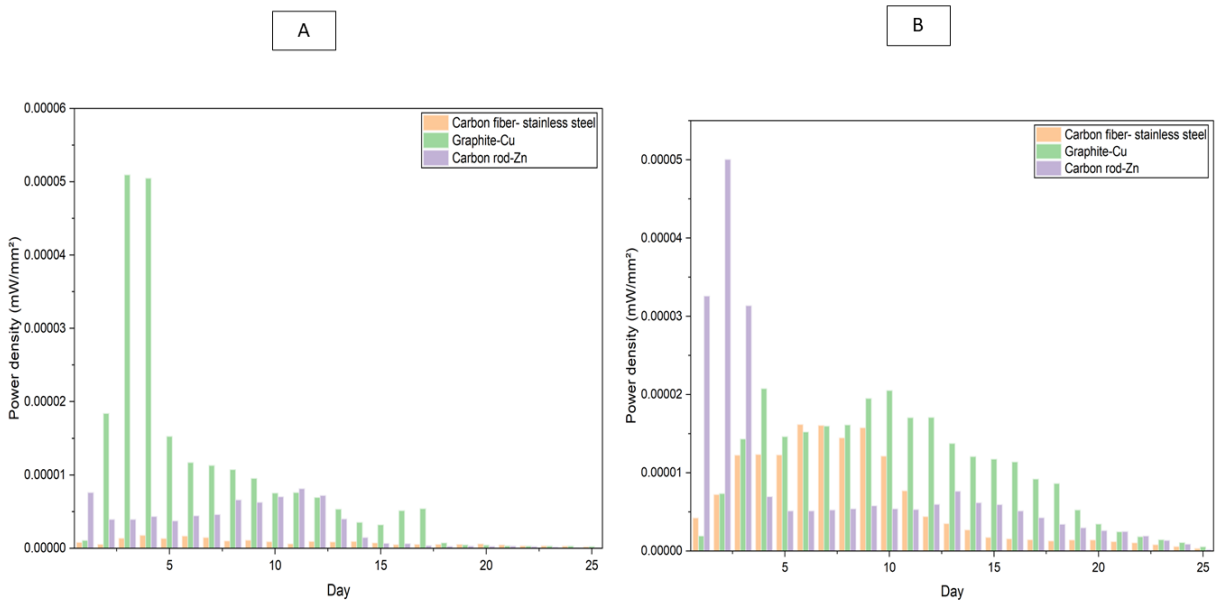


Figure 11. Power density variations of dairy farm (a) and river (b) wastewater MFCs using carbon fiber – stainless steel, Graphite-Cu, and carbon rod- Zn electrodes.

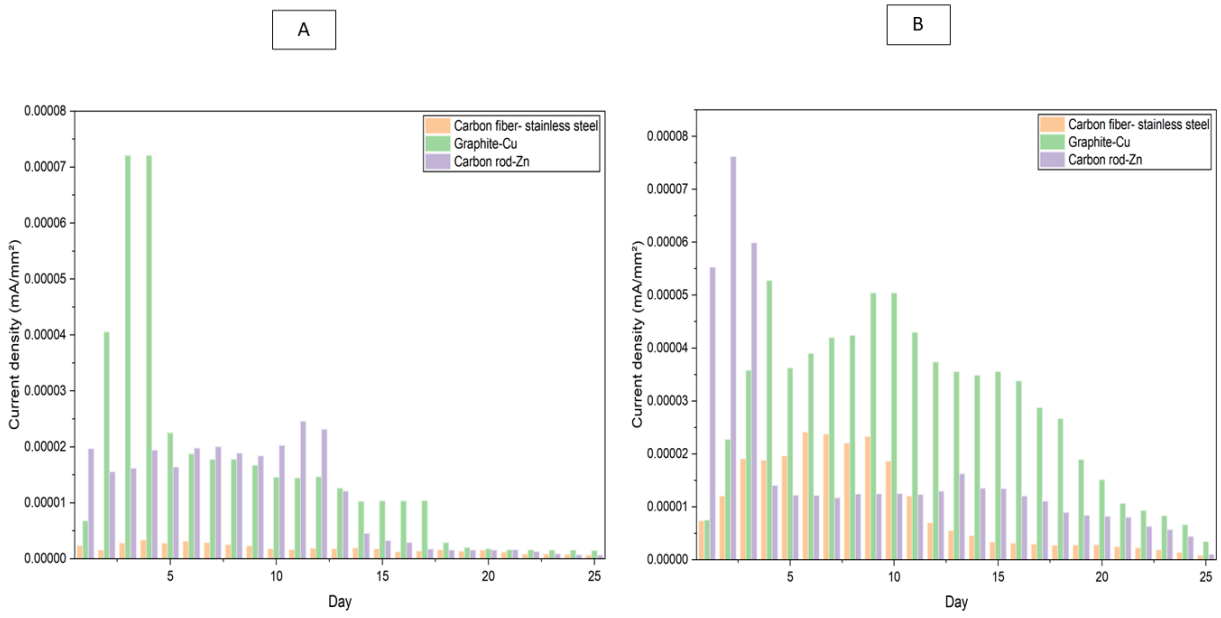


Figure 12. Current density variations of dairy farm (a) and river (b) wastewater MFCs using carbon fiber- stainless steel, Graphite-Cu, and carbon rod- Zn electrodes.

3.4. Wastewater treatment efficiency

The treatment performance of the microbial fuel cells was evaluated based on the reduction of major physicochemical parameters, namely chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), and turbidity. The variations in these parameters for dairy and river wastewater are illustrated in Figure 13.

For the dairy wastewater, significant improvement in water quality was achieved across all electrode combinations. The G-Cu MFC demonstrated the highest COD removal efficiency, 98.53%, followed by CR-Zn (85.33%) and Cf-SS (83.33%). Similarly, TDS reduction was highest for G-Cu (92.73%), while Cf-SS and CR-Zn achieved 90.91% and 80.45%, respectively. The TSS removal results indicated maximum efficiency for CR-Zn (99.19%), followed by Cf-SS (96.75%) and G-Cu (91.06%). A comparable trend was observed for turbidity reduction, with Cf-SS attaining 82.02%, G-Cu 80.74%, and CR-Zn 78.51%. These results collectively suggest that all MFC configurations effectively reduced the measured water quality parameters, although the best-performing electrode combination varied among the parameters.

In the Turag River water system, the overall pollutant removal efficiencies were comparatively lower, likely due to the reduced organic and nutrient load in the substrate. COD removal reached 70.59% for Cf-SS, 64.71% for G-Cu, and 55.88% for CR-Zn. TDS reduction was also moderate, with Cf-SS achieving 46.36%, CR-Zn 45.45%, and G-Cu 40%. The TSS removal trend showed maximum reduction with G-Cu (80%), followed by Cf-SS 70% and CR - Zn 30%. Similarly, the turbidity reduction rate was highest in Cf-SS (49.07%), with G-Cu and CR-Zn achieving 44.96% and 34.18%, respectively.

Overall, the pollution removal efficiency was high in the G-Cu MFC in dairy, with the highest COD and TDS removal rates of 98.53% and 92.73%, respectively. For river water, G-Cu showed a moderate performance; the CF-SS showed the highest pollutant removal rate for COD, TDS, and turbidity, 70.59%, 46.36%, and 49.07%, respectively, while G-Cu showed the highest TSS removal (80%). Overall, G-Cu showed high to moderate removal performance across both wastewater types. It achieved the highest COD and TDS removal in dairy wastewater and maintained moderate removal efficiencies in river wastewater. Although the other electrode

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configurations performed better for some individual pollutants, G-Cu showed comparatively consistent performance across the two wastewater types.

The pollutant removal rate was high as the organic substrate was degraded by microbial metabolism. The pollutant removal efficiency was high in the dairy sample, while the lower efficiencies in river wastewater were due to its lower organic load, which reduced microbial activity. The results from Figure 13 clearly demonstrate that the MFCs efficiently improved the quality of both wastewater types between the initial and post-treatment stages, with dairy farm wastewater showing higher removal efficiencies due to its richer organic composition and higher biodegradability.

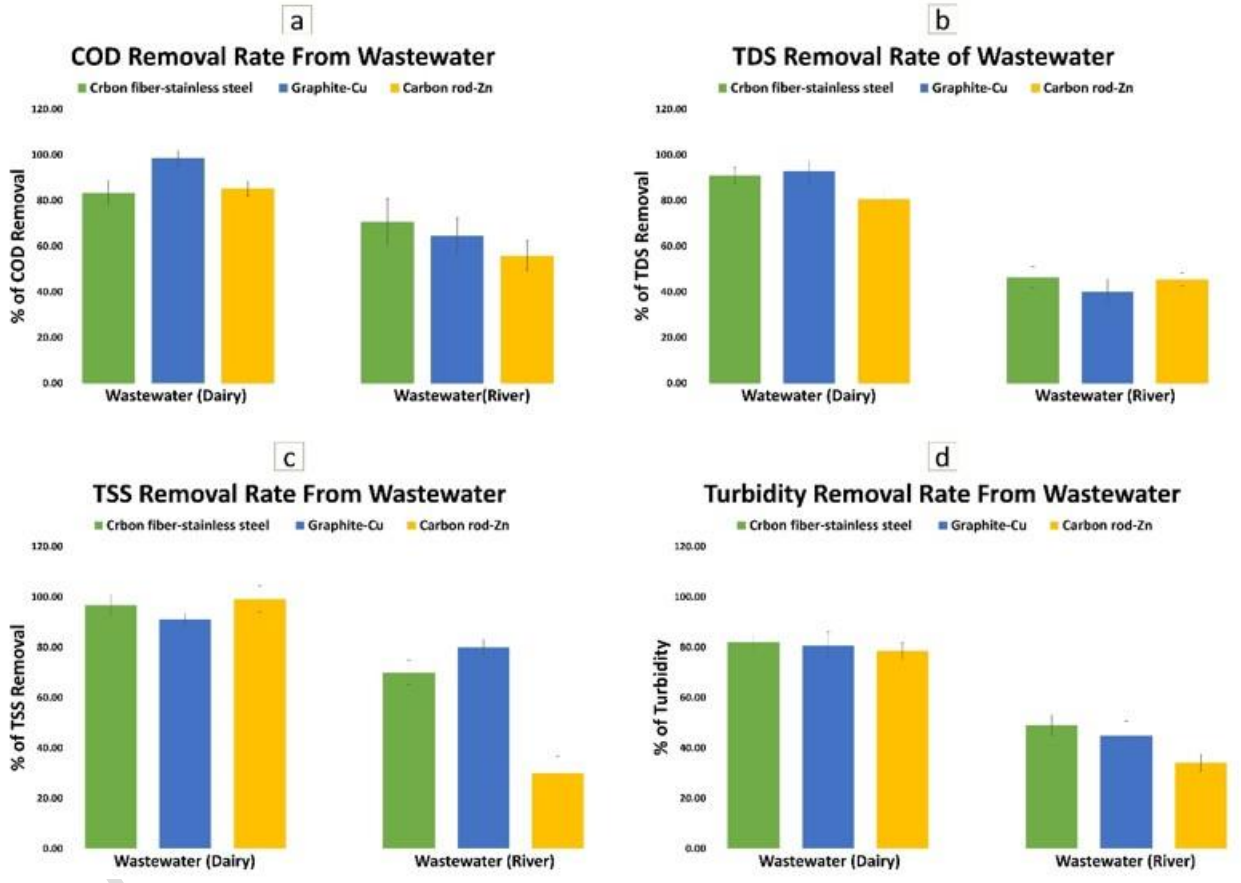


Figure 13. COD(a), TDS(b), TSS (c), and Turbidity (d) removal efficiency of MFCs treating dairy and Turag River water using different electrode combinations

Table 3: Maximum pollutant removal efficiencies achieved by different electrode combinations in dairy farm effluent and Turag River water after 25 days MFC operation.

Pollutant parameter	Dairy farm effluent	Turag River water
COD removal (%)	98.53 (G-Cu)	70.59 (Cf-SS)
TDS removal (%)	92.73 (G-Cu)	46.36 (Cf-SS)
TSS removal (%)	99.19 (CR-Zn)	80.00 (G-Cu)
Turbidity removal (%)	82.02 (Cf-SS)	49.07 (Cf-SS)

As shown in **Table 3**, the pollutant removal efficiency varied among the electrode combinations and wastewater types. In dairy farm effluent, G-Cu performed best in COD and TDS removal, while CR-Zn showed the highest TSS removal. In Turag River water, Cf-SS achieved the highest removal of COD, TDS, and turbidity, whereas G-Cu showed the highest TSS removal. These results indicate that the most effective electrode combination depends on the type of pollutant being treated and the characteristics of the wastewater.

3.5. Bacterial identification

Bacterial identification was performed to determine the predominant microbial species present in the MFC systems and explore their potential association with electrochemical activity.. The isolates obtained from both the Dairy farm and Turag River water were first examined by Gram staining for morphological characterization and then confirmed at the Quality Control Laboratory using the Bruker MALDI-MS Biotyper (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry). This is a highly precise method that identifies microorganisms based on protein mass spectra.

In the Dairy farm wastewater, four bacterial isolates were confirmed. Gram staining showed D1 and D4 as Gram-negative rods, D2 as a Gram-negative short rod, and D3 as a Gram–positive coccus. MALDI Biotyper analysis identified D1 as *Escherichia coli*, D2 as *Aeromonas hydrophila*, D3 as *Acinetobacter bereziniae,* and D4 as *Aeromonas caviae* (Table 4). Previous studies have reported electroactive potential in some bacterial species belonging to the genera identified in this study. Their presence may suggest potential involvement in microbial processes

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within the MFC systems. However, their individual contribution to electron transfer and electricity generation was not investigated in this study.

For the river sample, four isolates were identified: R1, R2, R3, and R4; all were Gram-negative, rod-shaped in morphology. Whereas R4 was a Gram-positive coccus. The MALDI Biotyper analysis identified R1 as *Aeromonas caviae*, R2 as *Escherichia coli*, R3 as *Acinetobacter Lwoffii,* and R4 as *Staphylococcus arlettae* (Table 5). *Escherichia coli* is a facultative electroactive bacterium capable of forming conductive biofilms and participating in electron transfer to the anode under stressed conditions (12). *Aeromonas cavia, Staphylococcus arlettae,* and *Acinetobacter Lwoffii* are all electroactive bacteria and can oxidize organic matter and transfer electrons to the anode.

Overall, bacterial identification results show that both dairy and Turag River water contained different types of bacteria, including species of *Aeromonas, Acinetobacter, Escherichia*, and *Staphylococcus*. Some of these bacteria have been reported in previous studies for their electroactive properties, making their presence relevant to understanding the microbial processes involved in MFC operation.

Table 4: Summary of bacterial identification and electrogenic potential in dairy farm wastewater

Sample no.	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**	**Gram reaction and morphology**	**Electrogenic properties**
D1	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Escherichia*	*E. coli*	Gram-negative (rod)	Electrogenic (facultative) (Nandy et al., 2016a;

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								Yoon et al., 2024)
D2	Pseudomonadota	Gammaproteobacteria	Aeromonadales.	Aeromonadaceae	*Aeromonas*	*A. hydrophila.*	Gram-negative (short rod)	Electrogenic (Pham et al., 2003)
D3	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*A. baumannii*	Gram-negative (cocci)	Electrogenic (De La Cruz-Noriega et al., 2023)
D4	Pseudomonadota	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	*Aeromonas*	*A. caviae*	Gram-negative (rod)	Electrogenic (Pham et al., 2003)

Table 5: Summary of bacterial identification and electrogenic potential in the Turag River water

Sample no.	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**	**Gram stain and morphology**	**Electrogenic properties**
R1	Pseudomonadota	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	*Aeromonas*	*A. caviae*	Gram-negative (rod)	Electrogenic (Pham et al., 2003)

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R2	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Escherichia*	*E. coli*	Gram-negative (rod)	Electrogenic (facultative) (Nandy et al., 2016a; Yoon et al., 2024)
R3	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*A. lwoffii*	Gram-negative (rod)	Electrogenic (Partipilo et al., 2025; Yoon et al., 2024)
R4	Bacillota	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	*S. aureus*	Gram-positive (Cocci)	Electrogenic(Marito et al., 2021; Tahernia et al., 2020)

4. Discussion

The performance of MFCs operated with dairy farm and Turag River water demonstrated the close interrelationship between electrode composition, microbial activity, and substrate characteristics. The results confirmed that MFCs can simultaneously generate bioelectricity and achieve significant pollutant removal, ensuring their potential as a sustainable wastewater treatment system. In this experiment, the G-Cu electrode pair exhibited the highest peak voltage and current in dairy farm wastewater, achieving a maximum voltage of 706.67 mV and a current of 0.2667 mA, while the Cf-SS and CR-Zn configurations performed well in the Turag River water with peaks of 676.67 mV voltage and 0.2667 mA current, respectively. These variations align with reports that electrode material significantly affects MFC efficiency through electron transfer rate, surface roughness, and biofilm formation (12;13). Carbon fiber provides a large, hydrophilic surface for bacterial attachment and stable voltage generation, whereas Zn paired with a carbon anode can facilitate rapid charge transport and higher current under low-strength alkaline conditions.

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The maximum power density obtained was 5.093×10^{-5} mW/mm^2 for Graphite-Cu in dairy farm wastewater and approximately 5.003×10^{-5} mW/mm^2 for Carbon rod-Zn in river water, demonstrating that both electrode configuration and substrate strength determine energy recovery potential. However, in the river wastewater system, the electrode that produces high voltage (Cf-SS) was different from the one that produces high current (CR-Zn). The overall power output depended on both voltage and current, as well as the internal resistance of the system. (18; 12;19).

The MFCs showed good pollutant removal along with electricity generation. In the dairy wastewater, the Graphite-Cu setup performed best, removing 98.53% COD and 92.73% TDS, while the CF-ss and CR-Zn systems also removed more than 80% COD. However, CR-Zn showed the highest TSS removal 99.19%, while CF-SS showed the highest turbidity removal 82.02%. This shows that in the dairy MFCs, electricity generation and pollutant removal were associated with the overall MFC treatment process. Microbial degradation of organic matter can contribute to COD reduction and electron generation, while other biological and physicochemical processes can contribute to the removal of suspended and dissolved substances. A similar trend has been observed in other studies for wastewater treatment in MFCs (13;20).

In the Turag River water, this relationship between electrical output and pollutant removal was less consistent. The Cf-SS produced a high voltage (676.67 mV) and showed the highest COD, TDS, and turbidity removal efficiencies at 70.59%, 46.36%, and 49.07%, respectively. The reason behind this may be the large surface area and biocompatibility, which allow bacteria to attach easily and form a stable biofilm for organic degradation. (13;16). Although the CR-Zn electrode generated a high current (0.2667 mA) in the initial phase, its pollutant removal efficiency was comparatively lower. This difference may be related to variations in electrode characteristics, wastewater composition, and microbial activity. The river wastewater also contained fewer nutrients and organic compounds, which limited the microbial activity. (21).

The comparatively high average current generated by the G-Cu electrode pair in both samples is likely due to graphite's excellent conductivity, chemical stability, and compatibility with microbial communities, which could favour biofilm development (22;23) Copper, used as the cathode, may also have influenced the oxygen reduction reaction, facilitating a higher electron flow and better energy recovery from organic matter. (24) This combination works well together, which helps produce a higher current than the other MFCs.

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Interestingly, all electrode systems produced more current in river water than in dairy wastewater, even though the dairy sample had a higher COD concentration. This finding suggests that excessive organic load in dairy wastewater may have caused substrate inhibition or unfavorable pH shifts, impairing microbial activity and electron transfer. (25). In contrast, the moderate organic content in river water likely provided a more optimal environment for electrogenic microbial activity, resulting in higher current densities.

The G-Cu showed comparatively better performance. The almost complete removal of COD and TSS from dairy and river wastewater indicates the strong biodegradation and solid separation capability of this electrode pair. Additionally, the decrease in turbidity and TDS levels in the effluent indicates that the particulate trapping and possible chemical reaction at the electrode surface contributed to pollutant removal. (26). In contrast, the CR-Zn configuration showed effective TSS removal. Visible corrosion of the zinc electrode was observed during the experimental period, This corrosion may have released zinc ions into the cathode chamber. Although zinc concentration was not measured, the release of zinc ions may have affected the cathodic environment and potentially influenced electron transfer and overall electrochemical performance of the MFC system. Previous studies also reported that excessive zinc ion concentration may potentially inhibiting microbial activity and long-term stability. (27). The Cf-SS system exhibited lower treatment performance, possibly due to poor catalytic activity at the stainless-steel cathode. (28). Overall, the results indicate that electrode performance varied according to wastewater type and the parameter considered. TheG-Cu showed comparatively strong performance, particularly dairy farm waste, achieving high electricity generation and pollutant removal efficiency, suggesting its potential as an effective electrode combination for testing wastewater with a high organic load.

The pH shifts and visual improvements further confirmed the treatment efficiency of the MFCs. In the dairy wastewater, pH decreased from 7.8 to 7.1-7.5, and in the river wastewater, from 8.6 to 7.2-8.0, indicating the proton release during microbial oxidation, which supports the bio-electrocatalysis (18). The color improvement from greenish to yellow in dairy wastewater and from light yellow to whitish in river water reflects the degradation of organic and suspended matter, which matches the decreasing trend of COD and TSS.

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The bacterial analysis confirmed the presence of several Gram-negative and Gram-positive bacteria in both dairy farm effluent and Turag River water samples. The isolates identified using the Bruker MALDI Biotyper included *Escherichia coli*, *Aeromonas hydrophila*, *Acinetobacter berezin*iae, and *Aeromonas caviae* in dairy farm wastewater and *Aeromonas caviae*, *Escherichia coli*, *Acinetobacter Lwoffii,* and *Staphylococcus arlettae* in river wastewater. The dominance of these bacteria supports the strong electrochemical activity observed in both MFC systems. *Aeromonas hydrophila* and *Aeromonas caviae* are well-known electrogenic bacteria that can transfer electrons directly to the anode through outer-membrane cytochromes and conductive pili, enabling simultaneous electricity generation and organic matter degradation. (29). *Acinetobacter bereziniae* and *Acinetobacter Lwoffii* also showed electrogenic potential. *Acinetobacter Lwoffii*, recently recognized as an emerging exoelectrogen, can survive in low-nutrient environments such as the Turag River and maintain long-term electrochemical activity. (30;31). *A. bereziniae* contributes to forming a stable biofilm, which supports steady current generation (19). These species can produce electricity even when the available organic concentration is low.

Although *E. coli* is not a strong electrogenic bacterium, it was abundant in both samples because it is a common intestinal bacterium originating from cow dung in dairy farm effluent and domestic waste in the river. As a facultative anaerobe, *E. coli* helps maintain the biofilm environment and supports other electroactive bacteria by producing metabolic intermediates and redox mediators. (12;30). The detection of *Staphylococcus arlettae* in the Turag River sample is likely due to anthropogenic pollution and industrial activities. (32). This bacterium is usually found on the skin of humans and animals and often enters water bodies through domestic sewage, animal waste, or factory discharges. (33). Its appearance in the river suggests contamination from both household and industrial sources, which agrees with earlier studies showing high levels of microbial pollution from textile, tannery, household, and other sources in the surface water. (34;32). Overall, the findings show that MFC performance depends on the combined effects of electrode material, wastewater characteristics, and microbial community. The Graphite-Cu electrode performed best in both dairy farm wastewater with higher organic content and river water in terms of electricity generation and pollutant removal. The presence of electrogenic bacteria such as *Aeromonas*, *E. coli*, and *Acinetobacter* played a key role in maintaining electricity generation and pollutant removal. These findings agree with recent studies that highlight the importance of optimizing electrode materials and microbial activity for real wastewater applications. (16;6;30).

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Despite the positive outcomes, additional studies are needed to address the remaining research gaps. This study was conducted under laboratory conditions, and further evaluation is needed to assess long-term stability, large-scale applicability, and economic feasibility. Additionally, temperature and pH optimization were not extensively studied. Future research should focus on the development of cost-effective, nanostructured electrode materials with enhanced conductivity, biocompatibility, and corrosion resistance to improve electron transfer efficiency. Further work should also investigate the microbial community and species-specific electrochemical activity using molecular and electrochemical approaches. Moreover, a factorial experimental design in which anode and cathode materials are varied independently would help separate the individual effects of each electrode material. For zinc-base electrode systems, future study should also investigate electrode degradation and quantify potential xzinc release during operation. Designing a scalable reactor system could further improve power generation and treatment efficiency under real operating conditions.

5. Conclusions

This research successfully applied MFC technology for the dual purpose of wastewater treatment and bioelectricity generation using dairy farm and Turag River water. The study confirmed that MFCs can utilize the organic content of real wastewater as a bioenergy source while improving overall water quality.

Among the tested electrode combinations, the Graphite-Cu pair showed comparatively better electrical performance, particularly in dairy wastewater, while Cf-SS and CR-Zn showed comparatively high voltage and current, respectively, in the Turag River water. These results indicate that electrode performance varied according to the wastewater type and the electrical parameter considered.

In term of pollutant removal efficiency, the Graphite-Cu system 98.53% COD and 92.73% TDS removal in dairy wastewater . However, its performance in Turag River water was comparatively different. Although G-Cu demonstrated favourable balance between electric output and pollutant removal, the results do not establish it as the universally superior electrode

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combination. Rather, these findings highlight the importance of selecting electrode combinations based on the characteristics of wastewater and the desired outcomes in term of electricity generation and pollutant removal.

Bacterial identification revealed the presence of *Aeromonas hydrophila*, *Acinetobacter berezin*iae, *Aeromonas caviae, Acinetobacter Lwoffii, Staphylococcus arlettae, and Escherichia coli*. The abundance of *E. coli* was expected, as samples originated from cow dung and domestic waste, common in both wastewater sources.The presence of these bacterial species suggests their potential involvement in biofilm formation and organic matter degradation within the MFC system. However, the individual contribution of these bacterial species to electricity generation and extracellular electron transfer was not directly investigated in this study. Therefore, their specific electrogenic roles cannot be confirmed from the present findings. The present study represents an initial investigation into the use of different electrode combinations for simultaneous wastewater treatment and bioelectricity generation under laboratory conditions. Although differences in electrical outputs and pollutant removal efficiency were observed, the findings are not sufficient to establish one electrode combination as universally superior. Further investigations involving repeated experimental trials, detailed microbial community analysis, and systematic evaluation of electrodes are necessary to better understand the interactions between electrode materials, microbial communities, and electricity generation. In addition, larger-scale experiments and reactor optimization are required to confirm the most suitable electrode combinations and assess their long-term performance and practical application.

Overall, the results demonstrate that MFCs offer sustainable and environmentally friendly technology for simultaneous wastewater purification and renewable energy generation. With optimization of electrode materials, system design, and microbial enrichment, MFCs hold strong potential for practical application in decentralized wastewater treatment systems.

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