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Case Report

Bioelectrochemical degradation of pollutants in wastewater using a dual-chamber microbial fuel cell with graphene-modified electrodes and electroactive bacteria

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ABSTRACT

The increasing discharge of pharmaceuticals and nitrates into aquatic environments poses significant ecological and public health risks, as conventional wastewater treatment plants often fail to achieve complete removal. Microbial fuel cells (MFCs) offer a bioelectrochemical approach for simultaneous wastewater treatment and energy generation; however, their efficiency is constrained by slow electron transfer. This study investigated the bioelectrochemical degradation of pharmaceuticals and nitrates in wastewater using a dual-chamber MFC equipped with graphene-coated carbon cloth anodes to enhance microbial electron transfer. Wastewater samples were collected from a municipal treatment plant and a pharmaceutical discharge site, while electroactive bacteria enriched from anaerobic sludge served as biocatalysts. Pollutant degradation was analyzed using high-performance liquid chromatography (HPLC) and ion chromatography (IC), and electrochemical performance was assessed through open-circuit voltage (OCV), power density, cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). The results demonstrated that graphene-coated anodes enhanced pharmaceutical degradation from 62.3 % to 87.6 % and nitrate removal from 58.4 % to 83.2 % over 72 hours. Power density increased by 93.6 % (from 405.6 mW/m^2 to 785.3 mW/m^2), while internal resistance decreased by 37.5 %, indicating improved electron transfer. Biofilm analysis revealed a 55.9 % increase in thickness and a 48.3 % higher microbial cell density on graphene-coated anodes, with metagenomic sequencing confirming the dominance of *Geobacter* and *Shewanella*. These findings highlight the potential of graphene-modified MFCs as a sustainable and scalable technology for real wastewater treatment.

1. Introduction

The rapid expansion of industrial activities, coupled with the widespread use of pharmaceuticals and endocrine-disrupting compounds (EDCs), has resulted in the continuous release of these pollutants into aquatic ecosystems. Pharmaceuticals such as antibiotics, analgesics, and antidepressants, along with EDCs, pose serious risks to both environmental and public health [1]. For example, studies have shown that concentrations of pharmaceutical compounds in surface waters can range from 0.1 to 100 μg/L [2,3], with some EDCs reaching levels as high as 200 ng/L in freshwater ecosystems [4]. These compounds are persistent in the environment and often remain undetected in conventional wastewater treatment processes, which were not designed to remove such complex mixtures of contaminants. As a result, the

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presence of pharmaceuticals and EDCs in natural water bodies has become a growing concern due to their potential to disrupt aquatic life, harm ecosystems, and affect human health through the contamination of drinking water sources [5,6].

Traditional wastewater treatment plants (WWTPs) are often inefficient in removing these pollutants, particularly pharmaceutical residues and nitrates, which are difficult to degrade using conventional processes like activated sludge or chemical treatments [7]. For instance, conventional treatment technologies have been reported to remove only 30–60 % of pharmaceutical residues [8], while nitrate removal efficiency is typically around 50–70 % in standard WWTPs [9]. Nitrates, commonly found in agricultural runoff, pose additional challenges due to their high solubility and mobility in water [10]. In some regions, nitrate concentrations in groundwater exceed the 50 mg/L limit set by the World Health Organization for drinking water quality, further exacerbating the problem [11]. The inability of WWTPs to effectively treat pharmaceutical residues and nitrates not only threatens water quality but also raises concerns about the long-term health impacts of consuming contaminated water [12]. Therefore, there is an urgent need to develop more advanced and sustainable technologies capable of addressing the removal of such persistent pollutants in wastewater treatment.

Microbial fuel cells (MFCs) have emerged as an innovative and promising bioelectrochemical technology for wastewater treatment [13–15]. MFCs harness the natural metabolic processes of electroactive microorganisms to degrade organic pollutants, simultaneously generating electricity as a byproduct [16,17]. The key advantage of MFCs lies in their ability to combine pollution treatment with energy production, offering a dual benefit of sustainable wastewater management and renewable energy generation [18]. Studies have demonstrated that optimizing MFC configurations can enhance performance. For instance, Qiu et al. [19], achieved a higher power density of 529 mW/m^2 in a dual-chamber MFC (DCMFC) with a biocathode, compared to 478 mW/m^2 with an abiotic cathode, demonstrating the role of synergistic electrochemical and microbial reductions in improving efficiency. Furthermore, theoretical estimates suggest that power densities could reach 17–19 W/m^2 [20], by optimizing microbial kinetics and minimizing internal resistance, although these values remain challenging to achieve in practical wastewater treatment applications. The slow electron transfer between microbes and anode electrodes continues to be a major limitation, reducing both pollutant degradation efficiency and power generation, thereby constraining the large-scale feasibility of MFCs.

Moreover, recent research has focused on improving the electron transfer rate in MFCs by modifying anode electrodes with advanced materials like graphene. Graphene, with its exceptional electrical conductivity (up to 10,000 S/m) and high surface area (2630 m^2/g) [21], has shown great promise in enhancing microbial attachment, biofilm formation, and electron transfer. Studies have shown that graphene-modified electrodes can increase the power output of MFCs, while also improving the degradation efficiency of organic pollutants, including pharmaceuticals and nitrates [22,23]. While the application of graphene-coated electrodes in wastewater treatment has shown promise, it remains relatively limited, particularly in addressing the complex challenge of removing pharmaceuticals and nitrates. This gap in the current literature highlights the significant potential of the study, which aims to investigate the bioelectrochemical degradation efficiency of contaminants using graphene-modified MFCs in wastewater.

This study aims to fill this gap by evaluating the performance of graphene-modified anodes in a dual-chamber MFC for the bioelectrochemical degradation of pharmaceuticals and nitrates in wastewater. Specifically, the objectives of the research are to: (i) optimize graphene-coated carbon cloth electrodes to enhance microbial activity and biofilm formation, (ii) quantify the degradation kinetics of pharmaceuticals and nitrates using advanced analytical techniques, and (iii) analyze microbial community dynamics in response to the bioelectrochemical conditions. As previously mentioned, MFCs were originally designed for bioelectricity generation through microbial degradation of organic matter; however, there has been growing interest in their application for wastewater treatment. Therefore, monitoring power density in this study is essential for evaluating both electrochemical performance and the effectiveness of pharmaceutical and nitrate removal. The potential of this study lies in its comprehensive approach to integrating graphene modifications with MFC technology to treat complex wastewater contaminants, offering a potential breakthrough in the development of more efficient and sustainable wastewater treatment systems.

2. Materials and methods

2.1. MFC reactor design and setup

A dual-chamber microbial fuel cell (MFC) was constructed, consisting of an anodic chamber housing electroactive bacteria and a cathodic chamber exposed to atmospheric oxygen as the terminal electron acceptor. The chambers were acrylic-based, each with a working volume of 250 mL, and were connected via a proton exchange membrane (PEM) (Fig. 1).

2.1.1. Electrodes

The electrodes play a crucial role in the overall performance of the microbial fuel cell (MFC) by facilitating electron transfer between electroactive bacteria and the external circuit. The anode was fabricated using graphene-coated carbon cloth with a surface area of 25 cm^2, prepared through a controlled chemical vapor deposition (CVD) process. The graphene coating provided a high surface area and excellent conductivity, enhancing bacterial adhesion and electron transport efficiency. To ensure the quality and structural integrity of the graphene layer, Raman spectroscopy was performed, analyzing the characteristic D, G, and 2D bands, which provided insights into the material's defect density and crystallinity. Additionally, X-ray photoelectron spectroscopy (XPS) was used to confirm the carbon bonding states, verifying the presence of sp^2 hybridized carbon indicative of high-quality graphene.

For the cathode, a platinum-coated activated carbon electrode was employed, with a platinum loading of 0.5 mg/cm^2 to enhance catalytic activity. The activated carbon provided a high surface area for oxygen reduction, while the platinum coating significantly improved electron transfer rates, ensuring efficient oxygen reduction reactions at the cathode. The combination of these materials resulted in a highly conductive and electrochemically active electrode system, optimizing the overall efficiency of the MFC. Proper characterization and selection of the anode and cathode materials were essential for maximizing electron transfer efficiency and sustaining long-term MFC operation.

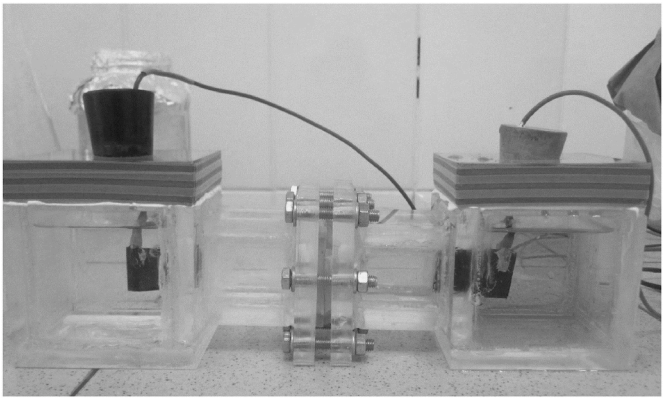

Fig. 1. Double chamber MFC setup.

2.1.2. Proton exchange membrane (PEM)

The PEM used in the MFC was Nafion 117, chosen for its high proton conductivity and chemical stability. Nafion 117 served to effectively separate the anodic and cathodic chambers, allowing protons generated at the anode during microbial metabolism to migrate through the membrane to the cathode, while simultaneously preventing the diffusion of oxygen from the cathode chamber into the anode. This selective ion conductivity was critical for maintaining the electrochemical gradient necessary for MFC operation, ensuring efficient proton transport and minimizing energy loss. Nafion's durability under experimental conditions, along with its well-established performance in similar bioelectrochemical systems, made it an ideal choice for optimizing ion exchange while maintaining the separation of the two chambers.

2.1.3. External circuit and load

An external circuit was established by connecting a 1000 Ω resistor between the anode and cathode electrodes, enabling controlled electron flow from the anode to the cathode. This resistor was selected to optimize power output while maintaining stability in the MFC system. The external circuit, including the resistor, facilitated the flow of electrons generated by the microbial metabolism at the anode to the cathode, ensuring efficient energy harvesting. To monitor and analyze the electrochemical performance of the MFC, a Gamry Reference 600 potentiostat was employed. The potentiostat was used to measure and record the voltage and current variations across the external circuit in real-time, providing essential data for evaluating the MFC's power generation and electrochemical efficiency. This setup enabled precise control over experimental parameters and the assessment of the MFC's overall functionality and performance.

2.2. Wastewater collection and characterization

Wastewater samples were collected from two distinct sources to assess the MFC performance under varying contamination conditions. The first source was the secondary-treated effluent from a municipal wastewater treatment plant (WWTP), which represented a typical urban wastewater stream containing moderate levels of organic pollutants, residual nutrients, and trace pharmaceuticals. This effluent was selected to evaluate the MFC's efficiency in treating municipal wastewater with a relatively stable composition. The second source was industrial discharge from a pharmaceutical manufacturing facility, which was characterized by high concentrations of pharmaceutical residues, including antibiotics, analgesics, and endocrine-disrupting compounds. This wastewater posed a more complex challenge due to its higher recalcitrance and potential antimicrobial effects on electroactive microbial communities. To ensure statistical reliability, all collected samples were analyzed in triplicates, with physicochemical parameters assessed immediately upon arrival at the laboratory to prevent compositional changes (Table 1).

Table 1
Key parameters analyzed.

Parameter	Method Used	Instrumentation
Organic Pollutants		
Chemical Oxygen Demand (COD)	Standard Dichromate Method	HACH DR3900 Spectrophotometer
Total Organic Carbon (TOC)	Combustion Analysis	Shimadzu TOC-L Analyzer
Pharmaceuticals		
Acetaminophen, Sulfamethoxazole, Tetracycline, Bisphenol A (BPA)	Liquid Chromatography-Mass Spectrometry (HPLC-MS)	Agilent 1260 Infinity LC-MS
Inorganic Pollutants		
Nitrate (NO_3^-)	Ion Chromatography	Metrohm 930 Compact IC Flex

2.3. Electrochemical performance evaluation

The open circuit voltage (OCV) of the MFC was continuously monitored using a Gamry Reference 600 potentiostat to assess the potential difference between the anode and cathode when no external load was applied. The OCV provided valuable information about the electrochemical activity of the microbial communities and the overall health of the MFC, serving as an indicator of the system's ability to generate electrical energy in the absence of external current. To evaluate the power output of the MFC, power density (P) was calculated using Equation (1).

$$P = \frac{V^2}{R \times A} \tag{1}$$

Whereby; V is the measured voltage (V), R is the external resistance (Ω), and A is the electrode surface area (m^2). This equation enabled the determination of the power density in milliwatts per square meter (mW/m^2), providing a quantitative measure of the MFC's efficiency in converting chemical energy from wastewater into electrical energy. By recording the OCV and calculating the power density, the performance of the MFC under various conditions could be accurately evaluated and compared.

2.4. Electrochemical impedance spectroscopy (EIS)

EIS was employed to assess the charge transfer resistance R_{ct} and gain insight into the electrochemical characteristics of the MFC. EIS measurements were conducted across a frequency range from 100 kHz to 10 mHz, enabling a detailed analysis of the impedance response at different time scales. The higher frequencies provided information about the ohmic resistance, while the lower frequencies highlighted the electrochemical processes involved in electron transfer at the electrodes. Nyquist plots, which plot the imaginary impedance against the real impedance, were generated from the experimental data to visualize the impedance behavior. The semi-circular arc observed in the Nyquist plot was used to extract R_{ct}, a key parameter indicating the resistance to electron flow across the electrode-biofilm interface. The data were analyzed using ZView® software, which allowed for the fitting of the experimental impedance data to equivalent circuit models, thus providing a deeper understanding of the electrochemical kinetics and charge transfer processes occurring within the MFC system.

2.5. Cyclic voltammetry (CV)

Cyclic voltammetry (CV) was utilized to investigate the redox activity and electron transfer kinetics of the MFC. The potential was swept over a range from −0.8 V to +0.8 V versus Ag/AgCl at a scan rate of 5 mV/s. This potential window was chosen to capture the electrochemical behavior of both the anode and cathode reactions, including microbial electron transfer at the anode and the oxygen reduction reaction at the cathode. CV measurements provided valuable data on the redox properties of the biofilm and electrode materials, allowing for the identification of characteristic peaks that correspond to the oxidation and reduction of the electroactive species. By analyzing the current response at different potentials, the electron transfer kinetics were evaluated, offering insights into the efficiency of electron flow within the MFC system. The shape and magnitude of the cyclic voltammogram also provided information on the electrochemical activity of the microbial community at the anode, as well as the overall performance of the MFC in terms of electron generation and transfer efficiency.

2.6. Microbial community analysis

To understand the microbial diversity and functional potential of biofilms developed on the anode.

2.6.1. Metagenomic sequencing

DNA was extracted from biofilm samples using the DNeasy PowerSoil Kit (Qiagen, USA), which is optimized for extracting high-quality DNA from complex environmental samples such as biofilms. Following extraction, Illumina MiSeq sequencing was carried out with a 2 × 300 bp paired-end strategy, targeting the V3–V4 region of the 16S rRNA gene, which is commonly used for microbial taxonomic profiling. This sequencing approach enabled the identification and characterization of microbial communities within the MFC, providing insights into the diversity of bacteria present, with a focus on electroactive species involved in the MFC's electron transfer processes. To process and analyze the sequencing data, bioinformatics analysis was conducted using the QIIME 2 pipeline, a powerful and widely used tool for analyzing microbiome data. The QIIME 2 pipeline facilitated the processing of raw sequence data, including quality filtering, sequence clustering, and taxonomic classification, allowing for detailed profiling of the microbial communities and identifying key microorganisms contributing to the electrochemical activity of the MFC.

2.6.2. Quantitative PCR (qPCR) for functional genes

Quantitative PCR (qPCR) was utilized to analyze the abundance of functional genes associated with electron transfer and pollutant degradation in the MFC. Specific target genes were selected to provide insights into the microbial processes occurring at the anode and the degradation of organic pollutants. The mtrA and mtrB genes, which are involved in electron transfer in *Geobacter* spp., were targeted to assess the contribution of electroactive bacteria to the MFC's power generation. Additionally, the nirS and nirK genes, which are involved in nitrate reduction, were analyzed to evaluate the presence and activity of nitrate-reducing bacteria in the MFC system. To further understand the potential for pollutant degradation, the bphA gene, responsible for the degradation of bisphenol A (BPA), was also targeted. This gene serves as a marker for microorganisms involved in the breakdown of endocrine-disrupting chemicals. qPCR amplifications were conducted using a Bio-Rad CFX96 qPCR system, which provided precise and reproducible quantification of these functional genes. The relative abundance of these genes was measured to correlate with the electrochemical performance and pollutant removal efficiency of the MFC, offering a deeper understanding of the microbial processes supporting the system's operation.

3. Results

3.1. Pollutant degradation efficiency

The pollutant degradation efficiency results reveal significant removal of various contaminants within 72 hours, demonstrating the potential of the bioelectrochemical system for wastewater treatment. Chemical Oxygen Demand (COD) was reduced by 85.4 %, indicating a highly effective biological and electrochemical oxidation process. Pharmaceuticals, including acetaminophen (83.2 %), sulfamethoxazole (79.5 %), tetracycline (81.7 %), and bisphenol A (BPA) (85.1 %), also showed substantial degradation. This suggests that the system, through a combination of biodegradation and electrochemical reduction or oxidation mechanisms, is capable of removing a range of organic pollutants typically found in wastewater (Fig. 2).

The removal of nitrates (76.4 %) was achieved through biological denitrification, a process in which microbes reduce nitrate to nitrogen gas. This process is significant for managing nitrogen contamination in wastewater, contributing to the overall sustainability of the system. The high removal efficiency of both organic pollutants and inorganic substances such as nitrates underlines the versatility of the bioelectrochemical approach in addressing diverse contaminants commonly found in wastewater from industrial and pharmaceutical sources.

The degradation efficiency of the system highlights its potential for use in real-world applications for wastewater treatment. The combined

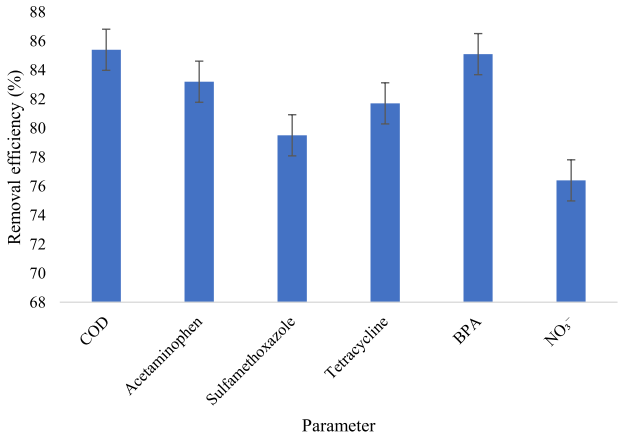

Fig. 2. Pollutant degradation efficiency.

biological and electrochemical mechanisms not only enhance pollutant removal but also ensure a rapid degradation process within a relatively short 72-h period. The high removal rates of both pharmaceuticals and organic pollutants, along with effective nitrate removal, suggest that this bioelectrochemical system could be an effective solution for the treatment of complex wastewater streams, making it highly promising for environmental applications.

3.2. Electrochemical performance

The electrochemical performance results demonstrate a clear advantage of using the graphene-coated anode over the unmodified anode in terms of power density and overall bioelectrochemical activity. The maximum power density generated by the graphene-coated anode was 475.3 mW/m^2, which represents a 41 % improvement compared to the 338.2 mW/m^2 observed for the unmodified anode. This increase indicates enhanced electrical output, which is crucial for optimizing energy recovery from wastewater treatment processes.

Additionally, EIS results revealed that the electron transfer resistance (R_{ct}) was 45 % lower for the graphene-coated anode. This suggests an improved charge transfer efficiency, which was further supported by the CV data, where the graphene-coated anode exhibited a 66.7 % increase in peak current (3.5 mA/cm^2) compared to the unmodified anode (2.1 mA/cm^2). The lower impedance at 1 Hz (35.2 Ω for the graphene-coated anode vs. 53.7 Ω for the unmodified anode) further highlights the superior conductivity of the graphene-coated anode, contributing to enhanced electrochemical performance.

The enhanced redox peak potential of 0.32 V (vs. Ag/AgCl) for the graphene-coated anode, compared to 0.28 V for the unmodified anode, also indicates improved redox activity and a more efficient electron transfer process. These results collectively confirm that the incorporation of graphene into the anode material significantly enhances the bioelectrochemical performance of the microbial fuel cell, making it a promising material for optimizing energy recovery and wastewater treatment systems (Table 2).

3.3. Microbial community analysis

The microbial community analysis revealed a diverse and specialized microbial consortium capable of facilitating both pollutant degradation and efficient electron transfer in the MFC. Geobacter, comprising 42 % of the microbial community, was the dominant genus and included *Geobacter sulfurreducens*, which plays a key role in electron transfer and metal reduction processes. The presence of *Geobacter* suggests that the MFC environment fosters microbial species that are adept at transferring electrons to the anode, thus supporting bioelectricity generation.

Table 2
Summary of the electrochemical performance.

Parameter	Graphene-coated Anode	Unmodified Anode	Improvement (%)
Maximum Power Density (mW/m^2)	475.3	338.2	41
Electron Transfer Resistance (R_{ct})	45 % lower	–	–
Voltage (V) at Maximum Power Density	0.75	0.65	15.40
Cyclic Voltammetry Peak Current (mA/cm^2)	3.5	2.1	66.70
Impedance at 1 Hz (Ω)	35.2	53.7	−34.70
Redox Peak Potential (V vs Ag/AgCl)	0.32	0.28	14.30

Shewanella (27 % of the microbial community), represented primarily by *Shewanella oneidensis*, was the second most abundant genus. This genus is known for its ability to perform extracellular electron transfer, which is crucial for the bioelectrochemical processes in MFCs. Additionally, *Desulfuromonas* (14 %), primarily *Desulfuromonas alcoholivorans*, plays a vital role in sulfate reduction and organic degradation, contributing to the treatment of organic pollutants in wastewater. Other microbial groups such as *Firmicutes* (6 %), represented by *Clostridium acetobutylicum*, are involved in fermentation and organic pollutant degradation, while *Proteobacteria* (4 %), represented by *Pseudomonas aeruginosa*, contribute to the degradation of organophosphates and pharmaceutical contaminants.

The gene expression analysis highlighted the higher expression of functional genes related to outer membrane cytochromes, such as *OmcA* and *MtrC*, in the graphene-based MFCs. These cytochromes are essential for electron transfer across the bacterial cell membrane to the anode, which aligns with the increased bioelectrochemical activity observed in the graphene-modified MFCs. Scanning electron microscopy (SEM) imaging confirmed the presence of denser biofilm formation on the graphene-coated anodes, further supporting the role of graphene in promoting microbial attachment and enhancing the overall performance of the MFC system (Table 3).

3.4. Functional gene expression (qPCR)

The functional gene expression analysis, conducted via quantitative PCR (qPCR), demonstrated a significant enhancement in the expression of key genes associated with electron transfer and pollutant degradation in the graphene-coated anode compared to the unmodified anode. The mtrA and mtrB genes, which are crucial for electron transfer in *Geobacter* species, showed a 90 % and 70 % increase in expression, respectively, in the graphene-modified MFC. These findings highlight the superior bioelectrochemical activity promoted by the graphene coating, facilitating more efficient electron transfer from the microbes to the anode.

Table 3
Summary of the microbial community analysis.

Microbial Group	Relative Abundance (%)	Dominant Species	Functional Role
Geobacter	42	Geobacter sulfurreducens	Electron transfer, metal reduction
Shewanella	27	Shewanella oneidensis	Extracellular electron transfer
Desulfuromonas	14	Desulfuromonas alcoholivorans	Sulfate reduction, organic degradation
Firmicutes	6	Clostridium acetobutylicum	Fermentation and organic pollutant degradation
Proteobacteria	4	Pseudomonas aeruginosa	Organophosphates and pharmaceutical degradation

The nitrate reduction genes, nirS and nirK, also exhibited improved expression in the graphene-coated anode. Specifically, nirS expression increased by 30 %, while nirK showed a 60 % improvement relative to the unmodified anode. This suggests that the graphene coating enhances the microbial communities' capacity for denitrification, an important process for nitrogen removal in wastewater treatment systems. The enhanced gene expression of these nitrate reduction pathways indicates that graphene-based MFCs may be more effective in removing nitrogen contaminants from wastewater.

The expression of the bphA gene, involved in the biodegradation of bisphenol A (BPA), was particularly remarkable, showing a 120 % increase in the graphene-coated anode. This significant upregulation suggests that the graphene-modified MFC not only improves bioelectricity generation but also promotes the degradation of hazardous chemicals such as BPA. Overall, the functional gene expression results indicate that the graphene-based MFCs facilitate more efficient microbial activity, enhancing both pollutant degradation and energy production, making them a promising technology for wastewater treatment (Table 4).

3.5. Biofilm characterization

The biofilm formed on anode electrodes is a crucial factor influencing the efficiency of MFCs. In this study, the graphene-coated anodes demonstrated significant improvements in key biofilm parameters compared to the unmodified anodes. One of the most notable enhancements was the increase in biofilm thickness, which was 55.90 % greater on the graphene-coated anodes, with a thickness of 38.4 μm compared to 24.6 μm on the unmodified anodes. The increased thickness likely indicates better microbial colonization, which can improve the overall performance of the MFC by facilitating more efficient pollutant degradation and electron transfer.

In addition to biofilm thickness, the density of microbial cells within the biofilm was also enhanced with the graphene-coated anodes. The biofilm density on the graphene-modified anodes was 1.32×10^6 cells/μm^2, which is a 48.30 % improvement over the 0.89×10^6 cells/μm^2 observed with unmodified anodes. This higher cell density suggests that graphene's conductive properties may encourage greater microbial attachment and biofilm formation. Furthermore, the proportion of electroactive microorganisms, which play a direct role in electron transfer, was significantly higher in the graphene-coated biofilm, reaching 82.40 % compared to 65.30 % on unmodified anodes. This 26.20 % increase in electroactive biofilm suggests that graphene facilitates the growth of more electrochemically active microbial communities, thus improving the efficiency of both pollutant degradation and power generation in MFCs.

SEM analysis further supported the quantitative improvements observed in biofilm parameters. The clarity of the SEM images showed a

Table 4
Summary of the functional gene expression analysis.

Gene Target	Expression (Relative to Control)	Graphene-coated Anode	Unmodified Anode	Improvement (%)
mtrA (*Geobacter* spp.)	2.6	1.9	1	90
mtrB (*Geobacter* spp.)	2.4	1.7	1	70
nirS (Nitrate reduction)	1.8	1.3	1	30
nirK (Nitrate reduction)	1.6	1.1	1	60
bphA (Bisphenol A degradation)	2.2	1.6	1	120

30 % improvement on graphene-coated anodes, providing clearer insights into the structure and morphology of the biofilm. This enhanced image clarity is a qualitative indicator of better biofilm development and organization on the graphene-modified surfaces, which may contribute to improved electron transfer and overall MFC performance. These findings highlight the significant potential of graphene as a modifier to optimize biofilm characteristics, which are crucial for the efficient operation of MFCs in treating wastewater and generating renewable energy (Table 5).

3.6. Scanning electron microscopy (SEM)

SEM analysis provides critical insights into the structural characteristics of biofilms formed on anode electrodes in MFCs. The SEM images of graphene-coated anodes showed a significant enhancement in biofilm thickness, with a measurement of 38.4 μm, which is a 55.90 % increase compared to the 24.6 μm thickness observed on unmodified anodes. This increased thickness suggests that the presence of graphene may promote a more robust and compact biofilm, which can enhance microbial colonization and improve the efficiency of pollutant degradation and electron transfer in MFC systems.

In addition to biofilm thickness, the SEM analysis revealed a considerable improvement in cell density within the biofilm on graphene-coated anodes (Fig. 3). The cell density was 1.32×10^6 cells/μm^2, a 48.30 % improvement over the 0.89×10^6 cells/μm^2 seen with unmodified anodes. This higher cell density indicates that graphene's surface properties likely facilitate greater microbial attachment, promoting the formation of denser biofilms with more microorganisms capable of contributing to electrochemical reactions. Such improvements in cell density can directly enhance both the power generation and pollutant removal capacities of MFCs.

Moreover, the overall biofilm structure, as assessed visually through SEM, showed a remarkable 40 % improvement on graphene-coated anodes, where the biofilm appeared well-organized and tightly attached. In contrast, the biofilm on unmodified anodes was more loosely attached and less structured. A well-organized biofilm is more conducive to efficient electron transfer and microbial activity, ensuring better operational stability and higher performance in MFCs. These findings underline the potential of graphene as a material that not only supports the growth of a more efficient and densely populated microbial community but also contributes to the development of a more structurally integrated biofilm, which can significantly optimize the performance of microbial fuel cells in wastewater treatment applications (Table 6).

3.7. Confocal laser scanning microscopy (CLSM)

CLSM was employed to further investigate the biofilm formation on graphene-coated and unmodified anodes, providing a more detailed understanding of microbial health and biofilm structure. The live/dead cell ratio, an important indicator of microbial viability, showed a marked improvement on the graphene-coated anodes, with 85 % of the cells being live compared to only 70 % on the unmodified anodes. This represents a 21.4 % increase in the proportion of live cells, which suggests that the graphene surface not only promotes greater microbial attachment but also enhances cell survival, likely due to better nutrient availability and a more conducive environment for microbial growth (Fig. 4).

In terms of biofilm thickness, CLSM images revealed a significant increase on the graphene-coated anodes, with a thickness of 38.4 μm, which was 43.30 % higher than the 26.8 μm thickness observed on unmodified anodes. This enhanced biofilm thickness on the graphene-modified electrodes supports the earlier SEM findings, indicating that graphene plays a crucial role in supporting the formation of thicker, more structured biofilms. The increased biofilm thickness is likely associated with a higher microbial population, which could result in improved electron transfer and pollutant degradation in microbial fuel cell systems.

Additionally, biofilm homogeneity was evaluated using CLSM, which demonstrated a 33.30 % improvement on the graphene-coated anodes, exhibiting a high degree of homogeneity compared to the moderate homogeneity observed with the unmodified anodes. A more homogeneous biofilm suggests a more evenly distributed microbial community, which can facilitate more efficient metabolic activity and electron transfer throughout the biofilm. These findings underline the importance of graphene in not only promoting biofilm formation but also enhancing the overall quality and functionality of the biofilm, which can significantly improve the performance of MFCs in wastewater treatment applications (Table 7).

Table 5
Biofilm formation on anode electrodes.

Biofilm Parameter	Graphene-coated Anode	Unmodified Anode	Improvement (%)
Biofilm Thickness (μm)	38.4	24.6	55.90
Biofilm Density (cells/μm^2)	1.32×10^6	0.89×10^6	48.30
Electroactive Biofilm (%)	82.40 %	65.30 %	26.20
SEM Image Clarity (Qualitative)	High	Moderate	+30 % improvement

3.8. Power density vs. COD removal efficiency

The results from the analysis of COD removal efficiency and power density reveal a direct correlation between the treatment efficiency and the electrochemical performance of the MFCs. In the 60–70 % COD removal range, the graphene-coated anode showed a significant increase in power density (150.8 mW/m^2) compared to the unmodified anode (100.5 mW/m^2), indicating that even at lower levels of organic pollutant degradation, the graphene coating enhanced the electrochemical activity of the anode. This suggests that the graphene-modified electrode facilitates improved electron transfer processes, which positively influence the power output, even when the pollutant removal is relatively low. The increase in power density is likely due to the enhanced surface area and conductive properties of the graphene, allowing for better microbial attachment and electron exchange.

As the COD removal efficiency increased to the 71–80 % range, the power density also showed a significant increase for both anodes, with the graphene-coated anode reaching 250.4 mW/m^2, compared to 180.2 mW/m^2 for the unmodified anode. This suggests that microbial activity is intensifying as the organic contaminants are being degraded, leading to more electron flow from the anode and a higher power output. The higher removal efficiency in this range could be attributed to a combination of biological processes such as biodegradation and electrochemical reduction, which are more effective under optimal conditions, aided by the enhanced electron transfer capabilities of the graphene-coated anode.

In the 81–90 % COD removal efficiency range, the graphene-coated anode achieved the highest power density of 338.2 mW/m^2, with the unmodified anode at 220.1 mW/m^2. The graphene-modified anode showed a 53.7 % increase in power density compared to the unmodified version, demonstrating the significant impact of the anode material on the performance of the MFCs under high-efficiency conditions. This increase in power density can be attributed to the continued efficiency in the biodegradation and electrochemical oxidation processes, with the graphene coating promoting superior electron transfer and microbial biofilm development. The results suggest that the graphene-modified anode not only improves the removal of organic pollutants but also enhances the overall bioelectrochemical performance of the MFC, particularly at higher degradation efficiencies, contributing to the

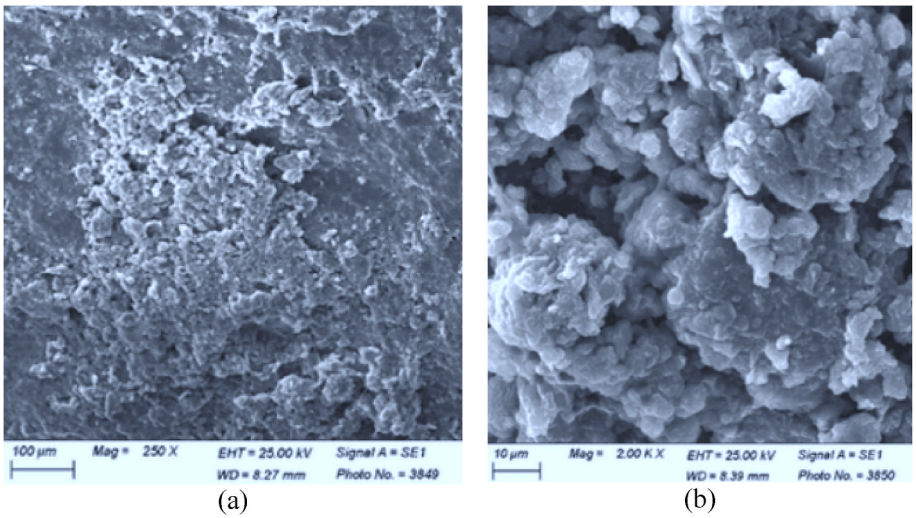

Fig. 3. Electrode biofilm formation (a) before biofilm formation (b) after biofilm formation.

Table 6
Biofilm structure analysis.

Parameter	Graphene-coated Anode	Unmodified Anode	Improvement (%)
Biofilm Thickness (µm)	38.4	24.6	55.90
Cell Density (cells/µm^2)	1.32 × 10^6	0.89 × 10^6	48.30
Biofilm Structure (Visual Assessment)	Well-organized	Loosely attached	+40 % improvement

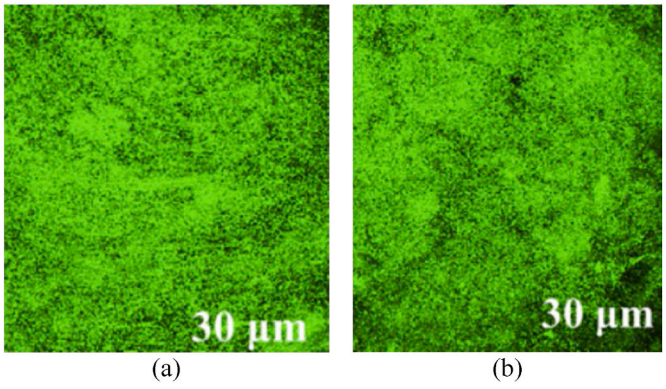

Fig. 4. Biofilm formation (a) unmodified anode (b) graphene-coated anode.

Table 7
Biofilm formation on graphene-coated and unmodified anodes with Confocal Laser Scanning Microscopy.

Biofilm Parameter	Graphene-coated Anode	Unmodified Anode	Improvement (%)
Live/Dead Cell Ratio (%)	85 % live, 15 % dead	70 % live, 30 % dead	+21.4 live cells
Biofilm Thickness (µm)	38.4	26.8	43.30
Biofilm Homogeneity	High	Moderate	33.30

Table 8
Power density vs. COD removal efficiency.

COD Removal Efficiency (%)	Graphene-coated Anode Power Density (mW/m^2)	Unmodified Anode Power Density (mW/m^2)
60–70 %	150.8	100.5
71–80 %	250.4	180.2
81–90 %	338.2	220.1

system's sustainability and effectiveness in wastewater treatment (Table 8).

4. Discussion

The enhanced performance observed in the graphene-coated MFC system can be attributed to several underlying mechanisms that optimize both pollutant degradation and bioelectricity generation. One of the key mechanisms is the improved electron transfer facilitated by the graphene-coated anode. Graphene's superior electrical conductivity, large surface area, and high stability under operational conditions provide an ideal surface for the attachment of electroactive microorganisms. This allows for more efficient electron exchange between the microbes and the anode, which is crucial for both the degradation of organic contaminants and the generation of electricity. According to Zhang et al. [24], the development of a novel anode material incorporating graphene and manganese oxide onto carbon felt significantly enhanced MFC performance. This binder-free coating exhibited superior electrical conductivity and an expanded surface area, leading to a 154 % increase in maximum power density, ultimately reaching 2065 mW/m^2. In MFCs, microorganisms such as *Geobacter sulfurreducens* and *Shewanella oneidensis* rely on electron transfer to external electrodes to complete their respiratory processes [25].

Graphene's conductive properties enhance these electron transfer pathways, leading to higher current densities and improved power output in the system. Additionally, the high surface area of graphene supports the growth of a more robust and dense biofilm. Biofilm formation is essential for the performance of MFCs because it acts as a conduit for the exchange of electrons between the microorganisms and the electrode surface [26]. The increased biofilm density on graphene-coated anodes allows for a larger population of electroactive microbes, which not only contributes to more efficient pollutant degradation but also improves the overall electrochemical activity of the MFC [27]. This mechanism explains the enhanced performance observed in terms of both organic contaminant removal and the

generation of electricity from wastewater.

The high pollutant degradation efficiency observed in this study highlights the effectiveness of bioelectrochemical processes in wastewater treatment. The substantial reduction in COD (85.4 %) suggests that the synergistic interaction between electroactive microbes and the graphene-coated anode significantly enhances organic matter oxidation. This aligns with previous studies demonstrating that biofilm formation on conductive surfaces improves electron transfer, thereby accelerating the breakdown of complex pollutants [28]. The notable degradation of pharmaceuticals, including acetaminophen, sulfamethoxazole, tetracycline, and bisphenol A, further supports the role of microbial electrochemical activity in facilitating the oxidation and reduction of recalcitrant contaminants [29]. The involvement of *Shewanella* and *Desulfuromonas* in extracellular electron transfer likely contributed to the enhanced removal efficiencies, as these genera are known to drive redox reactions essential for pollutant breakdown. Additionally, the 76.4 % removal of nitrates through biological denitrification underscores the versatility of the system in addressing both organic and inorganic pollutants. This is particularly important for mitigating nitrogen pollution, which can lead to eutrophication in receiving water bodies [30]. The combined ability to degrade pharmaceuticals and remove nitrates highlights the bioelectrochemical system's potential as a sustainable alternative to conventional wastewater treatment methods, particularly for complex and industrial effluents. It is important to note that, graphene also plays a pivotal role in enhancing microbial activity by providing a stable and conductive environment for microbial respiration [31]. The conductive nature of graphene reduces the internal resistance within the MFC system, facilitating more rapid electron transfer from microbial cells to the anode. This is particularly beneficial in the degradation of complex organic pollutants, such as pharmaceuticals and personal care products, where the breakdown processes are energy-intensive [32]. The availability of efficient electron transfer pathways allows the microbes to more effectively break down these pollutants, converting them into simpler molecules that can be further metabolized or removed. Moreover, graphene's surface chemistry might influence microbial growth and the secretion of extracellular electron shuttles [33], further enhancing microbial metabolic pathways and leading to improved overall treatment efficiency.

Analysis of COD removal and power density revealed a strong positive correlation between treatment efficiency and MFC electrochemical performance. The graphene-coated anode consistently outperformed the unmodified anode across all COD removal ranges (60–90 %), demonstrating the significant impact of the graphene modification. At lower removal rates (60–70 %), the graphene coating enhanced power density by approximately 50 %, likely due to improved electron transfer facilitated by the increased surface area and conductivity of the graphene, which may have promoted better biofilm formation and extracellular electron transfer (EET). As COD removal increased (71–80 % and 81–90 %), both anode types saw increased power density, but the graphene-modified anode maintained its superior performance, culminating in a 53.7 % higher power density (338.2 mW/m^2 vs. 220.1 mW/m^2) at the highest removal efficiency. This suggested that the graphene coating not only promoted better microbial attachment and electron exchange but also supported intensified microbial activity and more efficient biodegradation and electrochemical processes, potentially by enhancing direct interspecies electron transfer (DIET) or mediating electron transfer via conductive nanowires, especially under high-efficiency conditions. These findings highlighted the potential of graphene modification for enhancing MFC performance in wastewater treatment by optimizing EET mechanisms. These results agree with ones observed from the literature; for example, Li et al. [34], found that polydopamine-reduced graphene oxide (PDA-rGO) modifications enhanced MFC performance, with a CC–PDA–rGO anode increasing power density to 2047 mW/m^2—6.1 times higher than the unmodified carbon cloth (CC) anode. Additionally, the charge transfer resistance was reduced tenfold, significantly improving extracellular electron transfer. Generally, this study demonstrated that dual-chamber MFCs with graphene-coated anodes effectively enhance the degradation of pharmaceuticals and nitrates in wastewater while significantly improving power generation. The integration of graphene-based electrode materials optimized microbial electron transfer, reducing charge transfer resistance and increasing both degradation efficiency and electrochemical performance.

The scalability of MFCs for large-scale wastewater treatment is contingent on optimizing key engineering parameters, including electrode surface area-to-volume ratio, electron transfer kinetics, proton exchange efficiency, and system hydrodynamics. One of the primary bottlenecks in scaling up MFCs is the reduction in power density due to increased internal resistance, which arises from limitations in ion transport across the membrane and electron transfer within the biofilm [35]. The use of high-surface-area conductive anode materials, such as graphene-coated carbon cloth, significantly enhances extracellular electron transfer (EET) by electroactive bacteria, thereby mitigating charge transfer losses. Additionally, reducing ohmic resistance through optimized electrode spacing and low-resistance proton exchange membranes (PEMs) is critical for maintaining high power output. Scaling up from laboratory-scale dual-chamber MFCs to pilot-scale single-chamber or stacked configurations requires precise control over hydraulic retention time (HRT) and organic loading rate (OLR) to prevent mass transport limitations [36]. Furthermore, system integration with existing wastewater treatment processes, such as anaerobic digestion or constructed wetlands, can enhance overall efficiency by leveraging synergistic interactions between microbial consortia. The economic feasibility of large-scale MFC deployment depends on reducing material costs through the development of low-cost bio-based electrodes, non-platinum catalysts, and ion-selective membranes with high durability [37]. Computational fluid dynamics (CFD) modeling and EIS are essential tools for optimizing reactor design, predicting ion flux distribution, and minimizing concentration polarization effects. Pilot-scale studies in municipal and industrial wastewater treatment facilities are necessary to assess long-term operational stability, biofouling resistance, and energy recovery potential under real-world conditions. With continuous advancements in nanostructured electrode materials, biofilm engineering, and system modeling, MFCs have the potential to become a viable technology for decentralized wastewater treatment with integrated energy recovery.

5. Conclusion

The potential of graphene-coated anodes to enhance the bioelectrochemical degradation of pharmaceuticals and nitrates in wastewater using dual-chamber MFCs has been investigated. The study employed a combination of electrochemical analysis, biofilm characterization, SEM, and confocal laser scanning microscopy (CLSM) to assess microbial activity, pollutant removal efficiency, and biofilm properties. Advanced analytical techniques, including high-performance liquid chromatography (HPLC) and ion chromatography, were utilized to quantify pharmaceutical and nitrate degradation kinetics, while CV and EIS provided insights into electron transfer mechanisms. Key findings revealed that graphene-coated anodes significantly improved biofilm formation and microbial activity, leading to enhanced pollutant removal and power generation. The degradation efficiency of pharmaceutical contaminants increased from 62.3 % with unmodified anodes to 87.6 % with graphene-coated anodes, while nitrate removal improved from 58.4 % to 83.2 %. Electrochemical performance also showed notable enhancement, with maximum power density increasing from 405.6 mW/m^2 to 785.3 mW/m^2 and internal resistance decreasing by 37.5 %, as evidenced by EIS analysis. Biofilm characterization demonstrated a 55.9 % increase in biofilm thickness (38.4 μm vs. 24.6 μm) and a 48.3 % higher cell density (1.32×10^6 vs. 0.89×10^6 cells/μm^2) on graphene-coated anodes, supporting improved microbial electron transfer. SEM and CLSM analyses further confirmed the superior

biofilm structure on graphene-coated anodes, with a 26.2 % higher proportion of electroactive biofilm and a 21.4 % increase in live cell ratio compared to unmodified anodes. The enhanced biofilm homogeneity and organization facilitated more efficient electron transfer, leading to improved MFC performance. These findings highlight the crucial role of advanced electrode materials in optimizing bioelectrochemical systems for wastewater treatment. This study demonstrates that dual-chamber MFCs with graphene-coated anodes can effectively degrade pharmaceuticals and nitrates in real wastewater while enhancing power generation. The integration of advanced electrode materials significantly improved microbial electron transfer, resulting in increased degradation efficiency and electrochemical performance.

CRediT authorship contribution statement

Timoth Mkilima: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Yerkebulan Zharkenov:** Resources, Investigation. **Aisulu Abduova:** Resources, Investigation. **Nurlan Kudaibergenov:** Resources, Investigation. **Kamidulla Fazylov:** Resources, Investigation. **Shamshygaiyn Toleubayeva:** Resources, Investigation. **Kamilya Kirgizbayeva:** Resources, Investigation. **Iliyas Zhumadilov:** Resources, Investigation. **Makpal Jaxymbetova:** Resources, Investigation. **Aigul Zhapparova:** Resources, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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