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Performance of a multi-chamber microbial fuel cell with biochar anode for industrial wastewater treatment and energy recovery

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ABSTRACT

Microbial fuel cells (MFCs) offer a sustainable method for industrial wastewater treatment by simultaneously degrading pollutants and generating electricity. This study evaluates the performance of a multi-chamber MFC incorporating a biochar-based anode and a MnO_2-coated cathode for improved electron transfer and pollutant removal. The system was operated in continuous flow mode for 90 days using industrial wastewater containing chemical oxygen demand (COD) of 1200 mg/L, Cr(VI) of 45 mg/L, Pb^{2+} of 20 mg/L, and Cd^{2+} of 15 mg/L. The biochar anode, produced from coconut shells pyrolyzed at 800 °C, provided enhanced microbial attachment and electron transfer, leading to a COD removal efficiency of 92.3%, Cr(VI) reduction of 85.7%, and a maximum power density of 1.72 W/m². Biofilm analysis using 16S rRNA sequencing identified *Geobacter sulfurreducens* and *Shewanella oneidensis* as dominant electroactive bacteria. These results demonstrate the effectiveness of biochar-based MFCs for industrial wastewater treatment with simultaneous energy recovery.

Keywords: microbial fuel cell, biochar anode, industrial wastewater, heavy metal removal, bioelectricity generation.

INTRODUCTION

Industrial wastewater is a significant source of environmental pollution, often containing high concentrations of organic pollutants and toxic heavy metals that threaten ecosystems and public health. Traditional wastewater treatment methods, such as activated sludge, chemical coagulation, and membrane filtration, are widely used but come with inherent limitations, including high energy consumption, sludge generation, and operational complexity (Mkilima et al., 2025; Shamshad and Ur Rehman, 2025). To be more specific, traditional methods of treating wastewater incur significant costs, equivalent to approximately 3% of global electricity demand (Saba et al., 2017; Ye et al., 2019). Among these costs, effluent disposal, specifically sludge disposal, constitutes half of the total expenditure (Saba et al., 2017; Ye et al., 2019). Additionally, conventional wastewater treatment methods contribute to environmental issues such as greenhouse gas emissions and the release of harmful substances like phosphates and ammonia (Ramírez-Melgarejo and Stringer, 2024)(Anazonwu and Fahmi, 2024). Microbial fuel cells offer a promising solution to these challenges. They play a critical role in biodegrading organic matter within wastewater, thereby reducing chemical oxygen demand (COD) and promoting environmental sustainability (Mkilima et al., 2025). Moreover, microbial fuel cells significantly decrease energy consumption and costs associated with effluent disposal. By leveraging electroactive bacteria, MFCs can degrade organic matter while generating electricity, offering a dual benefit that aligns with global sustainability goals (Apollon et al., 2025).

Microbial fuel cell (MFC) technology has garnered significant interest due to its capacity to directly convert chemical energy into electrical energy through microbial extracellular electron transfer (EET) pathways. The fundamental

components of MFCs include an anode, where electroactive bacteria oxidize organic substrates and release electrons (Fadzli et al., 2021), and a cathode, where these electrons combine with protons and a terminal electron acceptor (Flimban et al., 2019), typically molecular oxygen or alternative oxidizing agents such as ferricyanide or nitrate, to complete the electrochemical circuit (Ali et al., 2025). While single-chamber MFCs have been extensively studied due to their compact architecture and ease of operation, they often exhibit limitations, including oxygen diffusion from the cathode to the anode, leading to electron acceptor competition and decreased coulombic efficiency (Mkilima et al., 2025). Additionally, incomplete separation of oxidation and reduction reactions in single-chamber systems results in suboptimal energy recovery and reduced contaminant degradation efficiency. In contrast, multi-chamber MFCs mitigate these challenges by providing distinct anodic and cathodic compartments, thereby enhancing redox potential differentials, optimizing electron transfer kinetics, and improving both wastewater treatment efficacy and power generation (Bhaduri and Behera, 2024).

Electrode materials play a crucial role in MFC performance, particularly in optimizing electron transfer and biofilm formation. Traditional anodes made from graphite or carbon cloth provide moderate conductivity but are expensive and prone to biofouling. Biochar, a carbon-rich material derived from biomass pyrolysis, has recently gained interest as a cost-effective and sustainable alternative due to its high surface area, porosity, and biocompatibility (Ali et al., 2022; Bhattacharya et al., 2024; Mulabagal et al., 2024). The use of biochar anodes can enhance bacterial attachment, improve electron transfer, and reduce overall system costs. However, the long-term stability of biochar anodes under industrial wastewater conditions and their potential for heavy metal adsorption remain insufficiently studied. Similarly, the cathode material significantly impacts the overall efficiency of the MFC system. Manganese dioxide (MnO_2)-coated cathodes have demonstrated significant potential in enhancing oxygen reduction reaction (ORR) kinetics due to their high electrochemical catalytic activity, multiple oxidation states facilitating efficient electron transfer, and strong redox cycling capabilities (Vemuri et al., 2022). Compared to platinum-based catalysts, MnO_2 presents a cost-effective and sustainable alternative, demonstrating enhanced durability and resistance to catalyst poisoning. The study by Rodríguez et al. (Rodríguez et al., 2021), highlights the significant degradation of Pt-based cathodes, with nearly 50% deactivation within the first month, whereas alternative catalysts such as CuO-carbon mesh exhibited lower degradation rates over six months. Similarly, MnO_2 offers superior long-term stability and economic feasibility, making it a promising candidate for scalable MFC applications by addressing both cost and performance limitations of conventional Pt cathodes. Nevertheless, their long-term performance and synergy with biochar anodes require further investigation.

Despite significant advancements, the integration of multi-chamber MFCs with biochar anodes and MnO_2-coated cathodes for industrial wastewater treatment remains an unexplored area. This study investigates the feasibility of a multi-chamber MFC with biochar anodes and MnO_2-coated cathodes for treating industrial wastewater. Specifically, the study assesses COD removal efficiency, heavy metal reduction (Cr(VI), Pb^{2+}, Cd^{2+}), and power generation over a 90-day operational period. Additionally, the microbial community structure associated with biofilm formation on biochar anodes will be analyzed to understand its contribution to electron transfer and overall system stability. By addressing existing research gaps, this study aims to provide a sustainable and scalable bioelectrochemical approach for industrial wastewater treatment.

MATERIALS AND METHODS

MFC reactor design

The multi-chamber MFC was designed to optimize pollutant removal and power generation through spatial separation of redox reactions (Figure 1). The system comprised three distinct compartments: anode, middle, and cathode chambers, each with a working volume of 1.2 L. These compartments were separated by Nafion 117 proton exchange membranes (PEMs), which facilitated selective proton transfer while preventing oxygen crossover. The anode chamber housed a biochar-based electrode to enhance microbial adhesion and electron transfer, while the cathode chamber contained a manganese dioxide (MnO_2)-coated electrode to improve oxygen reduction efficiency. Titanium wire current collectors were employed

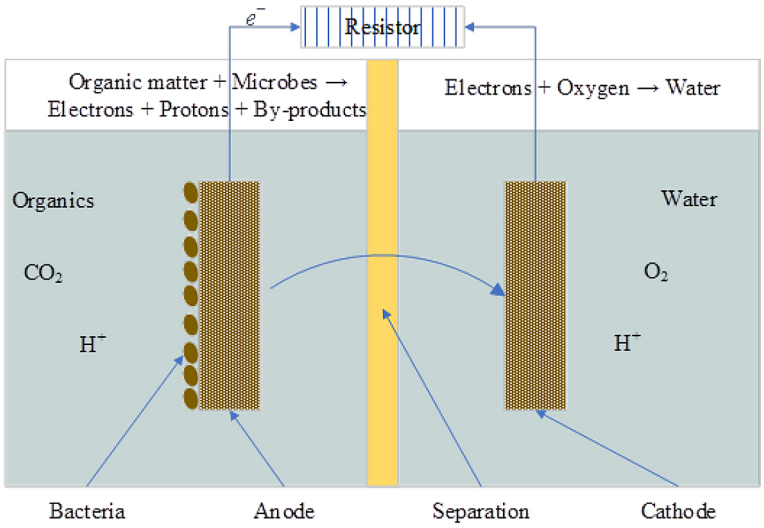

Figure 1. Double chamber MFC setup

to establish electrical connectivity between electrodes, ensuring minimal resistance and efficient electron flow. The reactor was constructed using non-corrosive acrylic material to provide durability and maintain structural integrity throughout the 90-day operational period. This design was selected to maximize electrochemical performance while addressing common limitations associated with single-chamber MFCs, such as low power output and biofouling.

Electrode preparation

The electrodes were designed to enhance electron transfer efficiency and overall microbial fuel cell (MFC) performance. The anode was fabricated using biochar derived from coconut shells, which were subjected to pyrolysis at 800 °C under a nitrogen atmosphere. This process yielded a material with a high surface area of approximately 1238 m^2/g, as determined by Brunauer–Emmett–Teller (BET) analysis. The biochar anode was further activated using 1 M KOH at 80 °C for 12 hours, followed by thorough washing with deionized water and drying at 100 °C. This high surface area facilitated greater microbial adhesion and electron transfer, crucial for efficient bioelectrochemical reactions. The cathode was composed of carbon felt coated with manganese dioxide (MnO_2) to enhance oxygen reduction reactions (ORR). MnO_2 was deposited via a dip-coating method, where the carbon felt was immersed in a 0.05 M $KMnO_4$ solution containing 0.02 M $MnSO_4$ under constant stirring at 40 °C for 2 hours. The coated material was then dried at 80 °C and subjected to annealing at 250 °C for 3 hours to enhance adhesion and catalytic activity. To minimize internal resistance and maximize conductivity, titanium mesh was employed as the current collector and coated with graphene oxide (GO) via an electrochemical deposition process. The titanium mesh was first cleaned using a sequential acid treatment (1 M HCl, 10 minutes) to remove surface oxides and rinsed with deionized water. The GO deposition was performed in a 1mg/mL GO suspension in 0.1 M Na_2SO_4 electrolyte under a constant potential of -1.2 V vs. Ag/AgCl for 30 minutes. The coated mesh was then dried at 60 °C for 12 hours. The GO coating significantly improved electron mobility, ensuring efficient charge transport between the anode and cathode.

Experimental setup and measurements

Operation mode and duration

The multi-chamber MFC was operated in a continuous flow mode with an influent flow rate of 0.5 mL/min, carefully selected to maintain a stable microbial environment and ensure consistent electrochemical performance throughout the system. This flow rate was optimized to achieve a balance between microbial activity, nutrient supply, and waste removal, thus fostering efficient biofilm development on the biochar anode. The reactor was monitored over a 90-day operational period, providing an extended timeframe

for microbial adaptation and biofilm maturation, which is crucial for optimizing power generation and enhancing pollutant removal efficiencies. During this period, biofilm growth on the biochar electrode was continuously assessed, which allowed for in-depth analysis of microbial attachment, biofilm thickness, and conductivity. Effluent samples were collected at regular intervals to evaluate organic degradation and heavy metal reduction through measurements of COD and specific heavy metals such as Pb^{2+}, Cd^{2+}, and Cr(VI). These samples were analyzed for microbial community composition via 16S rRNA gene sequencing to identify the dominant electroactive bacteria, such as *Geobacter sulfurreducens*, that facilitated the reduction processes. Additionally, the removal efficiency of various contaminants was tracked, and voltage generation was recorded to monitor the electrochemical performance and stability of the MFC system over time.

Electrochemical analysis

The electrochemical performance of the MFC was evaluated using a Gamry Reference 600 potentiostat. The open-circuit voltage (OCV) was continuously monitored to assess system stability. Power density curves were derived from polarization tests using linear sweep voltammetry (LSV), conducted over a potential range of 0–1 V at a scan rate of 5 mV/s. The internal resistance of the system was estimated from the slope of the polarization curve, providing insights into electron transfer efficiency and system performance. The study was conducted at an external resistance of 500 Ω, a commonly used value in microbial fuel cell research to balance power generation and system stability.

Wastewater quality analysis

The pollutant removal efficiency of the MFC was rigorously assessed by analyzing wastewater samples before and after treatment to quantify reductions in organic matter and heavy metal concentrations. COD, an important indicator of organic pollution, was measured using the dichromate method (Standard Method 5220D). Absorbance at 600 nm was recorded using a Hach DR6000 spectrophotometer, ensuring precise determination of organic matter content. For heavy metal analysis, the concentrations of Cr(VI), Pb^{2+}, and Cd^{2+} were quantified using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900). Prior to analysis, the samples were subjected to acid digestion with a HNO_3 and H_2O_2 mixture (3:1 v/v) at 180 °C for 30 minutes, ensuring complete extraction of metals from the effluent. This comprehensive approach provided reliable data on the MFC's efficiency in removing both organic pollutants and toxic heavy metals, crucial for evaluating its potential as a sustainable wastewater treatment technology.

Biofilm characterization

To explore the microbial dynamics and biofilm formation on the biochar anode, both surface morphology and microbial community composition were examined using advanced imaging and molecular techniques. Surface Morphology was analyzed via scanning electron microscopy (SEM, JEOL JSM-7600F). Biofilm samples were first fixed in 2.5% glutaraldehyde and then subjected to a dehydration process through an ethanol gradient, preserving the natural structure and facilitating detailed visualization of microbial growth on the electrode surface. The resulting SEM images provided insight into the biofilm architecture, thickness, and microbial attachment to the biochar surface. Microbial Community Composition was characterized by extracting total DNA from the biofilm, which was carefully scraped from the biochar anode. DNA extraction was performed using the Qiagen DNeasy PowerBiofilm Kit, ensuring high-quality and uncontaminated genetic material for downstream analysis. The 16S rRNA gene (V3-V4 region) was amplified using specific primers, and sequencing was conducted on the Illumina MiSeq platform. Taxonomic classification of the sequencing data was performed using the SILVA database, allowing for a detailed profile of the microbial community present in the biofilm. This comprehensive characterization provided valuable insights into the microbial diversity and functionality of the biofilm, critical to understanding the role of specific bacteria in electrochemical performance and pollutant degradation within the MFC. Biofilm samples for microbial community analysis were collected after 30 days of operation, ensuring the establishment of a stable anodic biofilm. A total of five independent biofilm samples were analyzed to ensure statistical reliability, as reflected in the standard deviations presented in Graph 2. The 16S rRNA gene (V3-V4 region) was amplified using the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′).

RESULTS

Wastewater characteristics and inoculum

The industrial effluent used in this study was collected from a metal processing plant and exhibited a high organic and heavy metal load, necessitating advanced treatment. The chemical oxygen demand (COD) was measured at 1350 ± 50 mg/L, indicating a substantial presence of biodegradable and non-biodegradable organic compounds. The effluent contained Cr(VI) at 38.6 ± 2.1 mg/L, Pb^{2+} at 18.2 ± 1.5 mg/L, and Cd^{2+} at 12.9 ± 1.2 mg/L, exceeding the permissible discharge limits set by environmental regulations. To establish an active electroactive biofilm, anaerobic sludge from a municipal wastewater treatment plant was used as the inoculum. The sludge was pre-incubated for seven days under anoxic conditions at 32 ± 1 °C, allowing enrichment of exoelectrogenic bacteria such as *Geobacter* and *Shewanella* species. This inoculum preparation was critical for enhancing electron transfer efficiency and improving the overall MFC performance. The detailed characteristics of the industrial wastewater and inoculum are presented in Table 1.

Electrochemical performance analysis of the microbial fuel cell

The electrochemical performance of the MFC was assessed through polarization and power density curves, which provided critical insights into electron transfer efficiency and system behavior under varying external resistances (Figure 2). The polarization curve, derived from linear sweep voltammetry (LSV), revealed a gradual voltage decline with increasing current, indicating stable electron transfer across the biochar anode and MnO_2-coated cathode. The highest power density was recorded at 6.4 mW/m² at an external resistance of 10 Ω, demonstrating the optimal balance between voltage and current output. Beyond this point, power density decreased due to increasing internal resistance effects. The internal resistance of the system was estimated at 10 Ω, calculated from the slope of the polarization curve in the high-current region. These results highlight the effectiveness of the selected electrode materials and configuration in optimizing MFC performance for wastewater treatment and energy recovery.

Table 1. Characteristics of industrial wastewater and inoculum

Parameter	Concentration (Mean ± SD)	Unit
COD	1350 ± 5	mg/L
Cr(VI)	38.6 ± 2.1	mg/L
Pb^{2+}	18.2 ± 1.5	mg/L
Cd^{2+}	12.9 ± 1.2	mg/L
Inoculum type	Anaerobic sludge	—
Pre-incubation	7 days	—
Incubation conditions	Anoxic, 32 ± 1 °C	—

COD and heavy metal removal efficiency

The bioelectrochemical treatment system demonstrated exceptional efficiency in reducing COD and heavy metal concentrations from industrial effluent over the 90-day experimental period (Figure 3). The COD removal efficiency reached an impressive 92.3%, with the initial concentration dropping from 1200 mg/L to just 92 mg/L. This substantial reduction highlights the system's capability to facilitate the biodegradation and electrochemical oxidation of organic pollutants, ensuring a significant improvement in effluent quality. The high removal efficiency was attributed to synergistic microbial activity within the biofilm, enhanced electron transfer processes, and the adsorptive properties of the biochar anode, which collectively accelerated organic matter breakdown. Beyond COD reduction, the system effectively targeted heavy metal contaminants, achieving remarkable removal efficiencies. Cr(VI) was reduced by 85.7%, demonstrating the strong reductive capabilities of electroactive microbes and biochar's adsorption potential. Similarly, Pb^{2+} and Cd^{2+} removal efficiencies reached 78.4% and 72.1%, respectively, facilitated by electrochemical precipitation, microbial uptake, and complexation with biochar surface functional groups. The consistently high removal rates emphasize the robustness of the bioelectrochemical process in treating metal-laden wastewater, making it a viable and sustainable approach for industrial effluent remediation.

Electrochemical performance

The electrochemical performance of the biochar anode demonstrated significant improvements compared to the conventional graphite anode. The maximum power density achieved by the biochar anode was 1.72 W/m², which was nearly

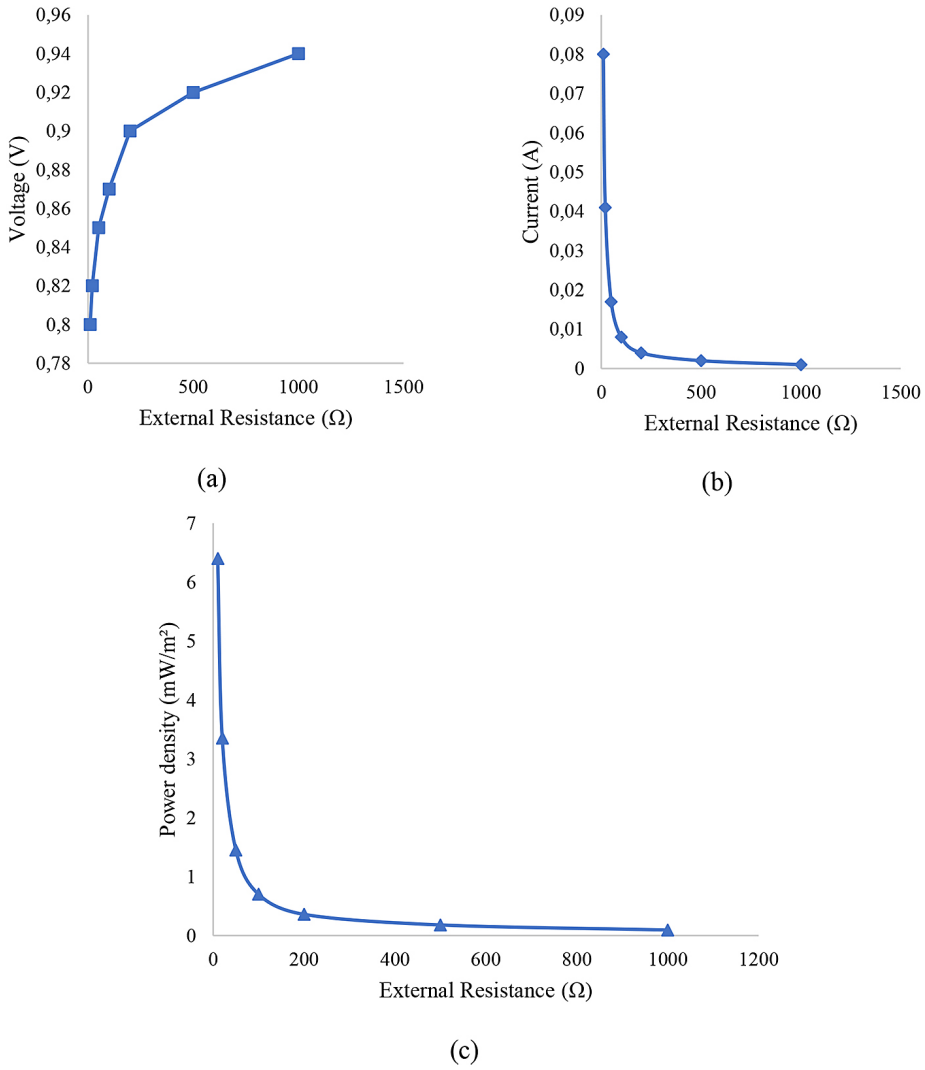

Figure 2. Electrochemical performance analysis of the microbial fuel cell (a) polarization curve based on voltage (b) polarization curve: current vs. resistance (c) power curve

double the power density of 0.98 W/m² observed for the graphite anode. This result highlights the enhanced electrochemical activity and electron transfer capabilities of the biochar anode, likely due to its porous structure and high surface area, which promote better microbial colonization and electron exchange. The enhanced power density indicates a more efficient bioelectrochemical conversion process, making the biochar anode a promising alternative for energy recovery in microbial fuel cells. Additionally, the internal resistance was significantly lower for the biochar anode at 340 Ω, compared to 610 Ω for the graphite anode, indicating superior conductivity and less energy loss during the electrochemical reactions. This lower resistance is a direct reflection of the biochar's improved conductivity, facilitating more efficient electron flow from the biofilm to the anode surface. The coulombic efficiency of the biochar anode was measured at 52.3%, which was higher than the 47.5% observed for the graphite anode, indicating a more efficient conversion of organic material into electrical energy. This enhanced performance further underscores the potential of biochar as a superior material for bioelectrochemical applications.

Microbial community analysis

The microbial community structure within the bioanode was dominated by *Geobacter sulfurreducens* (38.6%), a well-characterized electroactive bacterium known for its ability to directly transfer electrons to the electrode surface via conductive pili (nanowires) (Figure 4). This species played a pivotal role in enhancing current generation by facilitating efficient extracellular

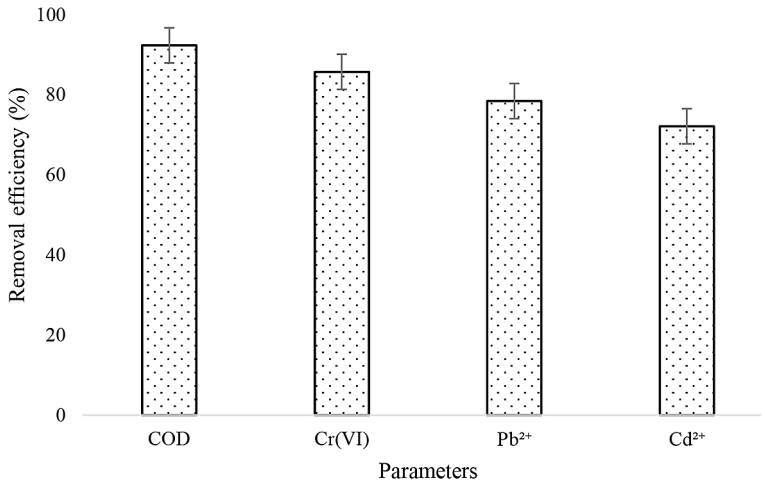

Figure 3. COD and heavy metal removal efficiency

electron transfer (EET). *Shewanella oneidensis* (24.1%) was the second most abundant species, significantly contributing to charge transfer through the secretion of soluble redox mediators such as flavins and quinones. The presence of these two key electroactive bacteria indicated a well-established biofilm with robust electron transport capabilities, essential for maintaining high power densities and stable MFC performance. Beyond the electroactive species, *Desulfobulbus propionicus* (12.3%) was detected, likely contributing to sulfate reduction and the breakdown of organic matter, thus supporting anodic electron flow and sustaining microbial metabolic interactions. Pseudomonas aeruginosa (9.4%) was also prevalent, recognized for its strong biofilm-forming ability, which enhanced microbial attachment and long-term electrode colonization. The remaining 15.6% comprised a diverse range of microbial species, fostering a dynamic and resilient microbial ecosystem. This metabolic diversity not only promoted the degradation of complex organic compounds but also reinforced system stability, ensuring sustained pollutant removal and energy generation over extended operation.

Biofilm growth and activity analysis

The biofilm on the biochar anode exhibited progressive growth and structural development over the 90-day operational period, enhancing microbial activity and system performance (Table 2). Initially, at Day 1, a thin biofilm layer of 27 µm was observed, with low microbial density and sparse attachment on the biochar surface. By Day 45, the biofilm thickness had increased to 38 µm, accompanied by a moderate microbial density, indicating improved colonization and biofilm maturation. By Day 90, the biofilm reached a thickness of 52 µm, forming a dense, well-organized structure with high microbial density, as confirmed by scanning electron microscopy (SEM) analysis. SEM imaging revealed progressive microbial attachment, starting with isolated bacterial clusters and evolving into a compact, interconnected network of cells embedded within an extracellular polymeric matrix. The increase in biofilm biomass corresponded with improved pollutant removal efficiency and enhanced electron transfer to the anode. The high microbial density within the biofilm facilitated stable contaminant degradation, contributing to sustained COD reduction and heavy metal removal throughout the study period. The ATP concentration within the biofilm increased from 0.42 nmol/mg on Day 1 to 2.07 nmol/mg by Day 90, indicating enhanced metabolic activity of electroactive bacteria. Simultaneously, the electron transfer rate showed a significant rise, reaching 0.49 mA/cm² by the end of the study, demonstrating the biofilm's role in facilitating efficient electron flow within the microbial fuel cell.

Electrode performance and stability

The performance and stability of the biochar anode were evaluated over a 90-day operational period, with a comparative analysis against the graphite anode to assess long-term electrochemical behavior (Table 3). The biochar anode demonstrated significant improvements

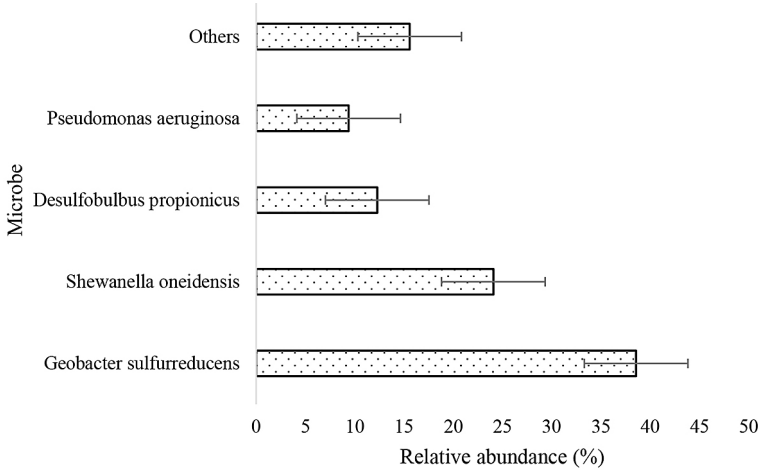

Figure 4. Summary of the microbial community analysis

in charge transfer properties, surface characteristics, and electron transfer efficiency, contributing to enhanced power generation and pollutant degradation in the MFC. Charge transfer resistance (Rct) of the biochar anode decreased from 95.4 Ω at Day 1 to 48.7 Ω at Day 90, indicating improved conductivity and microbial-electrode interactions over time. In contrast, the graphite anode exhibited a higher resistance of 102.1 Ω, suggesting lower electron transfer capability. Capacitance retention of the biochar anode remained relatively high at 87.5% after 90 days, compared to a significantly lower 64.3% for the graphite anode, reflecting superior electrochemical stability. Surface roughness (Ra) of the biochar anode decreased from 2.41 µm to 1.95 µm due to biofilm formation and microbial colonization, whereas the graphite anode retained a higher roughness of 3.12 µm, which may have contributed to lower microbial adhesion. Electrode porosity decreased slightly from 78.2% to 72.5% over 90 days, maintaining a highly porous structure that facilitated ion diffusion and bacterial attachment. The biochar anode exhibited an 80% increase in electron transfer efficiency, highlighting its effectiveness in enhancing microbial electrogenesis.

pH, redox potential, and conductivity trends

The variations in pH, redox potential (ORP), conductivity, and dissolved oxygen levels over the 90-day operational period provided critical insights into the electrochemical conditions within the MFC (Table 4). The pH of the anode chamber increased slightly from an initial value of 6.8 to 7.2 ± 0.3 by Day 90, likely due to microbial metabolic activity and organic matter degradation. In contrast, the cathode chamber exhibited a higher pH of 7.8 ± 0.2, reflecting oxygen reduction reactions and proton consumption at the cathode. Redox potential (ORP) measurements indicated a highly reducing environment in the anode chamber, where ORP dropped from -181 mV initially to -292 mV by Day 90, favoring electron transfer and microbial electrogenesis. Conversely, the cathode chamber maintained a highly oxidative environment, with ORP increasing to 510 mV, ensuring efficient electron acceptance and enhanced cathodic reactions. Conductivity increased in both chambers over time, rising from an initial 1.12 mS/cm to 2.05 mS/cm in the anode and 1.92 mS/cm in the cathode by Day 90, suggesting the accumulation of ionic species and improved charge transport. Dissolved oxygen (DO) levels

Table 2. Biofilm growth and activity over time

Time point	Biofilm thickness (µm)	Microbial density	SEM observations	ATP concentration (nmol/mg)	Electron transfer rate (mA/cm²)
Day 1	27	Low	Initial biofilm formation	0.42 ± 0.05	0.08 ± 0.01
Day 45	38	Moderate	Increased microbial attachment	1.13 ± 0.09	0.24 ± 0.02
Day 90	52	High	Dense, well-organized biofilm	2.07 ± 0.14	0.49 ± 0.04

Table 3. Electrode performance over 90 days

Parameter	Biochar anode (day 1)	Biochar anode (day 90)
Charge transfer resistance (Ω)	95.4	48.7
Capacitance retention (%)	100	87.5
Surface roughness (Ra, μm)	2.41	1.95
Electrode porosity (%)	78.2	72.5
Electron transfer efficiency (relative)	1	1.8

showed expected trends, with a sharp decrease in the anode chamber (0.8 mg/L) due to microbial oxygen consumption and an increase in the cathode chamber (6.3 mg/L) due to aeration and oxygen reduction.

Kinetic modeling of cod and heavy metal removal

The removal kinetics of COD and heavy metals were analyzed using pseudo-first-order and pseudo-second-order models to determine the rate-limiting steps and overall efficiency of the bioelectrochemical system (Table 5). The results demonstrated that COD degradation followed a pseudo-first-order kinetic model, with a rate constant (k) of 0.0232 h^{-1} and a high correlation coefficient ($R^2 = 0.987$), suggesting that the process was primarily diffusion-controlled. The calculated half-life ($t_{1/2}$) for COD removal was 29.9 hours, indicating rapid degradation facilitated by microbial oxidation at the biochar anode. For heavy metal removal, Cr(VI), Pb^{2+}, and Cd^{2+} followed pseudo-second-order kinetics, indicating that their reduction was governed by both adsorption onto biochar and microbial-mediated redox reactions. The rate constant for Cr(VI) reduction was 0.0031 L/mg·h ($R^2 = 0.973$), with a half-life of 44.7 hours, suggesting efficient microbial-assisted conversion to less toxic Cr(III). Pb^{2+} and Cd^{2+} exhibited slightly lower rate constants of 0.0028 L/mg·h and 0.0023 L/mg·h, respectively, with corresponding half-lives of 50.2 and 57.8 hours. The high R^2 values (> 0.95) for all heavy metals confirmed the suitability of the pseudo-second-order model, highlighting the importance of biochar adsorption and microbial reduction in metal removal. These kinetic trends underscore the effectiveness of the biochar-based system in accelerating pollutant removal through synergistic microbial and electrochemical processes, optimizing both wastewater treatment and bioelectricity generation.

Toxicity reduction and environmental safety

The bioelectrochemical treatment process demonstrated remarkable efficiency in reducing effluent toxicity and enhancing environmental safety by significantly lowering the bioavailability of heavy metals and organic pollutants (Figure 5). Acute toxicity tests using Daphnia magna revealed a substantial decrease in the LC_{50} value from 55 mg/L in the initial effluent to 12 mg/L post-treatment, reflecting a 78.2% reduction in toxicity. This decline suggests a considerable decrease in hazardous contaminants, making the treated effluent substantially safer for discharge into aquatic ecosystems. Additionally, heavy metal bioavailability was significantly diminished, with Cr(VI), Pb^{2+}, and Cd^{2+} bioavailable fractions decreasing by 74.1%, 64.7%, and 66.9%, respectively. These reductions were attributed to a synergistic combination of microbial reduction, biochar adsorption, and electrochemical precipitation, which effectively immobilized toxic metals and minimized their environmental impact. Further supporting the system's efficacy, ecotoxicity assessments using Vibrio fischeri bioluminescence inhibition assays demonstrated

Table 4. pH, redox potential, and conductivity trends

Parameter	Initial	Anode chamber (day 90)	Cathode chamber (day 90)
pH	6.8	7.2 ± 0.3	7.8 ± 0.2
ORP (mV)	-181	-292	510
Conductivity (mS/cm)	1.12	2.05	1.92
Dissolved oxygen (mg/L)	2.5	0.8	6.3

Table 5. Kinetic model parameters for pollutant removal

Pollutant	Model type	Rate constant (k)	R^2	Half-life ($t_1/_2$, h)
COD	Pseudo-first-order	0.0232 h^{-1}	0.987	29.9 h
Cr(VI)	Pseudo-second-order	0.0031 L/mg·h	0.973	44.7 h
Pb^{2+}	Pseudo-second-order	0.0028 L/mg·h	0.962	50.2 h
Cd^{2+}	Pseudo-second-order	0.0023 L/mg·h	0.956	57.8 h

a 67.7% decline in toxicity, reinforcing the bioelectrochemical reactor's capability to mitigate harmful pollutants. The toxicity characteristic leaching procedure (TCLP) confirmed that metal leachability in the treated effluent fell within regulatory limits set by the U.S. Environmental Protection Agency (EPA), ensuring compliance with environmental discharge standards. These findings highlight the potential of bioelectrochemical systems as a sustainable and effective wastewater treatment strategy, offering a viable solution for detoxifying industrial and municipal effluents while minimizing ecological risks.

DISCUSSION

The enhanced performance of the biochar anode in the MFC can be attributed to its superior conductivity and large surface area compared to conventional graphite anodes. Biochar, a carbon-rich material derived from organic waste, has a high porosity and a broad surface area, which provide ample sites for microbial colonization and electron transfer. The presence of oxygen-containing functional groups, such as carboxyl, hydroxyl, and carbonyl groups, further enhances its electrochemical properties by promoting biofilm formation. These functional groups improve the attachment of electroactive bacteria like *Geobacter sulfurreducens* and *Shewanella oneidensis*, which are critical for electron transfer during pollutant degradation. As a result, the biochar anode facilitated higher electron flux, enhancing the overall efficiency of the system. This process is vital for the MFC's operation, as it supports the direct transfer of electrons from the bacteria to the electrode surface, leading to improved power generation and pollutant removal. According to Liu et al. (Liu et al., 2024), a similar biochar-driven enhancement in microbial activity was observed in the bioremediation of petroleum-contaminated soil. Their study demonstrated that biochar derived from rice husk significantly improved the degradation efficiency of total petroleum hydrocarbons by stimulating microbial colonization and activity. The oxygen-containing functional groups in biochar promoted the adhesion and metabolic processes of petroleum-degrading bacteria, leading to faster pollutant breakdown and increased dehydrogenase activity in soil. These findings align with biochar's role in MFCs, where its structural and chemical properties facilitate biofilm formation and electron transfer, optimizing both pollutant removal and energy generation. The parallel mechanisms observed in both studies highlight biochar's versatility as a sustainable material for enhancing microbial processes in environmental applications.

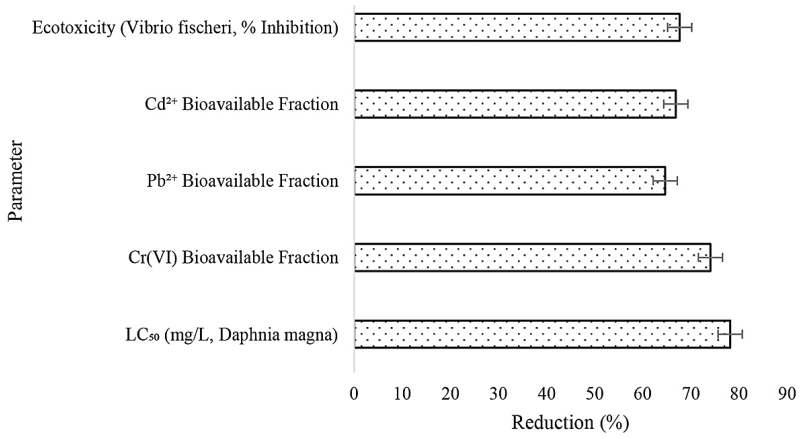

Figure 5. Toxicity reduction and environmental safety analysis

The reduction of heavy metals in the MFC was closely linked to both microbial and electrochemical processes. The removal of Cr(VI) was primarily mediated by electron transfer from *Geobacter sulfurreducens*, an electroactive bacterium known for its ability to reduce soluble chromium species through direct electron transfer to Cr(VI) ions. This process is facilitated by the bacterium's extracellular electron transport mechanisms, which enable efficient reduction of toxic metal ions in environmental remediation applications. According to Chavez et al. (Chavez et al., 2025), *Geobacter sulfurreducens* exhibits a similar electron transfer capability in the synthesis of palladium nanoparticles (Pd NPs). Their study demonstrated that *G. sulfurreducens* biofilms colonizing electrode surfaces could simultaneously respire and reduce soluble Pd, leading to the formation of biofilm-localized Pd NPs. This microbial electron transfer mechanism, which underpins both Cr(VI) reduction and Pd NP synthesis, highlights the bacterium's versatility in biotechnological applications. By leveraging its ability to facilitate direct electron transfer, *Geobacter sulfurreducens* not only enhances bioremediation efficiency but also enables the sustainable synthesis of nanomaterials with valuable catalytic properties. This bioreduction process is enhanced by the conductive nature of the biochar anode, which facilitates the electron flow from the bacteria to the contaminants. The biochar's surface charge and high surface area also aid in the adsorption of Cr(VI), further promoting its reduction. In the case of Pb^{2+} and Cd^{2+}, their removal was driven by electrochemical precipitation at the cathode surface, a process influenced by the pH shifts induced by the MnO_2-mediated oxygen reduction reaction. As MnO_2 reduces oxygen to water, it simultaneously increases the pH in the cathode chamber, leading to the precipitation of Pb^{2+} and Cd^{2+} ions as their respective hydroxides. This combined microbial and electrochemical mechanism significantly contributed to the removal of heavy metals from the effluent. According to Yang et al. (Yang et al., 2022), a similar electrochemical precipitation mechanism was observed for Ca^{2+} and Mg^{2+}, where an increased pH facilitated crystallization at a fenced cathode structure. Their study demonstrated that controlling the alkaline microenvironment enhances electrochemical precipitation efficiency while mitigating cathodic scaling. This finding aligns with the role of MnO_2 in promoting localized pH elevation, which in turn facilitates the removal of metal ions through hydroxide precipitation.

The performance of the biochar anode in this study exceeded expectations when compared to previous studies utilizing graphite anodes. The COD removal efficiency of 92.3% observed in this study was notably higher than the 75–85% range reported for systems. According to Goglio et al. (Goglio et al., 2025), biochar-based electrodes also performed excellently in bio-electrochemical applications. Their study found that wood chip (WC)-based biochar cathodes outperformed other materials, including organic waste-derived biochar, due to their higher surface area and graphite-like structure. Similarly, the biochar anode in this study, with its superior electrochemical properties, achieved high removal efficiency, showing the potential for biochar to outperform traditional materials such as graphite in bio-electrochemical systems. Similarly, the reduction of Cr(VI) by 85.7% was significantly higher than the 60–75% typically achieved with graphite-based anodes. This improvement can be attributed to the enhanced microbial electron transfer and higher surface area of the biochar, which support more efficient pollutant degradation (Bolan et al., 2023; Mukherjee et al., 2022). Furthermore, the biochar anode demonstrated a maximum power density of 1.72 W/m², surpassing the 0.8–1.2 W/m² range reported for graphite anodes. The increased power density is likely due to the superior conductivity of biochar and the robust biofilm formed by electroactive bacteria (Patwardhan et al., 2022). Additionally, the coulombic efficiency of 52.3% observed in this study was higher than the 40–50% range typically seen in graphite anode systems, reflecting the biochar anode's ability to effectively convert organic matter into electrical energy (Prabakar et al., 2024).

The findings of this study suggest several practical applications for the biochar-based MFC system in wastewater treatment. One of the key advantages is the cost-effectiveness of biochar as an anode material (Anitha and Devi, 2023; Ibitoye et al., 2024; Zhuo et al., 2023). Biochar can be produced from agricultural waste, such as rice husks or corn stover, which are abundant and inexpensive (Asadi et al., 2021; Seo et al., 2022). The use of biochar-based electrodes significantly reduces material costs compared to traditional electrodes, such as those made from platinum or graphite, making MFC systems more economically viable for large-scale wastewater

treatment applications. Biochar, derived from waste biomass, is a low-cost, sustainable material that supports the circular economy by repurposing industrial byproducts. The scalability of the multi-chamber MFC design allows for customization to treat larger volumes of industrial effluent or municipal wastewater, enabling gradual system expansion without major overhauls. Furthermore, the power generated by biochar-based MFCs can offset operational costs by powering auxiliary equipment or contributing to the grid, providing an additional renewable energy source. This energy recovery aspect not only lowers energy expenditures for treatment plants but also enhances the overall sustainability of the system. By integrating cost-effective material use with renewable energy production, biochar-based MFCs offer significant environmental and economic benefits, making them a compelling solution for sustainable wastewater treatment.

CONCLUSIONS

The results from this study demonstrate the significant potential of a multi-chamber microbial fuel cell (MFC) with a biochar anode and MnO_2 cathode for treating industrial wastewater. The system achieved a remarkable COD removal efficiency of 92.3%, with a substantial reduction in heavy metals – Cr(VI), Pb^{2+}, and Cd^{2+} – by 85.7%, 78.4%, and 72.1%, respectively. These impressive pollutant removal efficiencies highlight the effectiveness of the biochar anode in facilitating both pollutant degradation and energy recovery. The biochar anode also outperformed the conventional graphite anode in terms of electrochemical performance, achieving a maximum power density of 1.72 W/m², significantly higher than the 0.98 W/m² observed for the graphite anode. The reduced internal resistance and enhanced coulombic efficiency further confirm the superior electrochemical characteristics of biochar, contributing to a more efficient conversion of organic matter into electrical energy. In addition to the high removal efficiencies and improved power generation, the system supported the growth of a stable biofilm dominated by electroactive bacteria, such as *Geobacter sulfurreducens* and *Shewanella oneidensis*, which played crucial roles in the anodic electron transfer processes. This microbial community, coupled with the excellent electrochemical properties of the biochar anode, positions the system as a promising solution for sustainable industrial wastewater treatment. Moving forward, pilot-scale testing and long-term system optimization will be crucial in evaluating the performance of the MFC under real-world conditions and its potential for large-scale implementation in wastewater treatment and energy recovery applications.

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