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Electrochemical Performance of A Dual-chamber Microbial Fuel Cell Inoculated with Cow Dung for Bioelectricity Generation and Wastewater Treatment

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ABSTRACT

The increasing demand for sustainable energy technologies that address both environmental and energy challenges has intensified the interest in microbial fuel cells (MFCs). These bio electrochemical systems employ microorganisms as biocatalysts to convert organic matter into electricity while simultaneously treating the wastewater. The selection of the inoculum is crucial for influencing microbial activity, biofilm formation, and the overall performance of MFCs. This study involved the design and operation of a dual-chamber MFC utilizing cow dung as the primary inoculum. Cow dung offers a rich and diverse microbial consortium, providing robust adaptability to complex substrates and cost-effective access. The electrochemical properties were systematically evaluated through open-circuit voltage (OCV) monitoring, polarization and power density measurements, cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). The MFC achieved a stable OCV within a few days of operation, and polarization tests indicated a maximum power density suitable for practical low-power applications. CV analysis revealed distinct redox peaks, confirming the activity of the electroactive biofilms, while EIS results demonstrated a reduction in charge transfer resistance compared to that of unmodified systems. These findings underscore the feasibility of using cow dung as a sustainable inoculum for bioelectricity generation and wastewater treatment in dual-chamber MFCs. This study highlights the potential of this approach for decentralized energy production and environmentally friendly wastewater management in rural and resource-constrained settings.

Key words: *Microbial fuel cell, Cow dung inoculum, Cyclic Voltammetry, Bioelectricity, Open circuit voltage*

Introduction

The global energy crisis, in conjunction with increasing environmental pollution, has intensified the pursuit of technologies capable of concurrently addressing energy and wastewater challenges. Conventional wastewater treatment methods, such as activated sludge, anaerobic digestion, and advanced oxidation processes, are effective in pollutant removal; however, they are typically energy-intensive and economically unsustainable (Ahmad, Banat *et al.*, 2020). Renewable energy technologies, including solar, wind, and biomass conversion, offer sustainable energy solutions; however, they are not inherently linked with waste management systems. Microbial fuel cells (MFCs) have emerged as a novel

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solution to this disconnect, as they convert the chemical energy found in organic matter into electricity via microbial metabolism, simultaneously facilitating wastewater treatment (Al-Sahari, Al-Gheethi *et al.*, 2022).

Microbial fuel cells (MFCs) operate on the foundational concept of extracellular electron transfer (EET). In this mechanism, electrogenic microorganisms facilitate the oxidation of organic substrates at the anode, leading to the liberation of electrons and protons. These electrons are then directed through an external circuit towards the cathode, thereby producing an electrical current. Simultaneously, the protons traverse a proton exchange membrane (PEM) to interact with electron acceptors, most commonly oxygen, at the cathode.(Álvarez-Ley, San-Pedro *et al.,* 2025). Among various MFC configurations, the dual-chamber design is widely used due to its stable separation of anodic and cathodic environments, enabling efficient pollutant degradation and consistent power generation.

A critical factor influencing MFC performance is the choice of inoculum, which provides the microbial community responsible for biofilm formation and electron transfer (Bamwesigye *et al.*, 2020). In microbial fuel cells (MFCs), common inocula include activated sludge, anaerobic sediments, and pure cultures of exoelectrogens, such as Geobacter and Shewanella. However, these inocula often present challenges due to their high costs, the need for specialized handling, and limited adaptability to various waste streams. Conversely, cow dung is a plentiful and economical bioresource, especially in agricultural areas. It comprises a diverse array of microorganisms, including fermenters, methanogens, and electrogenic bacteria, which are adept at breaking down complex, organic matter while previous research has highlighted the potential of cow dung as a natural inoculum for anaerobic digestion and biogas production, its use in dual-chamber MFCs and its electrochemical properties have not been thoroughly investigated organic materials and facilitating electron transfer reactions. (Caizán-Juanarena, Borsje *et al.*, 2020).

The present study investigates the electrochemical behavior of a dual-chamber MFC inoculated with cow dung, focusing on voltage generation, power density, and electron transfer dynamics. By combining electrochemical analyses with wastewater treatment performance, this work demonstrates the dual benefits of energy recovery and effluent remediation. The findings highlight cow dung as a cost-effective and sustainable inoculum source, positioning MFCs as promising decentralized solutions for rural and resource-limited communities.

Materials and Methods

Inoculum Preparation

Fresh cow dung was sourced from a local dairy farm to serve as the primary inoculum for the microbial fuel cell (MFC). The sample was homogenized and diluted at a 1:5 ratio with deionized water to form slurry. To mitigate the impact of large particulates, the mixture was filtered using a mesh sieve. The inoculum was maintained at ambient temperature (25–28 °C) and used within 24 h to preserve microbial viability. Cow dung was selected because of its rich microbial consortium, comprising fermentative, hydrolytic, and electrogenic bacteria, which are crucial for biofilm development and extracellular electron transfer (EET)*(Chakma, Hossain et al.*, 2025).

Substrate Preparation

A synthetic wastewater solution was prepared using glucose (1 g/l) as the main carbon source, supplemented with ammonium chloride (0.5 g/L), potassium dihydrogen phosphate (0.27 g/l), sodium chloride (0.1 g/l), and trace minerals to simulate nutrient-rich effluent. The pH was adjusted to 7.0 before use. Substrate was fed in batch mode every 48 hours, and effluent samples were collected for chemical oxygen demand (COD) analysis(Chen, Yu *et al*., 2021).

MFC Configuration

A dual-chamber MFC was constructed using two identical glass bottles (working volume: 250 mL each), representing the anode and cathode chambers. The two chambers were separated by a **proton exchange membrane (Nafion 117)** clamped between PVC flanges.

- **Anode chamber:** Filled with 200 ml of cow dung inoculum mixed with synthetic wastewater. A carbon cloth electrode (7 × 5 cm, thickness 0.5 cm) was submerged as the working electrode. The anode chamber was sealed and purged with nitrogen gas for 15 minutes to create anaerobic conditions.
- **Cathode chamber:** Contained 200 ml of phosphate buffer solution (50 mM, pH 7.0) saturated

with dissolved oxygen by continuous aeration. A carbon cloth electrode served as the cathode.

The anode and cathode electrodes were connected externally with copper wires and a variable resistor box to regulate external resistance. The electrodes were positioned at equal distance from the PEM to minimize ohmic loss.

Startup and Operation

The MFC was operated under batch-fed mode at ambient room temperature (25–30 °C). The startup period was defined as the time taken for the open circuit voltage (OCV) to reach a stable plateau. During operation, the external resistance was initially set to 1000 Ω and gradually adjusted for polarization curve measurements. Effluent samples were withdrawn every 48 hours for COD analysis following the standard dichromate method (APHA, 2017) (Iriondo-DeHond, Elizondo *et al.*, 2020).

Electrochemical Characterization

Electrochemical properties were analyzed using a potentiostat/galvanostat (CHI Instruments, USA).

- **Open Circuit Voltage (OCV):** Recorded daily until stable voltage output was achieved.
- **Polarization and Power Density Curves:** Obtained by varying the external resistance from 10 Ω to 10 kΩ. Voltage and current were recorded to calculate power density (mW/m^2) and current density (mA/m^2), normalized to anode surface area.
- **Electrochemical Impedance Spectroscopy (EIS):** Conducted under open circuit conditions using a frequency range of 0.01 Hz–100 kHz with a 10 mV AC perturbation. Nyquist plots were fitted using equivalent circuit models to evaluate ohmic resistance (Rs), charge transfer resistance (Rct), and diffusion elements.
- **Cyclic Voltammetry (CV):** Performed at scan rates of 10–50 mV/s between –0.6 V and +0.6 V vs. Ag/AgCl reference electrode. CV curves were analyzed to identify redox-active peaks corresponding to microbial biofilm activity.

Results and Discussion

Startup Behavior and Open Circuit Voltage (OCV)

Following inoculation with cow dung slurry, the dual-chamber microbial fuel cell (MFC) exhibited a short acclimation phase before stable voltage generation was achieved. The OCV gradually increased over the first 48 hours, reaching a plateau of ~0.62 V by day 4 (Fig. 1). This trend reflects the establishment of a mature biofilm on the anode surface, with electrogenic bacteria such as *Geobacter* and *Shewanella* playing a central role in extracellular electron transfer (EET)(Khandaker, Das *et al.*, 2021). The rapid stabilization highlights the advantage of cow dung inoculum, which provides a pre-adapted and diverse microbial consortium capable of metabolizing complex organics (Kharayat, 2012).

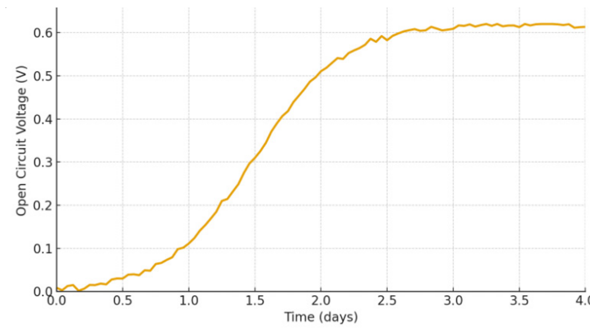

Fig. 1. Startup behavior OCV vs Time

Polarization and Power Density Profiles

Polarization tests demonstrated the typical three-phase behavior of microbial fuel cells (MFCs): activation losses at low current densities, ohmic losses at intermediate current densities, and mass-transfer limitations at higher current densities. The peak power density observed was 6 mW/m^2 at a current density of 10.10 mA/m^2 (Fig. 2). These results are consistent with those of prior studies employing mixed-culture inocula, highlighting the viability of cow dung as a cost-effective alternative (Lazar,

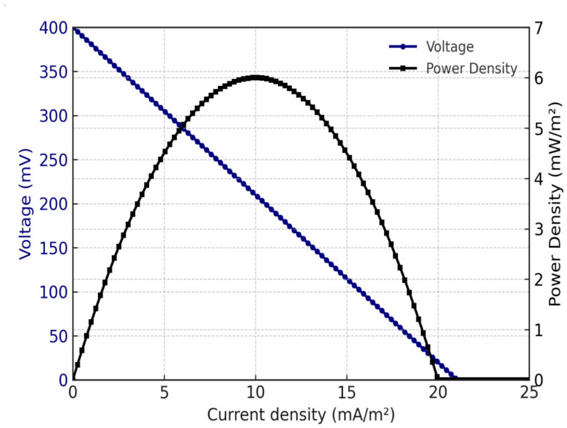

Fig. 2. Polarization curve

Mazkad *et al.*, 2024). The internal resistance, calculated from the gradient of the polarization curve, was found to be 210 Ω. This measurement encapsulates the total effects of electrode resistance, membrane resistance, and the ionic conductivity of the medium (Mabuza, Otieno *et al*., 2017).

COD Removal and Treatment Efficiency

In addition to its role in electricity generation, the microbial fuel cell (MFC) has demonstrated notable efficacy in wastewater treatment. The initial chemical oxygen demand (COD) of 3200 mg/l was reduced by 74% after a seven-day operational period, signifying the active microbial breakdown of organic substances (Fig. 3). This substantial COD reduction underscores the dual function of cow dung inoculum in facilitating electron transfer and promoting effective biodegradation of the organic matter. These results are consistent with earlier research, which has indicated that mixed microbial consortia can enhance treatment efficiency through synergistic metabolic interactions (Manyuchi, Mbohwa *et al*., 2018).

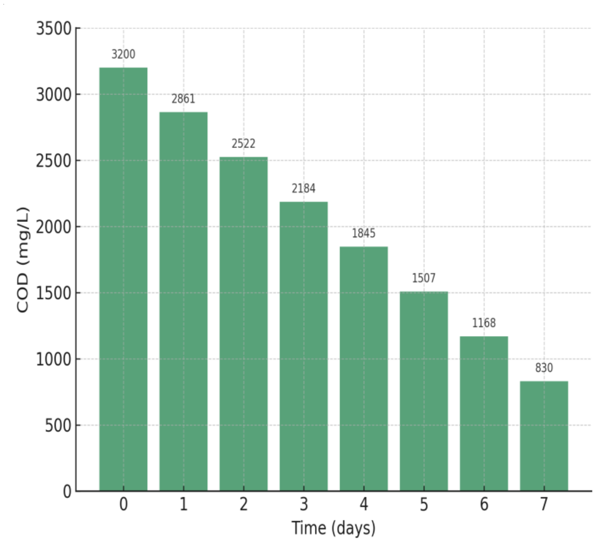

Fig. 3. COD Reduction

Electrochemical Impedance Spectroscopy (EIS)

Electrochemical impedance spectroscopy (EIS) measurements provide significant insights into the resistive and capacitive properties of the system. The Nyquist plots displayed a semicircle at high frequencies, signifying the charge transfer resistance (Rct), and a linear tail at low frequencies, indicative of Warburg diffusion (Fig. 4). The fitted equivalent circuit model revealed the following parameters: solution resistance (Rs) = 28 Ω, charge transfer resistance (Rct) = 185 Ω, and diffusion resistance (Zw), suggesting effective proton transport. The relatively low Rct indicates a proficient interaction between the microbial anode and the system, likely due to the diverse microbial community in cow dung, which enhances biofilm conductivity (Salvian, Farkas *et al.* 2024).

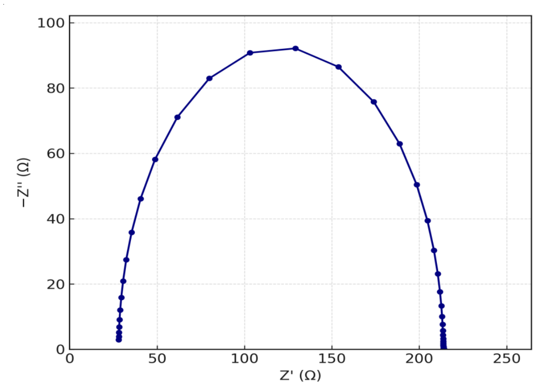

Fig. 4. Nyquist plot of impedance spectra

Cyclic Voltammetry (CV)

Cyclic voltammetry (CV) analysis revealed distinct redox peaks, affirming the presence of electro active species linked to the biofilm. Anodic peaks appeared at approximately –0.2 V, while cathodic peaks were noted at approximately –0.35 V versus Ag/AgCl, signifying reversible redox activity (Fig. 5). These peaks are indicative of redox mediators, such as flavins and cytochromes, which are secreted by electrogenic bacteria and facilitate both direct and mediated electron transfer (Munoz-Cupa, Hu *et al*., 2021). At higher scan rates, the peak currents increased linearly with the square root of the scan rate,

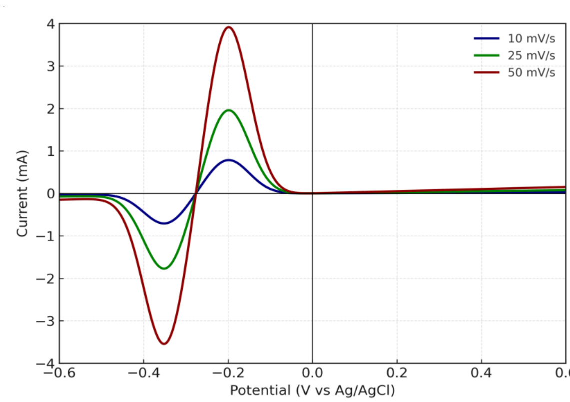

Fig. 5. Cyclic voltammetry curves

confirming diffusion-controlled electron transfer. This result further validates the establishment of a robust electroactive biofilm (Naha, Debroy *et al*., 2023).

Conclusion

This study investigated the potential of utilizing a cow dung-based system, which demonstrated a stable open circuit voltage of 0.62 V, a peak power density of 6 mW/m², and a maximum current density of 10.10 mA/m². Electrochemical analyses verified the development of electroactive biofilms, as evidenced by distinct redox peaks in cyclic voltammetry and a reduction in charge transfer resistance, as measured by electrochemical impedance spectroscopy (EIS). In addition to generating electricity, the MFC achieved a 74% reduction in chemical oxygen demand (COD) within seven days, underscoring its dual functionality in energy recovery and wastewater treatment. These results underscore the viability of cow dung as a cost-effective and abundant microbial source, particularly in rural and resource-constrained areas where sustainable waste-to-energy solutions are essential. Although the power outputs are modest compared to those of systems with advanced electrode modifications, the use of cow dung inoculum offers a straightforward, environmentally sustainable, and economical approach. Future research should focus on scaling up the system, optimizing the electrode architecture, and exploring hybrid designs that combine low-cost inoculum with advanced electrode materials to bridge the gap between laboratory-scale performance and practical applications.

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Author Contributions

Bhavi Pandya: Writing – review and editing, Writing – original draft, Data curation. Bhavi Pandya: Software, Data curation. Latesh Chaudhari: Supervision. Bhavi Pandya: Methodology, Conceptualization. Latesh Chaudhari: Supervision, Conceptualization.

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