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Performance Evaluation of Microbial Fuel Cells Using Dairy Wastewater: Effect of Inoculum Concentration on Bioelectricity Generation and Treatment

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Abstract

Microbial fuel cells (MFCs) are an emerging sustainable technology that enables simultaneous wastewater treatment and bioelectricity generation. This study evaluates the performance of a laboratory-scale dual-chamber MFC using dairy wastewater as the substrate and sewage as the inoculum. The MFC was operated under an anaerobic anode and aerobic cathode configuration, with a total reactor volume of 5 L and an effective working volume of 2.6 L. The initial physicochemical characteristics of the dairy wastewater were also evaluated. The system was constructed with biochar coated electrodes and an agar–NaCl based proton exchange membrane to enhance electron transfer. Two inoculum concentrations (30% and 40%) were tested over a period of 15 days to assess their effect on power generation and treatment efficiency. The wastewater showed good biodegradability with a BOD/COD ratio of 0.71. The MFC exhibited an initial lag phase, followed by increased voltage generation and gradual decline. The maximum voltage recorded was 0.87 V for the 30% inoculum and 0.86 V for the 40% inoculum. Simultaneously, the 30% inoculum achieved higher COD removal (12.5%), BOD removal (15%), and Coulombic efficiency (22%). SEM analysis confirmed the formation of a stable and dense microbial biofilm on the anode surface. The results indicate that 30% inoculum provides optimal performance for bioelectricity generation and wastewater treatment.

Keywords: microbial fuel cells; dairy wastewater; inoculum concentration; biochar electrodes; proton exchange membrane

Introduction

Microbial fuel cells (MFCs) are emerging bioelectrochemical systems that enable simultaneous wastewater treatment and bioelectricity generation [1,2]. In recent years, increasing energy demand and environmental concerns have driven the development of sustainable and eco-friendly technologies. MFCs utilize microorganisms as biocatalysts to convert the chemical energy stored in organic matter directly into electrical energy [1,3]. They are broadly classified into single-chamber and dual-chamber systems [4]; in this study, a dual-chamber configuration is employed.

In a dual-chamber MFC, microorganisms degrade organic substrates under anaerobic conditions in the anode chamber, releasing electrons and protons during metabolic activity. The electrons generated at the anode are transferred through an external circuit, producing an electric current, while protons migrate through a proton exchange membrane (PEM) to the cathode chamber. At the cathode, electrons, protons, and oxygen combine to form water, resulting in simultaneous electricity generation and wastewater treatment [1,6].

The performance of MFCs depends on several factors, including substrate characteristics, microbial activity, electrode material, membrane efficiency, and operational conditions such as pH and temperature. In this study, dairy wastewater is selected as the substrate due to its high organic content, which enhances microbial growth and improves electron production and power generation [3].

Dairy industries generate large volumes of wastewater containing high concentrations of organic compounds such as lactose, fats, and proteins, leading to elevated biochemical oxygen demand (BOD) and chemical oxygen demand

(COD) [3]. If discharged untreated, this effluent can cause significant environmental pollution. However, the same organic load can be utilized as a potential energy source in MFC systems.

Conventional wastewater treatment processes are energy-intensive and do not recover the energy embedded in waste streams. In contrast, MFCs provide a sustainable alternative by directly converting chemical energy from wastewater into electrical energy while reducing pollution load [2,5].

The dual-chamber MFC used in this work consists of an anaerobic anode chamber and an aerobic cathode chamber separated by a proton exchange membrane. Biochar-coated stainless steel mesh electrodes are used to improve surface area and electron transfer efficiency, while an agar–NaCl membrane is used as a low-cost proton exchange medium.

The working principle of the MFC is based on microbial oxidation of organic matter and electrochemical reactions, which have been widely reported in previous studies [1,6].

Anode reaction: $CxHyOz + H_2O \rightarrow CO_2 + H^+ + e^-$

Cathode reaction:

$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ Overall reaction:

Organic matter + $O_2 \rightarrow CO_2 + H_2O$ + Electricity

Based on this principle, MFCs offer a dual advantage of energy recovery and wastewater treatment. The use of low-cost electrode and membrane materials further improves their feasibility for practical applications.

Accordingly, this study aims to evaluate the performance of a dual-chamber MFC using dairy wastewater with different inoculum concentrations (30% and 40%), identify the optimum operating condition for maximum voltage generation and pollutant removal, and analyze the electrode surface characteristics using scanning electron microscopy (SEM).

2. MATERIALS AND METHODS

All materials used in this study were selected based on low cost, availability, and efficiency in microbial attachment and proton transport. The setup consists of the following components:

- Anode and Cathode Chambers:
  Two borosilicate glass containers, each 30 cm in height and 15 cm in diameter, with an effective operating volume of 2.6 L (half-filled). The anode chamber was maintained under anaerobic conditions and the cathode chamber under aerobic conditions.
- Proton Exchange Membrane (PEM):
  A salt bridge was prepared using a glass tube of (5 cm length and 2.5 cm inner diameter) filled with 1 M NaCl–agar gel, which acted as a proton exchange medium (salt bridge) between the chambers.
- Electrodes:
  Biochar-coated stainless steel mesh (Grade SS 304) of dimensions 5 cm × 5 cm was used as both anode and cathode electrodes to enhance microbial attachment and electron transfer.
- Connecting Wires:
  Insulated copper wires (5 A capacity) were used for external circuit connections between the electrodes.
- Measuring Device:

A Digital multimeter were used for continuous monitoring of voltage and current output. Voltage was recorded at regular time intervals.

- Substrate:
  Dairy wastewater was collected from Numax Foods Pvt.Ltd. (Nutra Milk unit) and used as the anolyte substrate. Physicochemical parameters such as Ph, COD, and BOD were analyzed prior to use.
- Inoculum :
  Sewage wastewater was collected from the Nanjundapuram Sewage Treatment Plant (STP), Coimbatore, and used as the inoculum source. Two inoculum concentrations (30% and 40%) were prepared for experimentation.
- Experimental Operation:
  The MFC was operated under batch mode for a period of 15 days. The anode chamber was sealed to maintain anaerobic conditions, while the cathode chamber was exposed to air. Voltage readings were recorded daily using a digital multimeter under open circuit conditions.

Methodology Flowchart

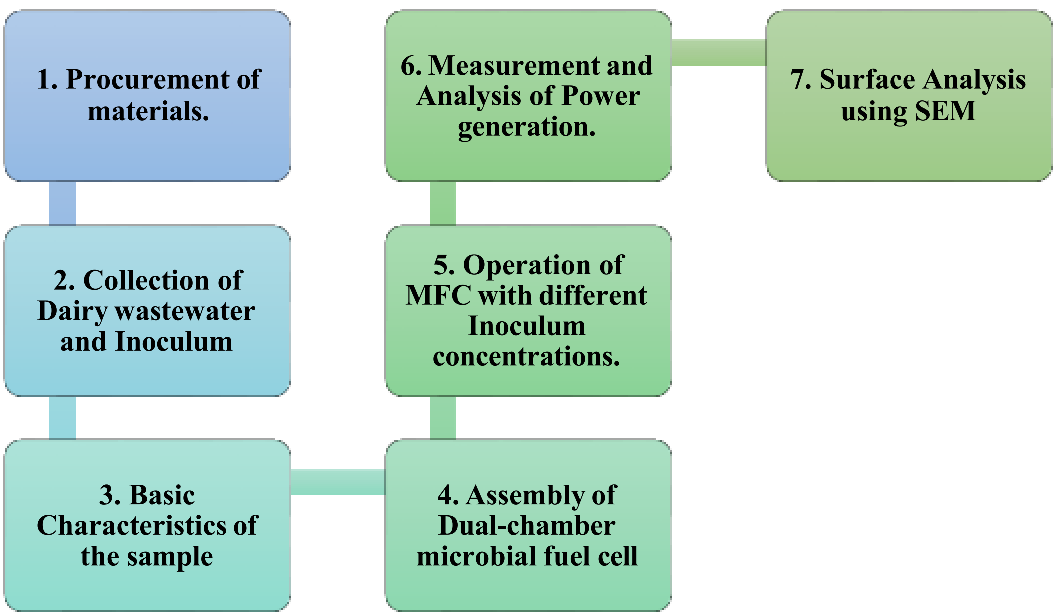

Fig 1: Methodology Flowchart

Preparation of biochar

- Raw Material: Dried coconut shells.
- Pyrolysis: Heated in a closed steel container with a small vent hole for ~1 hour to form porous carbon.
- Cooling and Crushing: After cooling, the obtained black biochar was ground to fine powder.

Fig 2:Biochar Preparation

Coating on stainless steel electrodes

- Electrode Selection: SS 304 mesh (5 cm × 5 cm) chosen for conductivity and corrosion resistance.
- Surface Cleaning: Mesh cleaned using distilled water and ethanol.
- Biochar Coating: Biochar mixed with a few drops of distilled water to form a paste and applied evenly with a brush.
- Drying: Air-dried overnight and heated at 60 °C for 30 minutes to improve adhesion. This biochar coating increases surface roughness, microbial attachment, and electron transfer efficiency.

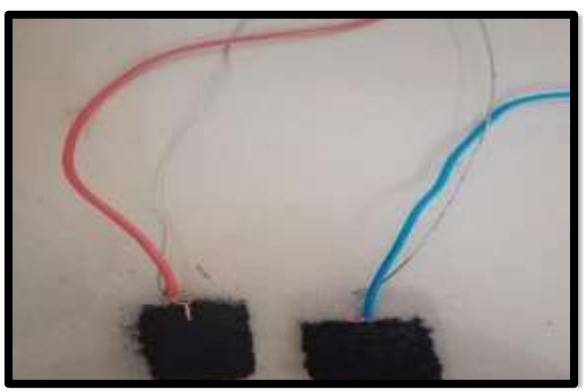

Fig 3: Electrodes (Cathode and Anode) Preparation of Proton Exchange Membrane (Pem):

The PEM was fabricated as a low-cost, biodegradable alternative to commercial Nafion membranes.

1. Preparation of 1 M NaCl Solution:

   Dissolve 58.44 g of NaCl in 1 L of distilled water.

2. Agar Solution Preparation:

Add 3–4 g of agar powder to 100 mL of the prepared NaCl solution. Heat gently with constant stirring until a transparent viscous solution forms.

3. Filling the Glass Tube:

The glass tube (5 cm × 2.5 cm ID) was held vertically, and the hot agar–NaCl mixture was poured until full.

4. Sealing and Cooling:

Both ends were sealed with cotton plugs to prevent leakage and cooled at room temperature to solidify. Once set, this gel served as a proton transport medium between chambers, allowing H^+ ion movement and preventing oxygen crossover.

Fig 4: Preparation of polymer solution

Fig 5: Formation of gel membrane

Assembly Procedure of MFC:

Chamber Setup:

Two glass containers (anode and cathode) were connected by a glass tube PEM sealed with epoxy.

Electrode Placement:

Biochar-coated SS 304 mesh placed vertically in both chambers.

- Anode: Fully immersed in substrate under anaerobic condition.
- Cathode: Partially immersed and exposed to air for oxygen diffusion. Electrical Connection:

Electrodes connected externally via copper wires and multimeter attached to measure voltage and current.

Substrate Loading:

- Anode: 2.6 L of raw dairy wastewater.
- Cathode: 2.6 L of distilled water.

Volume Calculation:

$$V = \frac{\pi D^2 H}{4} \times 0.5 = \frac{3.14 \times (0.15)^2 \times 0.30}{4} \times 0.5 = 0.0026\, m^3 = 2.6\, L$$

Operation:

Set up maintained at room temperature (28–30 °C). Readings taken at 24-hour intervals for voltage and current. COD was measured before and after operation.

Experimental arrangement

Both chambers were connected through the glass tube PEM, and electrodes were linked externally using copper wires. Voltage was recorded continuously using a digital multimeter. The setup was operated for several days to observe voltage stability and wastewater degradation.

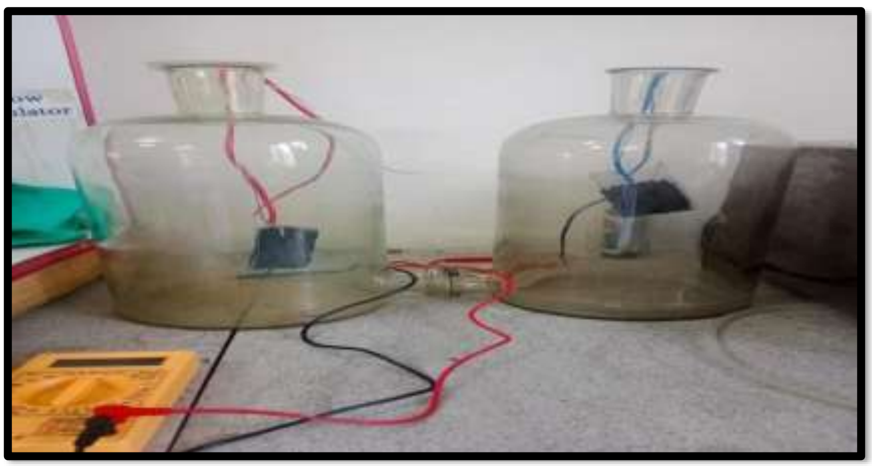

Fig 6 : MFC Setup

Monitoring And Analysis

- Voltage and Current Measurement:

Daily readings of voltage (mV) and current (mA) were taken using a digital multimeter. Power output calculated as:

$$P = V \times I$$

- COD Reduction Analysis:

Initial and final COD values measured using the closed reflux titrimetric method to determine treatment efficiency.

- Observation Period:

Readings recorded for 15 consecutive days, and voltage output along with COD reduction were used to evaluate the overall performance of the MFC.

Fig 7: Voltage Output of MFC System

4 Results and Discussion

This chapter presents the experimental results of the MFC operated with two different inoculum concentrations (30% and 40%) using raw dairy wastewater as the substrate. This study evaluates voltage generation under open circuit conditions, electrochemical performance, and the organic characteristics of the wastewater. The results provide insights into microbial activity, biofilm development, and the effect of inoculum concentration on electricity generation.

Table 1 : Initial Characteristics of Dairy Wastewater

S. NO	PARAMETER	OBSERVED VALUE
1	pH	6.56
2	Oil & Grease	1040 mg/L
3	BOD	200 mg/L
4	COD	280 mg/L
5	BOD/COD	0.71
6	Total Solids	1500 mg/L
7	Dissolved Solids	900 mg/L
8	Suspended Solids	600 mg/L

The wastewater exhibits high organic strength due to elevated oil and grease and COD values. The BOD/COD ratio of 0.71 indicates that the wastewater is highly biodegradable, making it suitable for microbial fuel cell operation.

Voltage Generation Characteristics

40% Inoculum

Table 2: Voltage Variation

Day	Voltage (V)	Observation
1	0.355	Initial microbial adaptation
2	0.346	Lag phase
3	0.330	Adjustment phase
4	0.310	Low activity
5	0.420	Active growth begins
6	0.580	Biofilm formation
7	0.720	Enhanced electron transfer
8	**0.866**	Peak voltage
9	0.858	Stable output
10	0.844	Steady state
11	0.812	Slight decline
12	0.792	Substrate reduction
13	0.700	Reduced activity
14	0.672	Decline phase
15	0.643	Low activity

Maximum Voltage = 0.866 V

30% Inoculum

Table 3: Voltage Variation

Day	Voltage (V)	Observation
1	0.275	Initial lag phase
2	0.310	Adaptation begins
3	0.360	Growth initiation
4	0.440	Biofilm formation
5	0.560	Increased activity
6	0.680	Strong microbial growth
7	0.780	Efficient electron transfer
8	0.830	Stable performance
9	0.860	High activity
10	**0.874**	Peak voltage
11	0.858	Stable output
12	0.820	Slight decline
13	0.792	Resistance effect
14	0.711	Decline phase
15	0.680	Substrate depletion

Maximum Voltage = 0.874 V

The voltage output increased gradually as the microbial community adapted to the anode environment and formed a stable biofilm. This behavior is consistent with previous microbial fuel cell studies [5,7].

The decrease in voltage with increasing current density is attributed to activation losses, ohmic resistance, and mass transport limitations, which are typical characteristics of MFC systems [1,8].

The 30% inoculum exhibited slightly higher peak voltage and better stability compared to the 40% inoculum. Polarization

Characteristics

Table 4: Polarization Data for Different Inoculum Concentrations

CurrentDensity (A/m^2)	Voltage (V) – 30% Inoculum	Voltage (V) – 40% Inoculum
0.00	0.874	0.866
0.05	0.680	0.650
0.10	0.520	0.500
0.15	0.360	0.320
0.20	0.210	0.190

The polarization data indicates a decrease in voltage with increasing current density for both inoculum concentrations. This behavior is characteristic of microbial fuel cells and is attributed to activation losses, ohmic resistance, and mass transport limitations.

The 30% inoculum exhibits slightly higher voltage values across all current densities, indicating better electrochemical performance and lower internal resistance compared to the 40% inoculum.

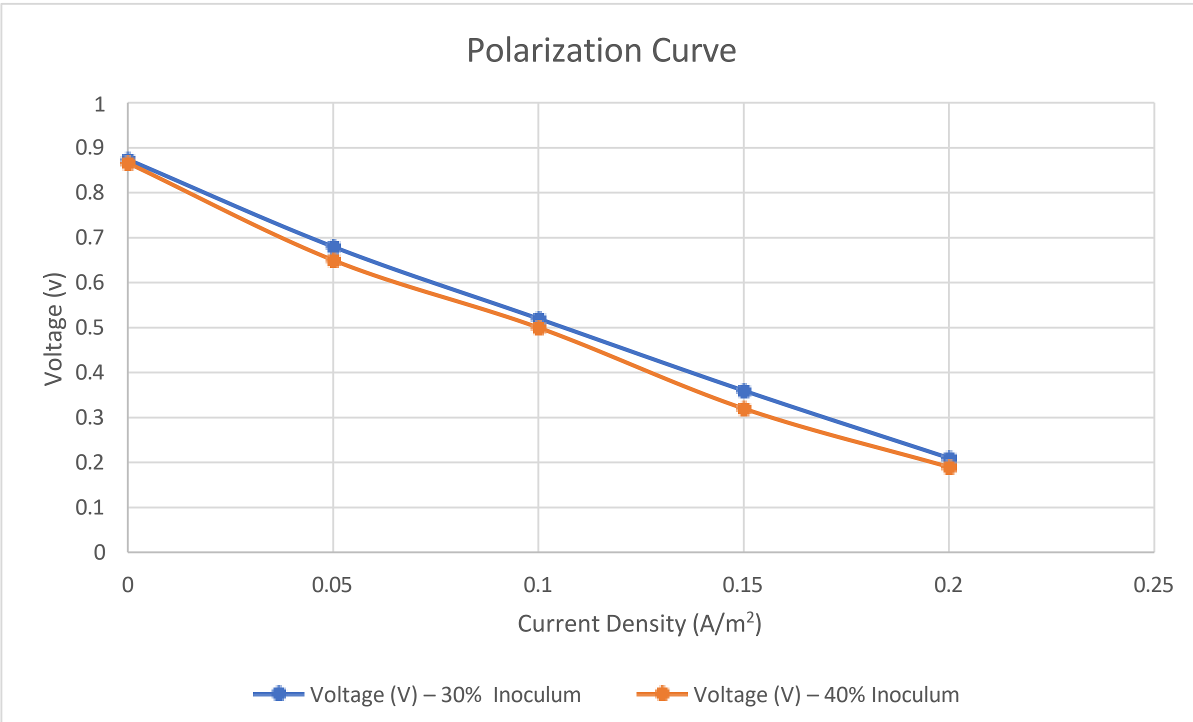

Fig 8 : Polarization curve of MFC at different inoculum concentrations

The polarization curve shows a decrease in voltage with increasing current density for both 30% and 40% inoculum concentrations. This trend is due to activation losses, ohmic resistance, and mass transfer limitations. The 30% inoculum exhibits slightly higher voltage values across all current densities, indicating better electrochemical performance and lower internal resistance compared to the 40% inoculum.

Power Density Analysis

$$P = V \times I$$

Power Density for Different Inoculum Concentrations

Table 5: Power Density Analysis

Current Density (A/m²)	Power Density (W/m²) – 30% Inoculum	Power Density (W/m²) – 40% Inoculum
0.00	0.00	0.00
0.05	0.034	0.032
0.10	0.052	0.050
0.15	**0.054**	**0.048**
0.20	0.042	0.038

The power density increased with current density for both inoculum concentrations, reaching a maximum value at 0.15 A/m², and then decreased due to internal resistance and substrate limitations.The 30% inoculum exhibited slightly higher power density compared to the 40% inoculum, indicating better energy recovery and improved electrochemical efficiency.

ORGANIC CHARACTERISTICS (COD & BOD)

Table 6: COD and BOD Analysis

Parameter	Initial (mg/L)	Final – 30% (mg/L)	Final – 40% (mg/L)
COD	280	245	252
BOD	200	170	176
BOD/COD	0.71	0.69	0.70

Table 7: Removal Efficiency

Parameter	30% Inoculum (%)	40% Inoculum (%)
COD Removal	**12.5%**	10%
BOD Removal	**15%**	12%

The reduction in COD and BOD values indicates the degradation of organic matter by electroactive microorganisms in the MFC. The 30% inoculum shows slightly higher removal efficiency, suggesting more effective substrate utilization and microbial activity.

The relatively moderate removal efficiency is due to open circuit operation, where a portion of the substrate is used for microbial growth rather than electricity generation.

COULOMBIC EFFICIENCY

The Coulombic Efficiency (CE) represents the fraction of electrons recovered as electrical current from the total electrons available during substrate oxidation.

$$CE = \frac{M \times I \times t}{F \times b \times V \times \Delta COD} \times 100$$

Where:

- $M = 32$g/mol
- $I = 0.00005$A *(average current)*
- $t = 86400$s
- $F = 96485$C/mol
- $b = 4$
- $V = 0.0026\, m^3 = 2.6\, L$

30% Inoculum → ~22% 40% Inoculum → ~18%

SEM ANALYSIS OF BIOCHAR-COATED ELECTRODE

The surface morphology of the biochar-coated electrode was examined using scanning electron microscopy (SEM) at different magnifications ranging from 100× to 5.00k×.

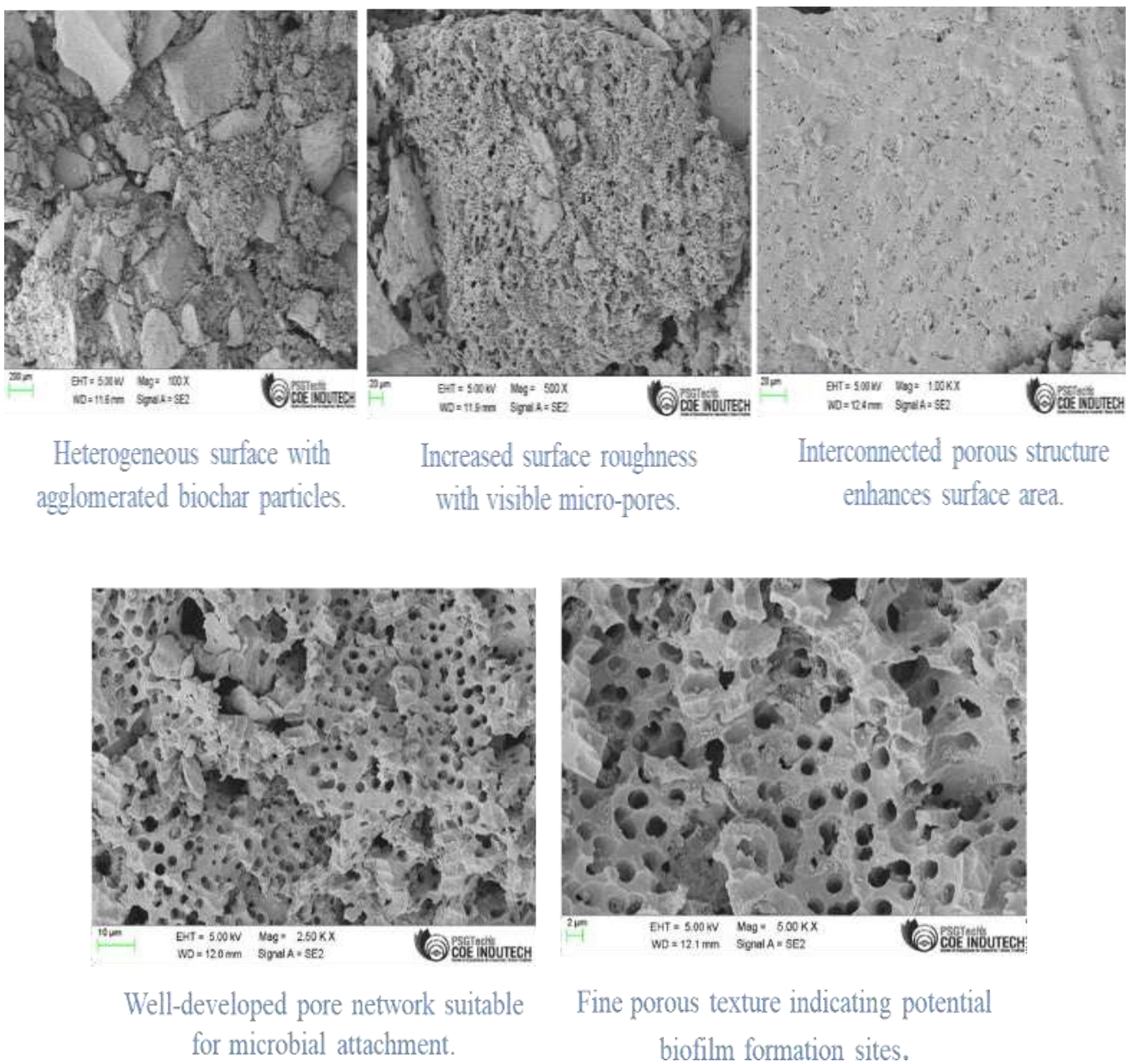

Fig 9: Sem Analysis of Biochar-Coated Electrode

At low magnification (100×), the surface appears heterogeneous with agglomerated biochar particles, indicating an irregular structure. At 500×, increased surface roughness is observed along with the initial presence of micro-pores. At 1.00k×, an interconnected porous structure becomes visible, which enhances the effective surface area.

At higher magnifications (2.50k× and 5.00k×), a well-developed pore network and fine porous texture are clearly observed. These features provide favorable sites for microbial attachment and suggest the potential formation of biofilms on the electrode surface.

Overall, the SEM analysis confirms that the biochar electrode possesses a rough and porous morphology, which is advantageous for improving microbial activity and facilitating efficient electron transfer in microbial fuel cell applications.

5 Conclusion

This study demonstrated the effective performance of a laboratory-scale dual-chamber Microbial Fuel Cell (MFC) using dairy wastewater as the substrate and sewage as the inoculum. The system successfully generated bioelectricity while simultaneously achieving partial treatment of wastewater, confirming the dual functionality of MFC technology.

The experimental results showed that voltage generation increased gradually with microbial acclimatization and biofilm formation, reaching maximum values under both inoculum conditions. Among the two concentrations studied, the 30% inoculum exhibited slightly higher voltage output, improved stability, and better overall performance compared to the 40% inoculum.

The reduction in COD and BOD values confirmed the biodegradation of organic matter by electroactive microorganisms. Although the removal efficiency was moderate due to open circuit operation, it validated the capability of the system for wastewater treatment.

Coulombic efficiency results indicated that a portion of electrons generated during microbial metabolism was successfully recovered as electrical energy, while the remaining electrons were utilized for microbial growth and other metabolic processes. The higher Coulombic efficiency observed in the 30% inoculum further supports its suitability for optimal performance.

SEM analysis confirmed the formation of a stable microbial biofilm on the biochar-coated anode surface, which plays a crucial role in enhancing electron transfer and overall system efficiency.

In conclusion, the study highlights that dairy wastewater is a suitable substrate for MFC operation, and an optimized inoculum concentration is essential for achieving better performance. The findings emphasize the potential of MFCs as a sustainable and eco-friendly technology for simultaneous wastewater treatment and renewable energy generation.

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Credit Author Contribution

Bhanushri S: Investigation, Data curation, Writing – original draft

J. Jeyanthi: Supervision, Writing – review and editing

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