Bio-Electrical Performance of a Tofu-Wastewater Dual-Chamber Microbial Fuel Cell with HCl-Activated Carbon Anodes for Renewable Bioelectricity Generation

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Abstrak

Tofu liquid waste is a high-content organic pollutant that has the potential to become a renewable energy source through Microbial Fuel Cell (MFC) technology. This study aims to characterize the bio-electrochemical performance of indigenous bacteria from tofu waste in a Dual-Chamber MFC (DCMFC) system using recycled carbon anodes from spent batteries. The innovation of this research lies in the electrode activation through a combination of thermal and chemical treatments (32% HCl for 12 hours) to increase surface porosity and biocompatibility. The system utilized a 17 cm salt bridge with a 7% (w/v) agar and 15% (w/v) NaCl matrix. The results showed a highly consistent increase in Open Circuit Voltage (OCV), rising from 48.0 mV to a peak of 231.0 mV at the 31st hour. Linear regression analysis indicated an average growth rate of 4.81 mV/hour with a very high coefficient of determination (R^2) of 0.9066, indicating excellent stability in ionic and electrical conduction. Qualitatively, the formation of a macroscopic, wax-like biofilm was observed on the anode surface at the end of the observation period, providing physical validation for the surge in the system's electrical performance. This study proves that engineered recycled electrodes and the utilization of local indigenous bacteria are capable of generating stable and progressive electrical energy.

Keywords: Microbial Fuel Cell; Tofu Waste; Indigenous Bacteria; Biofilm; Activated Carbon; Renewable Energy.

INTRODUCTION

The tofu industry in Indonesia currently provides a significant economic contribution to society; however, its operations generate liquid waste with a very high organic load. The characteristics of this wastewater include complex carbon compounds that have the potential to pollute aquatic ecosystems if not managed appropriately. Conversely, this abundant organic content actually holds renewable energy potential that can be converted into electrical power through the utilization of Microbial Fuel Cell (MFC) technology. An MFC system is a bio-electrochemical innovation that employs microorganisms as biocatalysts to oxidize organic substrates while directly harvesting electrons (Barakat et al., 2023).

The success of energy production in this system depends heavily on the bacteria's ability to transfer electrons to the anode surface. The use of indigenous bacteria naturally residing in tofu waste offers the advantage of more stable environmental adaptation compared to the use of pure external bacterial strains (Zater et al., 2024). Several studies have demonstrated that the native microbial populations in tofu waste possess electrogenic properties that can be optimized through a salt-bridge reactor design (Permana et al., 2024). In addition to biological factors, the selection of electrode materials plays a vital role in determining the resulting electrical performance (Shahid et al., 2022).

Carbon-based materials are the primary choice in MFC research due to their excellent chemical stability and biocompatibility for biofilm growth (Yoon et al., 2024). However, carbon rods require specific modifications to perform optimally. Concentrated acid treatment on the carbon surface is known to clean active sites and enhance reduction-oxidation reactions at the molecular level (Bao et al., 2024). These anode surface modification efforts aim to increase power density by accelerating electron transfer from the bacteria to the external circuit (Delgado et al., 2024). Nonetheless, the integration of activated recycled battery carbon with the potential of native tofu waste bacteria still requires more in-depth study.

Therefore, this research aims to characterize the bio-electrochemical profile of indigenous tofu waste bacteria in a Dual-Chamber MFC system. The study focuses on the effectiveness of recycled carbon anodes activated thermally and chemically using concentrated HCl to accelerate the start-up phase and enhance the stability of the electrical vol.

METHODOLOGY

DCMFC Reactor Design

The researcher employed a Dual-Chamber Microbial Fuel Cell (DCMFC) configuration consisting of two main compartments, each with a volume capacity of 500 mL. These two compartments were connected by a salt bridge, which serves as a pathway for proton migration (Permana et al., 2024). The salt bridge was constructed using silicone tubing with a diameter of 5/16 inch and an ionic path length of 17 cm. The researcher optimized the salt bridge matrix with a mixture of 7% (w/v) agar and 15% (w/v) NaCl to ensure stable ionic conductivity throughout the operational period (Kirmizakis et al., 2025).

FIGURE 1. Schematic Diagram of the Dual-Chamber Microbial Fuel Cell Circuit

Preparation and Modification of Recycled Carbon Electrodes

The anode in this study utilized carbon rods recycled from spent batteries. The researcher performed a series of preparation procedures to enhance the electrode performance:

1). Cleaning and Thermal Activation: The carbon core was extracted from the battery, physically cleaned, and then burned for 5 minutes to open the pore structure. The carbon surface was subsequently finely sanded to remove residual oxide layers that could inhibit electrical flow.
2). Chemical Activation: The carbon rods were immersed in a 32% hydrochloric acid (HCl) solution for 12 hours to remove metallic impurities from the active carbon sites (Bao et al., 2024).
3). Rinsing: The electrodes were rinsed thoroughly under running water to ensure that no acid residue entered the reactor system.

The final carbon rods had a diameter of 8 mm, with an immersion length in the anolyte of 3.3 cm (effective surface area of 0.829×10^{-3} m²). This surface modification step is crucial for strengthening the interaction between bacteria and the electrode to generate higher power (Delgado et al., 2024).

Circuit Installation and Measurement System

The researcher connected the anode and cathode using high-quality copper wire with a diameter of 1.5 mm. In the cathode compartment, a copper plate (6 x 3 cm) was used, connected to the wire via nuts and soldering to minimize contact resistance. Meanwhile, for the carbon anode, the end of the carbon rod was mechanically drilled to secure the copper wire firmly. The use of a stable current collector significantly influences the efficiency of electron conduction within the system (Moreno-Jimenez et al., 2024).

Operation and Data Collection

The researcher utilized diluted tofu liquid waste as the substrate, which simultaneously served as the source of indigenous bacteriamenggunakan limbah cair tahu encer sebagai substrat yang sekaligus menjadi sumber bakteri indigenous (Zater et al., 2024). The anode compartment was tightly sealed to maintain anaerobic conditions, while the cathode compartment was filled with a 0.5 M NaCl electrolyte solution. The bio-electrochemical performance was monitored through Open Circuit Voltage (OCV) values using a digital multimeter periodically over 31 hours (Barakat et al., 2023). This OCV measurement aimed to evaluate the initial bacterial colonization capacity on the anode surface before the system was subjected to an external load.

Visual-Electrochemical Correlation Analysis (Biofilm Formation)

The researcher did not rely solely on numerical data from the multimeter but also conducted a qualitative analysis through macroscopic observation of the anode surface at the end of the operational period. The appearance of a wax-like or slime-like layer on the submerged carbon surface was analyzed as the formation of an electrogenic biofilm.

The researcher correlated the thickness or presence of this physical layer with the recorded surges in OCV values. Theoretically, the formation of this biofilm serves as evidence of colonization by indigenous bacteria performing direct extracellular electron transfer (Zater et al., 2024). By combining the voltage increase data with physical evidence of the biofilm, the researcher can provide a stronger conclusion regarding the effectiveness of HCl activation in providing optimal microbial attachment sites on spent battery carbon.

RESULTS AND DISCUSSION

Electrical Performance of the DCMFC System

The researcher monitored the Open Circuit Voltage (OCV) values to evaluate the capability of indigenous tofu waste bacteria in generating electrical energy on the surface of the activated carbon anode. The observation data over 31 hours indicates a progressive upward trend in voltage, as summarized in **Table 1**.

TABLE 1. Voltage Profile (OCV) of the DCMFC System

Day	Time	Duration (Hours)	OCV (mV)	Increase Rate (mV/hour)	Microbial Growth Phase
1	13:00	0	48.0	-	Initiation & Penetration
1	20:16	7.25	135.3	12.04	Early Logarithmic Phase
2	05:00	16	140.2	0.56	Overnight Adaptation Phase
2	16:00	27	188.5	4.39	Continued Growth Phase
2	17:00	28	191.0	2.50	Biofilm Accumulation
2	**20:00**	**31**	**231.0**	**13.33**	**Peak Acceleration Phase**

At the initial stage (hour 0), the system recorded a basal voltage of 48.0 mV. The researcher argues that this relatively high initial value is a direct result of the chemical activation process using 32% HCl and thermal activation. This treatment successfully removed metallic impurities from the spent battery carbon and opened active pores, thereby facilitating immediate electron transfer after inoculation (Bao et al., 2024).

To ensure data integrity, the researcher performed visual documentation at each sampling stage. The documentation in Figure 2 demonstrates the correlation between the physical configuration of the circuit and the numerical values displayed on the digital multimeter for each data point.

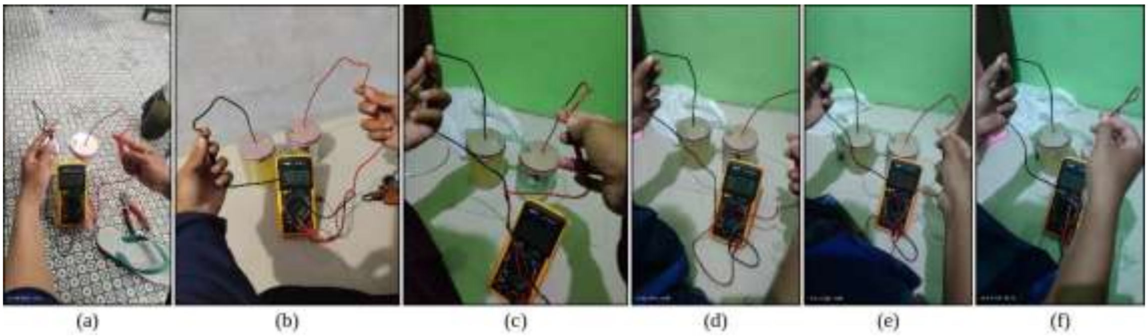

FIGURE 2. OCV measurement using a multimeter in the DCMFC system

Analysis Based on Documentation:

a). Reading Stability: The multimeter photograph displaying a value of 231 mV without significant fluctuations indicates that the connection system (nuts, soldering, and carbon bindings) is functioning effectively in conducting electrons from the biofilm to the current collector.

b). Circuit Integrity: The photograph of the circuit setup shows that the 17 cm salt bridge is installed securely (leak-free), ensuring that proton migration occurs through a single pathway via the agar matrix. This minimizes potential external disturbances that could distort the voltage readings.

Voltage Kinetics and Linearity Analysis

To understand the rate of electricity production, a linear regression analysis was performed on the voltage data over time (Figure 3). The visualization demonstrates a very strong positive correlation, represented by the trendline equation $y = 4{,}8103x + 68{,}079$.

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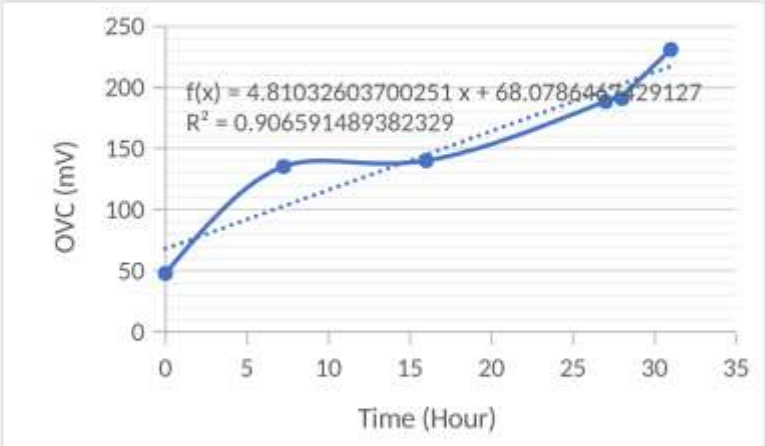

FIGURE 3. OCV Voltage Trend Graph over 31 Hours

The coefficient of determination value $R^2 = 0,9066$ indicates that 90.66% of the voltage increase is directly influenced by the system's operational duration. This demonstrates the stability of the DCMFC system in facilitating the metabolism of electrogenic bacteria. The average growth rate of 4.81 mV/hour reflects the efficiency of the 17 cm salt bridge in transferring protons to balance the electrical charge within the reactor.

The highest increase rate was recorded during the final observation period (from hour 28 to 31) at 13.33 mV/hour. This phenomenon indicates that the indigenous bacterial consortium in the tofu waste possesses strong electrogenic properties. The use of native bacteria provides the advantage of faster environmental adaptation, as reflected by the system's ability to surpass the lag phase in less than 24 hours (Zater et al., 2024).

Biofilm Observation (Wax-like Layer) and Physical Validation

Validation of the electrical data was conducted through physical observation of the electrode surface at the end of the study. The researcher discovered the formation of a white, wax-like layer covering the carbon rods submerged in the anolyte. This visual finding is presented in Figure 4.

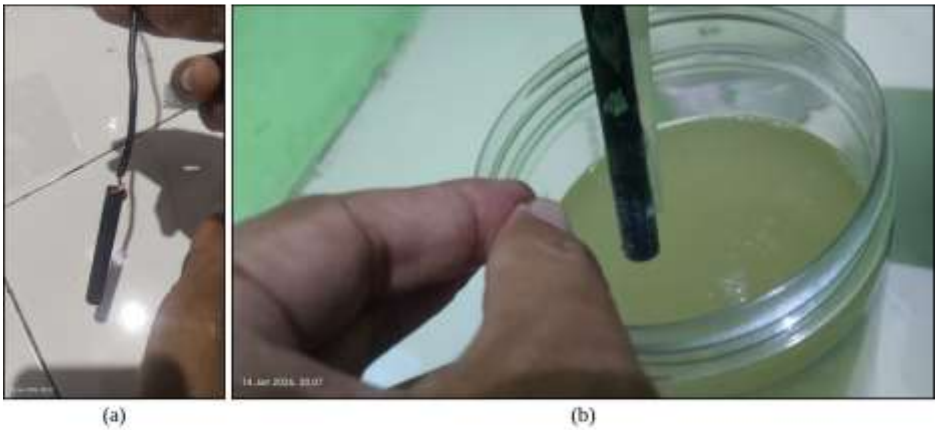

FIGURE 4. Documentation of Biofilm Observation and Physical Validation: (a) Carbon rod before immersion in tofu liquid waste (initial condition at 0 hours), (b) Carbon rod after 31 hours of immersion in tofu liquid waste, showing the emergence of a white, wax-like layer.

From a bio-electrochemical perspective, this layer represents a mature biofilm consisting of microbial cells and an extracellular polymeric matrix. The formation of this layer explains the voltage surge to 231 mV, indicating that the bacterial colonies have successfully established a stable electron conduction network on the anode surface (Yoon et al., 2024). This wax-like texture proves that the HCl activation method applied by the researcher successfully created excellent biocompatibility on the recycled carbon material, thereby allowing the bacteria to permanently "grip" the electrode (Delgado et al., 2024).

Effectiveness of the 17 cm Salt Bridge Design

The stability of the voltage increase until the end of the observation period proves that the salt bridge configuration, with a length of 17 cm and a 15% (w/v) NaCl matrix, functioned optimally. Despite the relatively long ionic pathway, the high salt concentration was able to provide an efficient proton migration path from the anode chamber to the cathode to maintain charge balance (Kirmizakis et al., 2025). This was further supported by the use of a 1.5 mm copper wiring system with soldered joints, which successfully minimized contact resistance, allowing the power generated by the biofilm to be accurately measured via the multimeter.

Role of HCl Activation and Local Substrates

The successful bacterial colonization, characterized by the "wax-like layer," confirms the effectiveness of using 32% HCl as an activating agent. Hydrochloric acid not only cleaned impurities from the spent battery carbon but also enhanced the surface biocompatibility of the electrode, allowing bacteria to adhere firmly (Delgado et al., 2024). The use of tofu liquid waste as the anolyte proved effective because it contains complex organic nutrients that support the growth of electrogenic bacteria without requiring additional external growth media.

CONCLUSION AND RECOMMENDATIONS

Conclusion

Based on the research results and data analysis conducted during 31 hours of Dual-Chamber Microbial Fuel Cell (DCMFC) operation using tofu liquid waste, the following conclusions are formulated:

1). Progressive Electrical Performance: The indigenous bacteria in tofu waste demonstrated superior bio-electrogenic activity, generating a peak voltage of 231.0 mV. Statistical analysis confirmed the linearity of the voltage increase with an R^2 value of 0.9066, indicating exceptionally high operational consistency within the system.

2). Electrode Activation Effectiveness: The recycling method for carbon electrodes involving 5-minute thermal activation and 12-hour immersion in 32% HCl proved highly effective. This treatment successfully created a biocompatible electrode surface, qualitatively evidenced by the formation of a wax-like biofilm on the anode surface.

3). System Design Optimization: The use of a 17 cm salt bridge with a 15% (w/v) NaCl concentration effectively facilitated proton migration without causing significant internal resistance, as evidenced by the average voltage increase rate of 4.81 mV/hour.

Recommendationsaran

For future research development and to enhance the quality of scientific data in subsequent publications, the researcher suggests the following:

1). Microbial Structure Analysis: It is recommended to perform Scanning Electron Microscopy (SEM) on the formed "wax-like layer" to observe the detailed physical interactions between bacterial cells and the activated carbon pores.

2). System Durability Testing: Given that the graph trend was still increasing at the 31st hour, future studies should extend the observation duration (e.g., 7–14 days) to reach a steady state and evaluate the biofilm's lifespan.

3). Power Density Measurement: Closed Circuit Voltage (CCV) testing with variations in external resistors should be conducted to calculate the polarization curve and the maximum power density the system can generate.

4). Reactor Scalability: Following the positive laboratory-scale results, it is advisable to conduct trials using larger reactor volumes to explore the potential for practical implementation within the tofu industry..

REFERENSE

Bao, W., Kubota, K. and Watanabe, T. (2024) "EFFECT OF ACID AND ALKALI TREATMENT ON OXYGEN REDUCTION REACTION ACTIVITY OF NIROGEN-DOPED ACTIVATED CARBON AS A CATHODE CATALYST IN AIR-BREATHING MICROBIAL FUEL CELLS," Journal of JSCE, 12(2), n/a. Available at: https://doi.org/10.2208/journalofjsce.24-25015.

Barakat, N.A.M. et al. (2023) "Synergistic advancements in sewage-driven microbial fuel cells: Novel carbon nanotube cathodes and biomass-derived anodes for efficient renewable energy generation and wastewater treatment," Frontiers in Chemistry, 11, 1286572. Available at: https://doi.org/10.3389/fchem.2023.1286572.

Delgado, Y. et al. (2024) "Effect of hydrochar-doping on the performance of carbon felt as anodic electrode in microbial fuel cells," Environmental Science and Pollution Research, 32(49), pp. 28253–28265. Available at: https://doi.org/10.1007/s11356-024-33338-2.

Kirmizakis, P. et al. (2025) "Microbial fuel cells to monitor natural attenuation around groundwater plumes," Environmental Science and Pollution Research, 32(4), pp. 2069–2084. Available at: https://doi.org/10.1007/s11356-024-35848-5.

Moreno-Jimenez, D.A., Hwang, M.-H. and Kim, K.-Y. (2024) "Impact of Current Collectors with Different Electrocatalytic Activities on Hydrogen Production in Microbial Electrolysis Cells with Flowable Cathodes," Energy & Fuels, 38(7), pp. 6085–6091. Available at: https://doi.org/10.1021/acs.energyfuels.4c00193.

Permana, D. et al. (2024) "Performance of salt-bridge microbial fuel cell (SB-MFC) with various microorganism cultures on the generation of electricity from tofu wastewater," Indonesian Journal of Biotechnology, 29(2), p. 48. Available at: https://doi.org/10.22146/ijbiotech.80928.

Shahid, K. et al. (2022) "Enhanced bioenergy and nutrients recovery from wastewater using hybrid anodes in microbial nutrient recovery system," Biotechnology for Biofuels and Bioproducts, 15(1), p. 19. Available at: https://doi.org/10.1186/s13068-022-02116-y.

Yoon, Y. et al. (2024) "Prevalence of Escherichia coli in electrogenic biofilm on activated carbon in microbial fuel cell," Applied Microbiology and Biotechnology, 108(1), p. 52. Available at: https://doi.org/10.1007/s00253-023-12829-1.

Zater, D.K. et al. (2024) "Investigating the bacterial consortia properties of electrogenic anodic biofilm in a double-chamber microbial fuel cell: Electrochemical, physical, biochemical and molecular characterization," Sustainable Environment Research, 34(1), p. 9. Available at: https://doi.org/10.1186/s42834-024-00215-z.