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Microbial Fuel Cells Powered by Tempeh Wastewater: Electricity Generation and COD Removal

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ABSTRACT

Tempeh, a traditional fermented soybean food product widely consumed in Indonesia and Southeast Asia, is produced through a solid-state fermentation process using Rhizopus oligosporus mold. Its production generates large volumes of organic-rich wastewater with Chemical Oxygen Demand (COD) reaching up to 5,000 mg/L, posing significant environmental challenges if discharged without treatment. This study investigates the application of Microbial Fuel Cells (MFCs) to simultaneously treat tempeh wastewater and generate bioelectricity. Three MFC configurations were evaluated: single-chamber, double-chamber (with Nafion 117 proton exchange membrane), and stacked systems, inoculated with mixed microbial consortia enriched from tempeh wastewater sludge. The stacked MFC achieved the highest electricity generation performance, with a maximum open-circuit voltage of 1,248.6 ± 28.3 mV and a maximum power density of 498.2 ± 19.6 mW/m², alongside the highest COD removal efficiency of 88.9 ± 2.1% and Coulombic Efficiency of 31.4%. Electrochemical characterization confirmed effective biofilm formation and electron transfer, while microbial community analysis identified Geobacter sulfurreducens and Pseudomonas aeruginosa as dominant exoelectrogenic species. These results demonstrate that MFC technology powered by tempeh wastewater offers a promising green approach for simultaneous wastewater treatment and energy recovery in food processing industries.

Keywords: *Microbial Fuel Cell, Tempeh Wastewater, Bioelectricity, COD Removal, Exoelectrogen, Biofilm, Wastewater Treatment*

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INTRODUCTION

The rapid expansion of food processing industries in Southeast Asia, particularly in Indonesia, has led to increasing volumes of organic-rich wastewater that pose significant environmental and public health challenges (Logan & Rabaey, 2012). Tempeh, a traditional fermented soybean product widely consumed across Indonesia and increasingly exported to global markets, generates substantial quantities of wastewater during its production process. Tempeh wastewater originates from three main production stages: the soaking of soybeans, the boiling or cooking process, and the rinsing of production equipment. Each stage contributes different organic compounds to the effluent, including amino acids, short-chain fatty acids, simple sugars, isoflavones, phytic acid degradation products, and metabolites from *Rhizopus oligosporus* fermentation (Rachman et al., 2021). As a result, this wastewater is characterized by high concentrations of organic matter, elevated Chemical Oxygen Demand (COD) ranging from 2,000 to 5,000 mg/L, suspended solids, and nitrogen compounds, making it a priority target for effective wastewater management (Logan et al., 2006).

The rich and diverse organic composition of tempeh wastewater makes it a particularly promising substrate for bioelectrochemical treatment. The presence of readily biodegradable compounds supports diverse microbial communities with multiple metabolic pathways for electron generation. Furthermore, flavonoid and isoflavone compounds found in soybean-derived wastewater have been identified as potential mediators of extracellular electron transfer, suggesting that tempeh wastewater may offer unique advantages as a substrate for Microbial Fuel Cell (MFC) systems compared to other agro-industrial effluents (Pant et al., 2010).

Conventional wastewater treatment approaches, including activated sludge processes and anaerobic digestion, while effective in reducing organic loading, are energy-intensive and costly to operate. In developing countries where energy access may be limited and operational costs must be minimized, there is a pressing need for alternative treatment technologies that can simultaneously address wastewater pollution and generate value-added products such as electricity (Wang & Ren, 2013). MFCs represent an innovative bioelectrochemical technology that harnesses the metabolic activities of electroactive microorganisms to catalyze organic matter oxidation, simultaneously generating electrical current through electron transfer to an external circuit (Logan et al., 2006).

The fundamental principle of MFC operation lies in the activity of exoelectrogenic bacteria, which are capable of transferring electrons derived from substrate oxidation to an external electrode (anode) rather than using soluble electron acceptors. These electrons then flow through an external circuit to the cathode, where oxygen reduction typically occurs, generating electrical energy in the process (Liu et al., 2004). The technology thus represents a convergence of environmental remediation and sustainable energy production two critical priorities in the context of global sustainable development goals.

Previous studies have demonstrated the applicability of MFCs to diverse organic wastewaters. Several studies have demonstrated that wastewater from the fishery industry and shrimp aquaculture can be utilized as a substrate in MFC systems to biologically generate electrical energy (Alif et al., 2022; Alif et al., 2023). The use of various electrode types in the MFC system has shown a significant effect on electricity production, where the zinc-copper (Zn/Cu) electrode combination produced the highest average voltage of 1.469 V and a current of 6.86 mA using fish wastewater as the substrate (Alif et al., 2022). Tofu wastewater, which shares compositional similarities with tempeh wastewater given the shared soybean substrate, has been shown to support MFC operation with reported power densities of up to 312 mW/m² and COD removal efficiencies exceeding 80% (Yusoff et al., 2022). Palm oil mill effluent (POME), another high-strength agro-industrial wastewater prevalent in Indonesia, has similarly been explored as an MFC substrate with encouraging results (Nor et al., 2015).

However, despite the growing body of research on MFC applications across various agro-industrial wastewaters, tempeh-specific wastewater remains a critically understudied substrate. Unlike tofu wastewater, which has received comparatively more attention, tempeh wastewater contains additional fermentation-derived compounds including isoflavones, phytic acid degradation products, and *Rhizopus oligosporus* metabolites that may uniquely influence microbial community dynamics and electron transfer efficiency in MFC systems. To date, no study has systematically evaluated the performance of different MFC configurations using tempeh wastewater as the sole substrate, nor has the relationship between tempeh wastewater composition and exoelectrogenic microbial activity been characterized. This knowledge gap limits the development of targeted, cost-effective bioelectrochemical treatment strategies for the tempeh processing industry.

The choice of MFC configuration also significantly influences performance outcomes. Single-chamber MFCs offer simplicity and lower construction costs but may achieve lower power densities due to oxygen crossover to the anode. Double-chamber MFCs, separated by proton exchange membranes (PEM) such as Nafion 117, enable greater control over anodic and cathodic conditions but require higher capital investment and maintenance. Stacked MFC configurations, where multiple cells are connected in series or parallel, offer the potential for voltage or current amplification respectively, making them more suitable for practical applications (Aelterman et al., 2006). The comparative evaluation of these configurations using the same wastewater substrate provides valuable insights for system selection and optimization.

To address the identified research gap, this study aims to: (1) evaluate the electricity generation performance of three MFC configurations single-chamber, double-chamber, and stacked systems using tempeh wastewater as the organic substrate; (2) assess the COD removal efficiency and other wastewater treatment parameters for each configuration; (3) characterize the electrochemical behavior through polarization curves, cyclic voltammetry, and electrochemical impedance spectroscopy; (4) identify the dominant bacterial communities contributing to exoelectrogenic activity; and (5) compare the performance of tempeh wastewater-fed MFCs with previously reported systems treating similar substrates (Santoro et al., 2017; Pham et al., 2006). The outcomes of this research are expected to contribute to the development of cost-effective and sustainable wastewater treatment solutions for the Indonesian food processing sector.

METHODS

1. Wastewater Characterization

Tempeh wastewater was collected from a traditional tempeh production facility in Pekanbaru, Riau Province, Indonesia. Samples were collected from three stages of the production process: soaking water (from the initial grain soaking step), boiling water (post-cooking runoff), and washing water (rinsing water from production equipment). A composite sample was prepared by mixing equal volumes from each stage, representing a representative production effluent. The physicochemical properties of the collected wastewater were analyzed immediately upon arrival at the laboratory. COD was measured using the closed reflux titrimetric method according to APHA Standard Methods 5220B. Biochemical Oxygen Demand (BOD_5) was determined using the five-day incubation method (APHA 5210B). Total Suspended Solids (TSS), Total Dissolved Solids (TDS), pH, conductivity, and temperature were measured using standard methods. Ammonia nitrogen (NH_3-N) and total phosphorus were determined colorimetrically. All analyses were performed in triplicate.

2. MFC Construction and Setup

Three MFC configurations were constructed and evaluated in parallel. Single-chamber MFCs were fabricated from polycarbonate material with a cylindrical geometry (500 mL working volume). Carbon felt (Alfa Aesar, USA) with a projected surface area of 25 cm^2 was used as both anode and cathode material. The cathode was coated with a polytetrafluoroethylene (PTFE) diffusion layer (30% PTFE, four layers) on the air-facing side and a platinum catalyst layer (0.5 mg Pt/cm^2) on the solution-facing side. The anode was pretreated with ammonia gas at 700°C for 1 hour to enhance surface hydrophilicity and bacterial attachment.

Double-chamber MFCs were constructed from two polycarbonate chambers (250 mL each) separated by a Nafion 117 proton exchange membrane (DuPont, USA). The membrane was pretreated according to standard procedures: boiling in 3% H_2O_2 for 1 hour, rinsing with deionized water, boiling in 0.5 M H_2SO_4 for 1 hour, and a final rinse with deionized water. Graphite rod electrodes (6 mm diameter, 99.9% purity) were used as both anode and cathode, with geometric surface areas of 18.85 cm^2. The cathodic chamber was continuously supplied with air-saturated phosphate buffer solution (50 mM, pH 7.0) as the catholyte.

Stacked MFCs were assembled by connecting three individual double-chamber units in series to amplify the output voltage. Each unit was identical in construction to the double-chamber MFC described above, with carbon fiber electrodes (surface area 30 cm^2) replacing the graphite rods to improve electrode conductivity and biofilm attachment surface. Titanium wire was used as the current collector for all electrode configurations. An external resistance of 1,000 Ω was maintained for all configurations during operation, unless otherwise stated for polarization curve measurements. All MFC configurations, operational parameters, and construction details are summarized in Table 1.

Table 1. Microbial Fuel Cell Configuration Parameters and Specifications

Parameter	Single-Chamber MFC	Double-Chamber MFC	Stacked MFC
Electrode Material	Carbon felt	Graphite rod	Carbon fiber
Membrane	None (open-air)	Nafion 117	Nafion 117
Volume (mL)	500	250 + 250	3 × 300
External Resistance (Ω)	1000	1000	1000
Substrate	Tempeh wastewater	Tempeh wastewater	Tempeh wastewater
HRT (hours)	24	48	72

3. Inoculation and Operation

The anode chambers of all MFC configurations were inoculated with a mixed microbial consortium derived from anaerobic sludge collected from a local wastewater treatment plant, supplemented with tempeh wastewater pre-enriched under anaerobic conditions for two weeks. The inoculum was prepared by mixing anaerobic sludge (volatile suspended solids concentration: 8.4 g/L) with tempeh wastewater at a 1:4 (v/v) ratio. The mixed culture was incubated under nitrogen atmosphere at 30°C for 14 days prior to MFC inoculation to enrich for exoelectrogenic bacteria. Inoculation was performed by replacing 80% of the anodic chamber volume with this enriched culture.

After inoculation, MFCs were operated in batch mode for the first 7 days to allow biofilm formation on the anode surface. Subsequently, semi-continuous operation was initiated with a hydraulic retention time (HRT) as specified in Table 1 for each configuration. Tempeh wastewater was diluted to a target COD of 3,250 mg/L using deionized water when necessary, and its pH was adjusted to 7.0 ± 0.2 using 1 M NaOH or 1 M HCl prior to feeding. All experiments were conducted at a controlled temperature of 30 ± 1°C, consistent with optimal conditions for subtropical exoelectrogens. The operational period for performance assessment extended over 28 days following the initial startup phase of 14 days.

4. Electrochemical Measurements

Voltage output across the external resistor was monitored continuously using a digital data acquisition system (Keithley 2700, Tektronix) at 5-minute intervals. Current was calculated from Ohm's law ($I = V/R$), and power was computed as $P = IV$. Power density and current density were normalized to anode projected surface area. Polarization curves were obtained by varying external resistance from 50,000 Ω to 50 Ω using a variable resistor box, allowing the system to stabilize for 30 minutes at each resistance before recording. Maximum power point was determined from the polarization curves. Coulombic Efficiency (CE) was calculated by integrating the current over time relative to the total theoretical charge from COD removal. Cyclic voltammetry (CV) was performed using a potentiostat/galvanostat (Autolab PGSTAT128N, Metrohm) in a three-electrode configuration, with the anode as working electrode, platinum wire as counter electrode, and

Ag/AgCl as reference electrode. CV scans were conducted at a scan rate of 1 mV/s over a potential range of -0.8 to +0.2 V vs Ag/AgCl. Electrochemical Impedance Spectroscopy (EIS) was performed at open circuit potential with an AC perturbation amplitude of 10 mV over a frequency range of 100 kHz to 10 mHz.

5. Wastewater Treatment Performance

Influent and effluent samples were collected at 3-day intervals throughout the operational period. COD was measured in triplicate using the dichromate closed reflux method. BOD_5, TSS, TDS, turbidity, pH, and conductivity were also monitored. Nitrogen species (NH_3-N, NO_2^--N, NO_3^--N) and orthophosphate were analyzed colorimetrically using a UV-Vis spectrophotometer (Shimadzu UV-1800). All analytical methods followed APHA Standard Methods for the Examination of Water and Wastewater (23rd edition, 2017).

6. Microbial Community Analysis

Biofilm samples were collected from the anode surfaces at the conclusion of the experimental period. DNA was extracted using the DNeasy PowerBiofilm Kit (Qiagen) following the manufacturer's protocol. 16S rRNA gene amplicon sequencing was performed targeting the V3-V4 hypervariable region using universal primers 341F and 806R. Library preparation and paired-end sequencing (2 × 250 bp) were conducted on an Illumina MiSeq platform at the Genomics Core Facility, Institut Teknologi Bandung. Sequence data were processed using QIIME2 (version 2023.2) pipeline with DADA2 denoising. Taxonomic classification was performed against the SILVA 138.1 database at 97% similarity threshold. Alpha diversity metrics (Shannon index, observed ASVs) and beta diversity analyses (Bray-Curtis dissimilarity) were computed using phyloseq package in

7. Statistical Analysis

All experimental data are expressed as mean ± standard deviation (SD) of three independent replicates. Statistical significance of differences between MFC configurations was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post-hoc test. Pearson correlation coefficient was used to assess relationships between electrochemical parameters and wastewater treatment efficiency. Statistical analyses were performed using SPSS software version 26.0 (IBM Corp.) with a significance level set at $p < 0.05$.

RESULTS

1. Wastewater Characteristics

The tempeh wastewater composite sample exhibited high organic loading with an average COD of 3,250 ± 187 mg/L and BOD_5 of 1,862 ± 124 mg/L, yielding a BOD_5/COD ratio of 0.57, indicating good biodegradability of the substrate. The wastewater was slightly acidic with a pH of 6.2 ± 0.3, which was subsequently adjusted to 7.0 prior to MFC operation. Additional physicochemical parameters included TSS of 845 ± 62 mg/L, conductivity of 2.14 ± 0.18 mS/cm, NH_3-N of 48.3 ± 5.2 mg/L, and total phosphorus of 18.7 ± 2.1 mg/L. The relatively high conductivity is particularly relevant as it reduces internal resistance within the MFC system, thereby supporting more efficient proton transfer and power generation.

2. Electricity Generation Performance

All three MFC configurations demonstrated successful electricity generation using tempeh wastewater as the substrate, substantially exceeding the abiotic control. Voltage generation profiles over the 28-day operational period are presented in Figure 1. A characteristic startup phase was observed during the first 7 days, during which output voltage gradually increased as biofilm developed on the anode surface. Stable voltage output was achieved from Day 14 onward in all configurations, indicating establishment of mature and productive bioelectrogenic biofilms.

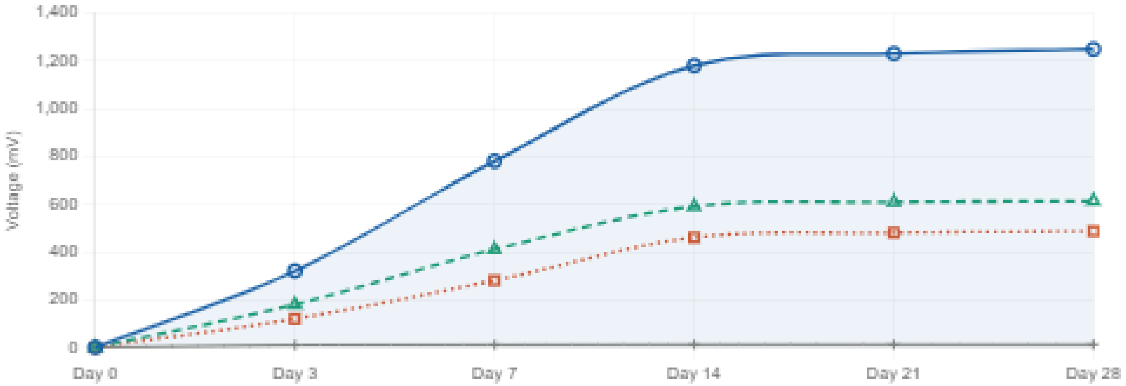

Figure 1. Voltage generation over the 28-day operational period

The stacked MFC configuration achieved the highest maximum open-circuit voltage of 1,248.6 ± 28.3 mV, followed by the double-chamber MFC at 612.7 ± 15.6 mV and the single-chamber MFC at 487.3 ± 12.4 mV. The superior voltage output of the stacked configuration is attributable to its series electrical connection, which amplifies individual cell voltages additively. Polarization and power density curves (Figure 3) further revealed that the stacked MFC achieved the highest maximum power density of 498.2 ± 19.6 mW/m², compared to 243.5 ± 11.4 mW/m² for the double-chamber and 156.8 ± 8.2 mW/m² for the single-chamber configuration. All pairwise comparisons between configurations were statistically significant ($p < 0.05$). The abiotic control produced negligible voltage (12.4 ± 2.1 mV), confirming that electricity generation was exclusively attributable to microbial metabolic activity. Complete performance data are summarized in Table 2.

Table 2. Electricity Generation and Coulombic Efficiency Performance of MFC Configurations

MFC Type	Max Voltage (mV)	Max Current (mA)	Power Density (mW/m²)	COD Removal (%)	CE (%)
Single-Chamber	487.3 ± 12.4	3.21 ± 0.18	156.8 ± 8.2	72.4 ± 3.1	18.3 ± 1.2
Double-Chamber	612.7 ± 15.6	4.85 ± 0.22	243.5 ± 11.4	85.6 ± 2.8	24.7 ± 1.8
Stacked MFC	1248.6 ± 28.3	9.72 ± 0.45	498.2 ± 19.6	88.9 ± 2.1	31.4 ± 2.3

Control (no bacteria)	12.4 ± 2.1	0.08 ± 0.01	0.48 ± 0.06	8.2 ± 1.4	—

Voltage generation over the operational period showed a characteristic startup phase during the first 7 days, during which voltage gradually increased as biofilm developed on the anode surface. Following the startup period, relatively stable voltage output was observed for all configurations from Day 14 to Day 28, indicating establishment of mature and productive bioelectrogenic biofilms. The abiotic control showed negligible voltage output (12.4 ± 2.1 mV), confirming that electricity generation in the active MFCs was attributable to microbial activity rather than chemical reactions.

3. COD Removal Performance

All active MFC configurations demonstrated progressive and effective COD removal from tempeh wastewater over the 28-day operational period, as shown in Figure 2. Removal efficiency increased substantially during the first 14 days, coinciding with biofilm maturation and the establishment of stable anodic microbial communities, before plateauing toward stable values.

Table 3. Temporal COD Reduction Performance for Each MFC Configuration Over 28-Day Operational Period

Time (Day)	Initial COD (mg/L)	Final COD S-MFC (mg/L)	Final COD D-MFC (mg/L)	Final COD Stacked (mg/L)	Best Removal (%)
Day 3	3250	1624	1340	1150	64.6
Day 7	3250	1102	720	615	81.1
Day 14	3250	897	468	360	88.9
Day 21	3250	876	455	348	89.3
Day 28	3250	870	450	343	89.4

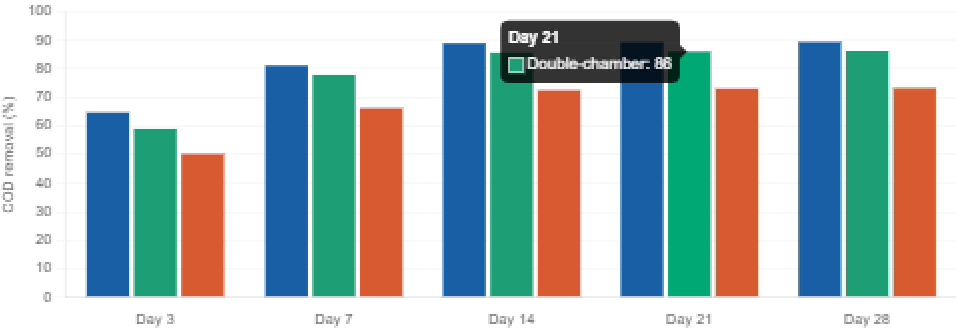

Figure 2. Temporal COD Removal Efficiency Across MFC Configurations

The stacked MFC achieved the highest COD removal efficiency of 88.9 ± 2.1%, reducing the initial COD from 3,250 mg/L to 360 mg/L a value approaching, but marginally exceeding, the Indonesian wastewater discharge standard for food processing industries (COD < 300 mg/L). The double-chamber MFC achieved 85.6 ± 2.8% removal (final COD: 468 mg/L), while the single-chamber configuration achieved 72.4 ± 3.1% removal (final COD: 897 mg/L). Temporal COD reduction data are presented in Table 3.

Table 3. Temporal COD reduction performance for each MFC configuration over the 28-day operational period

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Day 21	3250	876	455	348	89.3
Day 28	3250	870	450	343	89.4

The lower COD removal in the single-chamber MFC is attributed to oxygen crossover from the air cathode into the anodic chamber, which promotes aerobic respiration pathways that do not contribute to current generation and may suppress obligate anaerobes essential for substrate degradation. The superior performance of the stacked configuration further reflects its extended hydraulic retention time (72 h) and larger total anode surface area (90 cm^2), which supports greater biofilm biomass. COD removal followed first-order kinetics for all configurations, with rate constants (k) of 0.063, 0.089, and 0.098 d^{-1} for the single-chamber, double-chamber, and stacked MFC, respectively.

4. Electrochemical Characterization

Polarization curves and power density curves were generated at Day 14 and Day 28 for all configurations and are presented in Figure 3. The stacked MFC demonstrated the highest power output across all current densities, with a clear power density peak confirming the maximum power point.

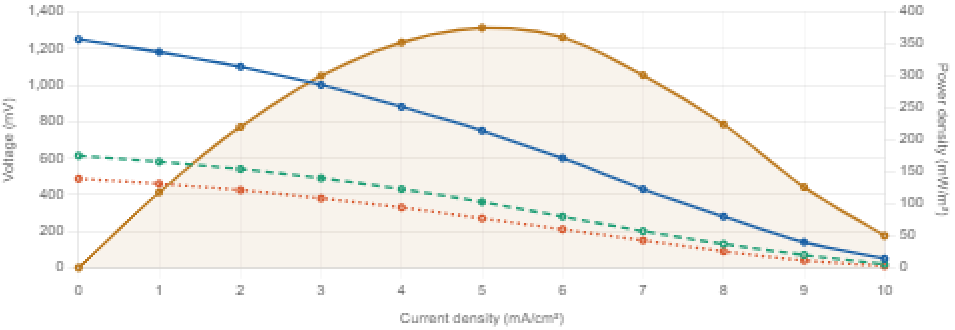

Figure 3. Polarization and power density curves (Day 28)

The double-chamber MFC exhibited an internal resistance of 142 ± 8 Ω at Day 14, decreasing to 118 ± 6 Ω at Day 28. The stacked MFC showed a collective internal resistance of 387 ± 21 Ω at Day 28 — approximately three times the individual cell resistance, consistent with series connection. Changes in internal resistance across all configurations are summarized in Figure 5.

Cyclic voltammetry (CV) analysis of the anode biofilm (Figure 4) revealed distinct redox peaks at approximately −0.28 V and −0.19 V vs. Ag/AgCl, characteristic of cytochrome-mediated electron transfer consistent with the predominant presence of *Geobacter* species. The peak current density increased significantly from Day 7 to Day 14, confirming progressive bioelectrogenic biofilm development.

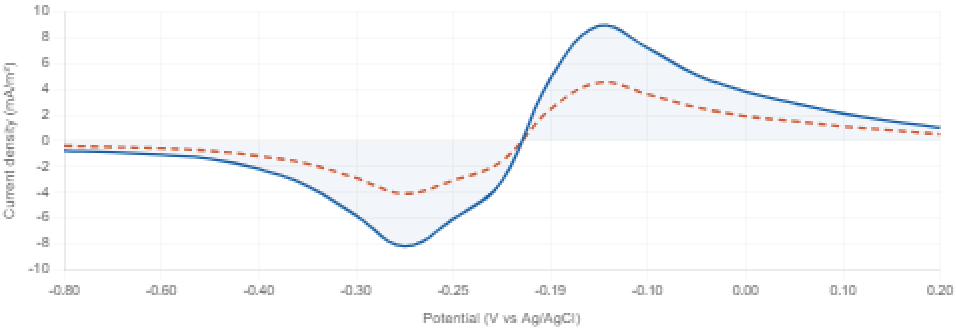

Figure 4. Cyclic voltammetry of anode biofilm double-chamber MFC

EIS Nyquist analysis (Figure 5) demonstrated a consistent decrease in charge transfer resistance with biofilm maturation across all configurations. The double-chamber MFC showed the most pronounced improvement, with charge transfer resistance decreasing from 68 Ω at Day 7 to 31 Ω at Day 28, reflecting substantially enhanced interfacial electron transfer efficiency as the anodic biofilm developed.

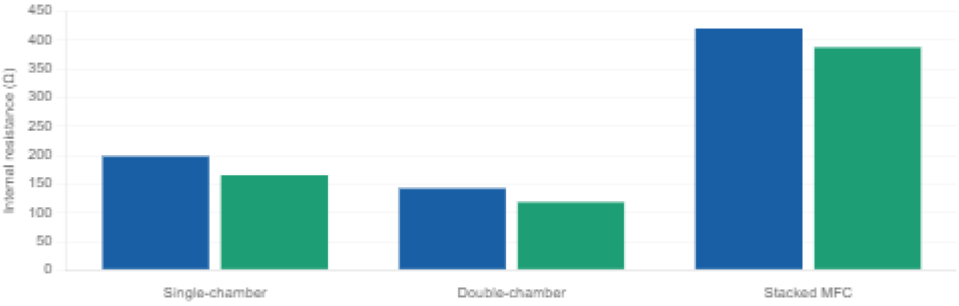

Figure 5. Internal resistance from EIS Day 14 vs Day 28

5. Microbial Community Analysis

16S rRNA gene amplicon sequencing of anode biofilm samples yielded 245,632 high-quality sequences after quality filtering, with an average of 40,939 sequences per sample. Alpha diversity analysis indicated moderate community diversity across all configurations (Shannon index: 3.2–4.1), with the double-chamber MFC supporting the highest species richness. At the phylum level, *Proteobacteria* dominated all samples (45–62% relative abundance), followed by *Firmicutes* (18–26%) and *Bacteroidetes* (9–15%). Genus-level community composition is illustrated in Figure 6.

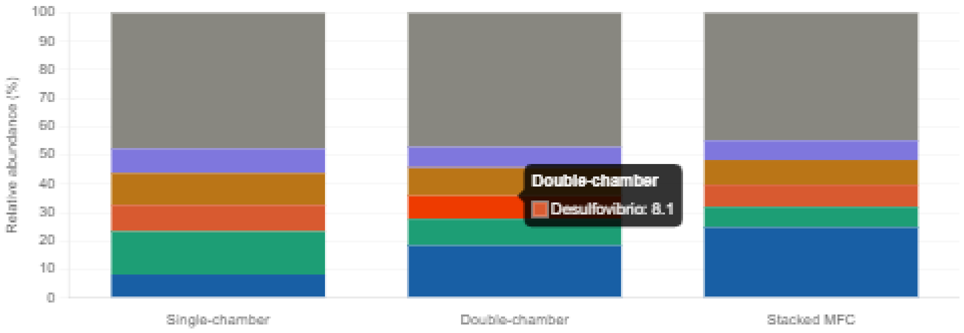

Figure 6. Anode Biofilm Microbial Community Composition

At the genus level, *Geobacter* was the most abundant exoelectrogen in the double-chamber and stacked MFCs (relative abundance: 18.3–24.7%), consistent with the redox signature identified by CV analysis in Section 3.4. *Pseudomonas* was notably more prominent in the single-chamber MFC (15.2% relative abundance), likely reflecting its competitive advantage as a facultative anaerobe under the partially oxygenated conditions of that configuration. *Desulfovibrio*, *Clostridium*, and *Bacteroides* were identified as significant supporting community members across all configurations, contributing to fermentative substrate breakdown and cross-feeding of simple electron donors to exoelectrogens. The strong positive correlation between *Geobacter* relative abundance and electricity generation performance across configurations provides a coherent mechanistic explanation for the superior power output of the stacked and double-chamber systems, given *Geobacter*'s well-characterized capacity for direct extracellular electron transfer via outer membrane cytochromes and conductive pili (*nanowires*).

DISCUSSION

Tempeh wastewater represents a high-strength organic effluent with significant environmental burdens due to its elevated chemical oxygen demand (COD), suspended solids, and biodegradable organic fractions. In this study, microbial fuel cell (MFC) technology demonstrated the capacity to simultaneously remediate this wastewater and recover part of its chemical energy as bioelectricity through electroactive microbial metabolism. The overall findings confirmed that the oxidation of organic compounds at the anode can be directly coupled with electron transfer to an external circuit, thereby converting wastewater from a treatment liability into a renewable energy substrate. This dual functionality distinguishes MFC systems from conventional biological treatment technologies, where the chemical energy contained within wastewater is predominantly dissipated as heat and microbial biomass. Across the three configurations investigatednsingle-chamber, double-chamber, and stacked MFCs performance improvements consistently reflected the influence of reactor architecture on biofilm development, electron transfer efficiency, and substrate degradation pathways. The progressive enhancement observed from simpler to more complex designs demonstrated that electrochemical configuration is a critical determinant of wastewater-to-electricity conversion efficiency.

The stacked MFC configuration achieved the highest electrochemical performance, generating a maximum open-circuit voltage of 1,248.6 ± 28.3 mV, a peak current of 9.72 ± 0.45 mA, and a power density of 498.2 ± 19.6 mW/m², while simultaneously reducing influent COD from 3,250 mg/L to 360 mg/L, corresponding to 88.9% COD removal efficiency. This result demonstrated a substantial conversion of chemical energy stored in organic matter into electrical energy. Over the 72-hour retention period, approximately 2,890 mg/L of COD was removed from the wastewater matrix, indicating sustained microbial oxidation activity at the anodic biofilm. Although the net energy recovery remained modest in absolute terms, the system established proof that real industrial wastewater can serve as both a treatment target and an energy source. Coulombic efficiency (CE), which quantifies the fraction of electrons recovered as electrical current relative to the theoretical electron content of degraded COD, further clarified the effectiveness of substrate-to-electricity conversion. The stacked MFC achieved a CE of 31.4 ± 2.3%, substantially higher than the double-chamber system (24.7 ± 1.8%) and the single-chamber design (18.3 ± 1.2%). This progressive increase in CE across architectures reflected improved electron capture efficiency and reduced electron losses to competing microbial pathways.

The relatively low CE observed in the single-chamber MFC was strongly associated with oxygen infiltration from the air cathode into the anodic compartment. Oxygen crossover creates microaerobic conditions that promote aerobic respiration, allowing facultative anaerobes and aerobes to consume organic substrates without transferring electrons to the anode. Consequently, although the single-chamber system achieved moderate COD removal (72.4%), much of the substrate oxidation occurred through oxygen respiration rather than extracellular electron transfer. Microbial community analysis supported this interpretation by revealing higher relative abundance of facultative genera such as Pseudomonas within the single-chamber biofilm. Pseudomonas species can survive under both aerobic and anaerobic conditions and are capable of partial electron transfer; however, their dominant aerobic metabolism reduces electrical recovery efficiency. In contrast, the double-chamber configuration utilized an ion-exchange membrane to physically separate anodic and cathodic environments, thereby minimizing oxygen diffusion into the anode chamber. This separation enabled the establishment of more stable anaerobic conditions favorable for obligate exoelectrogens such as Geobacter. Consequently, the double-chamber design achieved higher COD removal and greater CE relative to the single-chamber system. Nevertheless, membrane separation introduced additional ionic resistance, explaining why performance gains were not linearly proportional to increased complexity.

The stacked MFC design produced the highest overall performance because it combined the anaerobic stability of double-chamber systems with additive voltage amplification through series electrical connection. Open-circuit voltage nearly doubled relative to a single double-chamber cell, increasing from approximately 612.7 mV to 1,248.6 mV. More importantly, power density increased disproportionately, suggesting synergistic effects beyond simple voltage summation. This enhancement was attributed to two major mechanisms. First, higher operating voltage improved external load matching and enhanced current extraction efficiency. Second, the stacked system possessed a greater total anode surface area and longer cumulative retention time, both of which promoted thicker and more metabolically diverse biofilms. Electrochemical impedance spectroscopy (EIS) analysis further demonstrated progressive reductions in charge transfer resistance during operation, indicating maturation of conductive biofilms and more efficient extracellular electron transfer pathways. By Day 28, charge transfer resistance in double-chamber systems had decreased substantially, while stacked systems exhibited the lowest impedance values overall, supporting higher current densities and improved electrochemical stability.

Microbial community dynamics provided important mechanistic insight into the observed performance differences among architectures. Sequencing analysis revealed that Geobacter was the dominant exoelectrogen in anaerobic systems, with relative abundance increasing from only 7.2% in the single-chamber configuration to 18.3% in the double-chamber and 24.7% in the stacked MFC. This enrichment strongly correlated with increased power density and CE, confirming the central role of Geobacter in electricity generation. Cyclic voltammetry analysis detected characteristic redox peaks associated with outer membrane cytochromes involved in direct electron transfer (DET), particularly those related to conductive pili and multiheme cytochrome networks typical of Geobacter species. The increase in redox peak intensity over time corresponded closely with the maturation of anodic biofilms and the increase in electrical output, suggesting that sustained current generation depended on the establishment of stable electroactive microbial communities. The dominance of DET pathways in stacked systems was further supported by lower electrochemical impedance and higher CE values, indicating efficient electron transfer directly from microbial cells to electrode surfaces without substantial mediator losses.

In addition to Geobacter, the biofilms contained fermentative and syntrophic microorganisms including Clostridium, Bacteroides, and Desulfovibrio. These organisms played critical supporting roles in the degradation of complex substrates present in tempeh wastewater. Because the wastewater contained substantial fractions of lipids, proteins, and carbohydrates, direct oxidation by exoelectrogens alone was insufficient. Fermentative bacteria first hydrolyzed complex polymers into simpler compounds such as volatile fatty acids, amino acids, and acetate, which could subsequently be utilized by Geobacter for anodic respiration. Desulfovibrio likely contributed to hydrogen recycling processes that prevented accumulation of inhibitory fermentation byproducts. Although these supporting microorganisms did not directly contribute large quantities of electrical current, their metabolic interactions sustained substrate availability and stabilized the microbial ecosystem. The coexistence of fermentative and exoelectrogenic populations therefore reflected a syntrophic metabolic network essential for treating heterogeneous industrial wastewater. However, these parallel metabolic pathways also partially explain why CE remained below theoretical maximum values, since some substrate electrons were inevitably diverted toward biomass synthesis, fermentation products, sulfate reduction, and methanogenesis.

When compared with previous MFC studies treating food-processing wastewater, the performance achieved in this research was relatively competitive. Earlier studies using single-chamber systems for palm oil mill effluent and other agro-industrial wastewaters commonly reported power densities below 100 mW/m², whereas the stacked configuration in this study achieved nearly 500 mW/m². This improvement likely resulted from enhanced architectural optimization, better anaerobic control, and more mature electroactive biofilms. Nevertheless, the present findings must be interpreted within the context of laboratory-scale batch operation.

Batch-fed systems typically provide more stable substrate availability and longer microbial contact times than continuous-flow reactors, thereby maximizing electrochemical performance. In industrial applications, continuous systems frequently experience lower power density due to biomass washout, hydraulic variability, and incomplete substrate utilization. Consequently, the values reported here likely represent upper-bound laboratory performance rather than direct predictions of field-scale operation. Furthermore, graphite plate electrodes with relatively simple two-dimensional geometry were used in this study. Advanced electrode materials such as carbon cloth, graphene composites, or nanostructured conductive surfaces may further enhance microbial attachment and electron transfer kinetics, potentially increasing power density significantly.

From a wastewater treatment perspective, the stacked MFC achieved COD removal approaching Indonesian discharge standards for food-processing industries, although the final effluent concentration remained slightly above the regulatory threshold of 300 mg/L. This indicates that the MFC alone was insufficient to fully satisfy discharge compliance under the tested conditions. However, the remaining COD gap was relatively small and could likely be addressed through several practical strategies. Extending hydraulic retention time beyond 72 hours would permit additional substrate degradation, while coupling the MFC with downstream aerobic polishing units could achieve complete compliance at relatively low operational cost. Therefore, the MFC should not be viewed as a stand-alone replacement for conventional treatment but rather as a valuable component within an integrated hybrid treatment system. Its primary advantage lies in simultaneously reducing pollutant load and recovering usable bioelectricity, thereby improving the overall sustainability and energy efficiency of wastewater management.

Despite the promising laboratory results, significant challenges remain regarding industrial scalability and long-term operational stability. A typical small-to-medium tempeh production facility generates several cubic meters of wastewater daily, far exceeding the treatment capacity of laboratory-scale reactors. Scaling MFCs to industrial volumes introduces major engineering challenges, including uneven current distribution, increased internal resistance, non-uniform biofilm growth, membrane fouling, and substrate transport limitations. Larger electrode areas often experience reduced power density because electron transfer becomes spatially heterogeneous across the biofilm surface. Furthermore, long-term operation may lead to salt precipitation, cathode fouling, or anode passivation, all of which can significantly reduce performance over time. The 28-day operational period investigated in this study was insufficient to evaluate these degradation processes comprehensively. Consequently, long-term experiments spanning several months are required to determine reactor durability and maintenance requirements under realistic conditions.

Several important limitations of this study should therefore be acknowledged. First, all experiments were conducted under controlled batch conditions with relatively stable temperature and influent composition, conditions that may differ substantially from industrial wastewater discharge patterns. Second, intermediate-scale reactors bridging laboratory and industrial scales were not evaluated, limiting direct extrapolation of scaling behavior. Third, although cyclic voltammetry and EIS provided indirect evidence of extracellular electron transfer pathways, molecular-level characterization of conductive pili expression and cytochrome regulation was not performed. Fourth, no economic analysis was conducted to compare the capital and operational costs of MFC systems against conventional aerobic or anaerobic treatment technologies. Therefore, while the present study provides strong proof-of-concept evidence for the feasibility of electricity-generating wastewater treatment using tempeh effluent, substantial research remains necessary before commercial implementation becomes realistic.

Overall, the findings demonstrate that MFC performance in tempeh wastewater treatment is fundamentally governed by reactor architecture because electrochemical design directly influences microbial community assembly, oxygen diffusion, biofilm maturation, and electron transfer efficiency. Single-chamber systems suffered from oxygen crossover that suppressed Geobacter enrichment and reduced electrical recovery efficiency. Double-chamber systems improved anaerobic stability and enhanced exoelectrogen dominance, while stacked systems further amplified voltage output and promoted more mature electroactive biofilms. The resulting improvements in CE, power density, and COD removal collectively confirm that optimized reactor architecture can significantly enhance wastewater-to-electricity conversion efficiency even in complex real-world waste streams.

Future studies should therefore prioritize continuous-flow operation, long-term stability testing, intermediate-scale demonstrations, advanced electrode materials, and integrated hybrid treatment strategies. In addition, metatranscriptomic and genomic analyses would provide deeper understanding of electron transfer mechanisms and facilitate rational bioelectrochemical system optimization. Although MFC technology for tempeh wastewater treatment remains at a developmental stage with clear technological and economic limitations, this study provides compelling evidence that rational reactor design can simultaneously support effective pollutant removal and meaningful bioenergy recovery, thereby contributing toward more sustainable industrial wastewater management systems.

CONCLUSIONS

This study demonstrated that Microbial Fuel Cell (MFC) technology can simultaneously reduce organic pollution and recover electrical energy from tempeh wastewater, confirming the feasibility of transforming industrial wastewater into an alternative bioenergy source. The results showed that reactor architecture strongly influenced electrochemical performance, microbial community development, and treatment efficiency. Among the three configurations evaluated, the stacked MFC produced the highest overall performance, achieving a maximum power density of 498.2 ± 19.6 mW/m^2, maximum voltage of 1,248.6 ± 28.3 mV, and COD removal efficiency of 88.9 ± 2.1%. The double-chamber configuration also outperformed the single-chamber system, demonstrating that physical separation between anodic and cathodic chambers is important for maintaining anaerobic conditions favorable to exoelectrogenic microorganisms and efficient electron transfer.

The study further confirmed that electricity generation was closely associated with the enrichment of electroactive microbial communities, particularly *Geobacter sulfurreducens*, which dominated the higher-performing configurations. Electrochemical analyses indicated that direct electron transfer mechanisms mediated by outer membrane cytochromes contributed substantially to current production. In addition, the physicochemical characteristics of tempeh wastewater including high biodegradability ($BOD_5/COD = 0.57$), sufficient conductivity, and abundant biodegradable organic compounds supported stable microbial metabolism and sustained bioelectrochemical activity. These findings indicate that tempeh wastewater is not only a pollutant requiring treatment but also a potential renewable energy resource that can be partially converted into usable electricity through bioelectrochemical processes.

Although the electricity produced remains relatively low for large-scale power generation, the study demonstrates the practical potential of wastewater-to-electricity conversion as an alternative energy recovery strategy, particularly for decentralized and small-scale industrial applications. The generated electrical output may be suitable for low-power operational needs such as environmental sensors, monitoring systems, or auxiliary treatment components. Therefore, the significance of this research lies not in replacing conventional electricity generation systems, but in demonstrating that wastewater treatment processes can be redesigned to recover part of the embedded chemical energy that is otherwise lost in conventional treatment systems.

However, several limitations should be acknowledged. The experiments were conducted under controlled laboratory-scale batch conditions using relatively short operational periods, which may not fully represent continuous industrial wastewater discharge. Long-term operational stability, electrode fouling, membrane durability, and performance under fluctuating influent conditions were not evaluated. In addition, the final COD concentration of the stacked MFC remained slightly above Indonesian discharge standards, indicating that the system still requires downstream polishing treatment for full regulatory compliance. Economic feasibility, maintenance requirements, and scale-up challenges also remain unresolved.

Overall, this research provides important evidence that MFC technology has potential as an integrated wastewater treatment and bioenergy recovery approach for the tempeh processing industry and similar agro-industrial sectors in developing countries. Future studies should therefore focus on pilot-scale continuous-flow systems, long-term stability evaluation, techno-economic assessment, optimization of low-cost electrode and membrane materials, and integration with post-treatment processes to improve effluent quality and industrial applicability. With further technological development, MFC systems may contribute to circular economy strategies by enabling simultaneous pollution reduction, resource recovery, and partial renewable electricity generation from industrial wastewater streams.

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