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Research Paper

Performance of Nickel Foam (NF) Cathode in Microbial Electro-synthesis System for Generating Methane and Acetate Production from CO_2

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Abstract
The cathode material is one of the key factors in enhancing the overall performance of microbial electrosynthesis system (MES). Nickel-based materials are the best option for cathodes in MES due to their excellent catalytic activity. This study aims to evaluate the performance of nickel foam (NF) as self-cathode material in MES for acetate production from CO_2. A biocatalyst at the cathode was provided using a mixed-culture of anaerobic sludge from palm oil mill effluent (POME). The field emission scanning electron microscopy (FE-SEM) was used to analyze the cathode surface morphology, while high-performance liquid chromatography (HPLC) was used to quantify the volatile fatty acids (VFAs) in the effluent. The results indicate that the self-cathode NF exhibited excellent performance, achieving an acetate production rate (QAcetate) of 46.0 mM/d, compared to 41.7 mM/d with a graphite felt (GF) cathode at a cathode potential of -0.8 V. Additionally, the self-cathode NF in the MES system demonstrated a coulombic efficiency (CE) of approximately 22.9%. Moreover, the type of cathode material and the microbial community attached to the cathode surface significantly influenced MES performance.

Keywords
Mixed-culture, Biocatalyst, Nickel Foam, CO_2, Acetate Production

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1. INTRODUCTION

The microbial electrosynthesis system (MES) is a type of bio-electrochemical technology (BET) that presents a promising approach for reducing and converting CO_2 into value-added products (Anwer et al., 2021). MES consists of two main compartments which are the anode and the cathode. At the anode, water is oxidized, generating protons, electrons, and oxygen, while at the cathode, CO_2 and bicarbonate (HCO_3^-) are reduced in the presence of protons and electrons to produce organic acids, alcohols, and/or biogas Equation 1. Since CO_2 is thermodynamically stable, additional energy input is required to drive the synthesis of these products Equation 2. Theoretically, the reduction of CO_2 into acetate requires an additional voltage of -1.10 V Equation 3, (Brachi et al., 2024).

Anode :

$$4H_2O \rightarrow 8H^+ + 8e^- + O_2 \quad E^\circ = 0.82 \text{ V} \tag{1}$$

Cathode :

$$CO_2 + HCO_3^- + 8H^+ + e^- \rightarrow CH_3COO^- + 3H_2 \quad E^\circ = -0.28 \text{ V} \tag{2}$$

Overall :

$$4H_2O + CO_2 + HCO_3^- \rightarrow CH_3COO^- + 3H_2 + O_2 \quad E^\circ_{cell} = -1.10 \text{ V} \tag{3}$$

Beyond the need for additional energy, cathode materials and inoculum also play a crucial role in facilitating CO_2 reduction into valuable products. Carbon-based materials, such as graphite granules (Shakeel et al., 2020), graphene

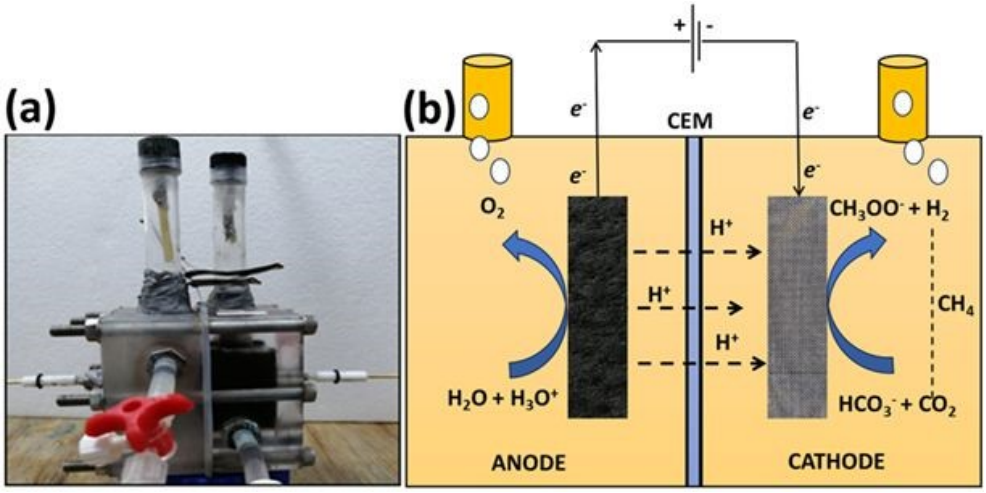

Figure 1. Photograph (a) and Schematic (b) of Microbial Electrosynthesis System (MES) Which Was Used in This Study

paper (Hui et al., 2023), carbon cloth/carbon nanotubes (Ragab et al., 2020), and graphite felt (Koul et al., 2022), are commonly used as cathodes in MES due to their advantages, including good conductivity, high porosity, resistance to fouling, affordability, and commercial availability. However, the acetate production rate (QAcetate) in MES remains relatively low. For example, MES systems using carbon-based cathodes with pure cultures (e.g., *Sporomusa ovata*) have achieved acetate production rates of approximately 51.1 g/m^2·d (Yang et al., 2021), while systems with mixed cultures produced around 49.2 mg/L·d of acetate from CO_2 (Mateos et al., 2019). To enhance MES performance, variations in cathode materials and inoculum can be simultaneously explored. For instance, Labelle et al. (2020) demonstrated that an MES using a single culture with a composite graphite felt–stainless steel (GF-SS) cathode achieved an acetate production rate of 2.4 mM·C/d, whereas a system utilizing a graphite plate and sulphate-reducing bacteria produced 2.9 mM·C/d of acetate.

As mentioned above, cathode potential is a crucial factor influencing biocathode performance. The metabolic pathways and end products of MES are highly dependent on the applied cathode potential (Hui et al., 2023). According to Xiang et al. (2017), cathode potential influences species competition within the biofilm. Moreover, the presence of microorganisms as a biocathode can lower the cathode potential and enhance the conversion of CO_2 into valuable products (Ge et al., 2022; Ibrahim et al., 2023). Microorganisms such as *Sporomusa, Clostridia*, and *Morella* sp. function as biocatalysts and can directly utilize electrons from the cathode to reduce CO_2 at a cathode potential of -0.6 V (vs. Ag/AgCl) (Agostino et al., 2020; Madjarov et al., 2022). In addition to cathode potential, current also influences autotrophic biocathode performance. Therefore, cathode materials should possess high conductivity, low overpotential, and minimal toxicity to support microbial activity

Recent advancements have explored metal-based cathodes to improve MES performance. Metal materials such as stainless-steel mesh combined with graphite (GF-SS) (Bajracharya et al., 2015), lead (Pb) (Dessì et al., 2021), and nickel (Ni) nanowires anchored to graphite (GF/Ni) (Brachi et al., 2024; Kim et al., 2018) have been studied as cathode materials in MES to enhance CO_2 reduction into acetate. So far, GF/Ni has achieved an acetate production rate of 282 mM·C/m^2·d, significantly higher than GF-SS (2.4 mM·C/d). Additionally, nickel-based materials such as Ni powder, Ni alloys, and nickel foam (NF) have been utilized in microbial electrolysis cells (MEC) for hydrogen production due to their excellent catalytic activity, lower overpotential, and environmental friendliness. Despite significant advancements in cathode materials for MES, limited studies have explored the potential of nickel foam (NF) in long-term MES operation with a mixed-culture inoculum. Therefore, this study investigates the use of NF as a cathode in a dual-chamber MES with a mixed-culture inoculum and provides a comparative analysis using a graphite felt (GF) cathode. The long-term batch operation of MES was evaluated based on acetate production rates and current efficiencies.

2. EXPERIMENTAL SECTION

2.1 Microbial Electrosynthesis System (MES) Setup

The dual-chamber MES consisted of two acrylic frames (3 cm × 4 cm × 4 cm) separated by a cation exchange membrane (CEM, CMI-7000s, Membrane International Inc., USA), which facilitated proton transfer from the anode to the cathode while minimizing product loss during the experiment. A photograph and schematic of the MES setup are shown in Figure 1. The anodic chamber operated under aerobic conditions, whereas the cathodic chamber was strictly anaerobic (Dessì et al., 2021). Graphite felt (GF, Sigma Aldrich, Malaysia BHD) and nickel foam (NF, Xiamen TOB

Table 1. General Characterization of POME Sludge Used for Inoculation

Parameters	Value
pH	4.6 ± 0.1
Water content (%)	93 ± 2
Total suspended solids (TSS) (g/L)	65.0 ± 5.9
Volatile suspended solid (VSS) (g/L)	10.2 ± 1.3
Chemical oxygen demand (COD) (g/L)	44.0 ± 3.1
Ash (g/L)	54.9 ± 5.9

New Energy Technology, China), each measuring 4 × 4 cm, were used as the anode and cathode, respectively. To remove surface contaminants, both GF and NF underwent a pretreatment process involving sequential washing with 1 M HCl and 1 M NaOH for one hour, followed by rinsing with deionized water. Titanium (Ti, Sigma Aldrich, Malaysia BHD) was used as the current collector and was connected to both electrodes. The net volumes of the anode compartment (NAC) and cathode compartment (NCC) were 40 mL each.

Table 2. Summary of Applied Parameters During Inoculation and Production

Phase	Inoculation	Production
Time (day)	30	90
Influent [a]	Synthetic media with glucose	Synthetic media with HCO_3^- and CO_2
pH control [b]	7.0 ± 0.1	7.0 ± 0.1

a = Carbon source in synthetic media was glucose during inoculation while the addition of $NaHCO_3$ and use of CO_2 gas bubbling until saturation;
b = pH was controlled and kept at 7.0 with 1 M HCl during MES operation.

2.2 Microbial Inoculation and Reactor Operation

Microbial inoculation was performed based on the modified method as described by Batlle-Vilanova et al. (2016). Anaerobic sludge from palm oil mill effluent (POME) as inoculum for mixed-culture was collected from POME pond Dengkil, Malaysia, which was mixed with the synthetic medium as described by Ragab et al. (2020). The characteristics of POME sludge are presented in Table 1. The anode was not inoculated and utilized water as the electron donor. Before introducing 40 mL of the mixture into the cathode chamber, the inoculum source was mixed and diluted (1:2) with a synthetic medium. The cathode was inoculated under closed circuit voltage (CCV) mode at a cathode potential of -0.6 V for 30 days. To promote microbial attachment to the cathode surface, a recirculation loop (7 mL/min) was maintained throughout this period. A summary of the applied parameters during microbial inoculation is provided in Table 2. After inoculation, the biocathode was flushed with a synthetic medium to remove soluble organic matter. Once the cathode was fully inoculated, the MES system was operated in batch mode for three months, with the cathode potential set at -0.8 V.

Table 3. Typical Characterization of Electrolytes Used in This Study

Parameter	Value
Anolyte	
pH	3 – 4
Dissolved oxygen (DO), (%Sat)	39.2 ± 0.3
Conductivity, (mS/cm)	24.7 ± 0.1
Catholyte	
pH	6.5 – 7.0
Dissolved oxygen (DO), (%Sat)	25.7 ± 0.2
Conductivity, (mS/cm)	7.4 ± 0.1

2.3 Electrolytes: Anolyte and Catholyte Preparations

The preparation was conducted using the method as described by Bajracharya et al. (2015). The catholyte or synthetic medium was composed of the following (all in g/L): 0.33 KH_2PO_4 0.45 K_2HPO_4, 1.0 NH_4Cl, 0.10 KCl, 0.8 NaCl, 0.20 $MgSO_4{\cdot}7H_2O$, 1.0 yeast extract, 10.0 mL vitamin solution, 10.0 mL trace element solution, and 4.0 $NaHCO_3$. Before the experiment, the medium was prepared anaerobically by heating it to just below boiling, followed by rapid cooling on ice while being bubbled with high-purity nitrogen (N_2, 99.99%) During the inoculation phase, 20 mM of glucose was used as the substrate. To maintain anaerobic conditions, 1 M $Na_2S{\cdot}9H_2O$ was added as an oxygen scavenger. In the production phase, glucose was removed, and sodium bicarbonate was used as the sole substrate. The pH of the sodium bicarbonate medium was maintained between 6.5 and 7.0. To ensure saturation, the synthetic medium was bubbled with CO_2 for 20 minutes. Meanwhile, a phosphate buffer solution (PBS) was used as the anolyte. The PBS composition (all in g/L) included 2.72 K_2HPO_4, 4.68 KH_2PO_4, and 8.76 NaCl, with the pH adjusted to 3-4 using 1 M HCl (Satar and Permadi, 2022). The electrolyte characteristics are summarized in Table 3.

2.4 Chemical and Gas Analysis

The analysis of the volatile fatty acids (VFA) was performed based on the method described by Bajracharya et al. (2015). Catholyte samples (10-15 mL) were collected from the MES system for analysis. The samples were first filtered using a

0.45 μm syringe filter (Minisart, Sartorius Stedim Biotech) and acidified with 0.5 mL of 1:1 (v/v) H_2SO_4. A total of 80 μL of 2-methyl hexanoic acid (6 mg/L) was added as an internal standard, followed by 1-2 mg of NaCl. Volatile fatty acids (VFAs) were then extracted using 2 mL of diethyl ether. The samples were vortexed (IKA*VORTEX 3, Sigma Aldrich, Malaysia) for 2 minutes and centrifuged at 1900 g (FORCE 1418, USA) for 3 minutes. The supernatant was then transferred into a vial for VFA analysis using high-performance liquid chromatography equipped with an ultraviolet detector (HPLC-UV, HPLC 1100, Agilent, USA). A PHENOMENEX ROA column (300 mm) was used at an operating temperature of 60°C. The carrier solution consisted of 0.005 N sulfuric acid (H_2SO_4) with a constant flow rate of 0.6 mL/min, and the retention time was set to 50 minutes. VFA composition was determined based on peak areas in the chromatogram. For alcohol analysis, D6-ethanol was used as an internal standard. The concentrations of ethanol, propanol, and butanol in the samples were analysed using HPLC-UV, with the composition of each alcohol compound determined based on its peak area in the chromatogram.

Whereas, gas samples from the system were initially collected using a syringe and transferred into a 25 mL septum bottle filled with 5% H_2SO_4. The gases, including H_2, O_2, N_2, CH_4, and CO_2, were then analyzed using gas chromatography (GC) equipped with a 2 m Porapak Q column packed with a molecular sieve 5A (80/100 mesh) and a thermal conductivity detector (GC-TCD, 7890D, Agilent, USA). Helium and air were used as carrier gases at flow rates of 15 mL/min and 150 mL/min, respectively. The composition of each gas (v/v) was determined based on its peak area in the chromatogram.

2.5 Chemical and Gas Calculation

The calculations in this study are based on the amount of VFA (i.e., acetate) and gas produced from the system, as described by Molinuevo-Salces et al. (2024). To calculate mol of acetate at any time (t) can be calculated using Equation 4, as follows;

$$n_{\mathrm{Acet},t} = \frac{V_{\mathrm{NCC}} \times (C_{\mathrm{Acet},t} - C_{\mathrm{Acet},t0})}{MW_{\mathrm{Acet}}} \tag{4}$$

The $n_{(Acet,t)}$ is the number of mol of acetate produced at t of the experiment, V_{NCC} is the volume of the net catholyte compartment, $C_{(Acet,t)}$,and $C_{(Acet,t0)}$ are the concentrations of acetate (mg/L) in the effluent, and fresh synthetic medium and MW_{Acet} is the molecular weight of acetate (60.0 g/mol). In this study, the production rate of acetate (Q_{Acet}) was presented in mmol of carbon per liter of NCC per day (mmol C/L/d or mM/d)) as shown in Equation 5 as follows;

$$Q_{\mathrm{Acet}} = \frac{n_{\mathrm{Acet},t}}{V_{\mathrm{NCC}} \times t} \tag{5}$$

The cathode electron (or current) efficiency (CE, %) is the efficiency of capturing the electron from the electrical circuit to the products. In this study, the CE was determined by using Equation 6, as follows;

$$CE_{\mathrm{t}} = \frac{n_{\mathrm{Acet},t} \times f_{\mathrm{Acet}} \times F}{\int_{t_0}^{t} I\, dt} \times 100 \tag{6}$$

where f_{Acet} is the molar factor conversion of acetate which was $8\bar{e}$ equivalent per mol acetate, F is Faraday's constant (96485 C/mol $\bar{e}$) and I dt is the amount of current for the certain t (second) of the experimental run. Whereas, the energy efficiency of MES (J_{MES},kWh/mol) can typically be determined based on the amount of energy invested into the system per unit product (mol). The J_{MES} can be calculated according to Equation 7. The ($E_{(Cat-)}$ E_{an}) is measured during the MES operation.

$$J_{\mathrm{MES}} = \frac{(E_{\mathrm{cat}} - E_{\mathrm{an}}) \times \int_{t_0}^{t} I\, dt}{n_{\mathrm{Acet},t}} \tag{7}$$

2.6 Morphology of Cathode Surface

The biofilm on the NF cathode surface was analyzed using field emission scanning electron microscopy (FE-SEM, JEOL JSM 5800) as described by Bajracharya et al. (2015) and Satar et al. (2018). After 90 days of operation, the NF cathode was removed from the system and cut into 0.25 cm^2 sections for analysis. Before imaging, the specimen was fixed in 4% glutaraldehyde in sterile PBS for at least 1 hour, then rinsed three times with PBS. It was subsequently stored at 4°C overnight and dehydrated using a graded ethanol series (20%, 30%, 50%, 70%, 90%, and 100%), with each step lasting 10 minutes. Next, the specimen was then dried at a CO_2-critical point and mounted on an SEM stub coated with gold. Different sections of the sample were examined using scanning electron microscopy (SEM) at an acceleration voltage of 20 kV and a working distance of 10 mm. Digital images were captured at a resolution of 1280 × 960 pixels with a dwell time of 160 s.

3. RESULT AND DISCUSSION

3.1 Methane and Acetate Productions During the Inoculation Phase

The microbial electrosynthesis system (MES) relies on multiple interdependent factors, including the supply of protons and electrons from water oxidation at the anode, the presence of a mixed-culture biocathode, and the application of external energy to overcome thermodynamic barriers. These elements collectively facilitate the reduction of substrates such as glucose and bicarbonate (HCO_3^-) into valuable products. During the inoculation phase, CH_4 and CO_2 were generated alongside acetate (CH_3COO^-), indicating the simultaneous activity of both fermentative and methanogenic

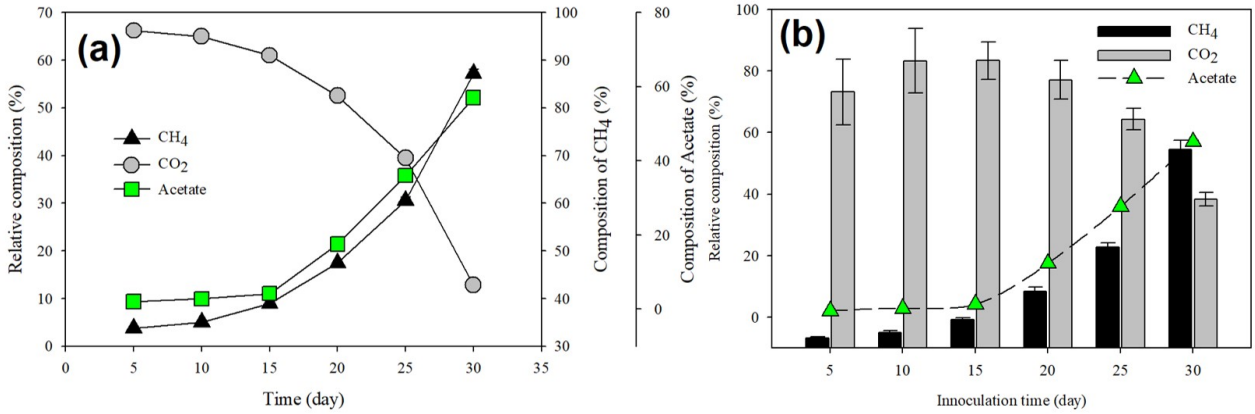

Figure 2. Trends of Gases Compositions (a) and Volume (b) During Inoculation Phase at -0.6 V vs Ag/AgCl of Cathode Potential

pathways. The production of acetate, CO_2, and H_2 was primarily driven by fermentative bacteria, which break down glucose into intermediate metabolites (Satar et al., 2017). Meanwhile, methanogens utilized CO_2 and H_2 through hydrogenotrophic methanogenesis to produce methane. The presence of CH_4 in the system can be attributed to the lack of an inhibitor, such as sodium 2-bromoethanosulfonate (NaBES), which is commonly used to suppress methanogenic activity (Liu et al., 2018). During the first week of the inoculation stage, CH_4 and CO_2 compositions were measured at 0.64 ± 0.08 mmol/LNCC/d (3.8%) and 17.1 ± 0.2 mmol/LNCC/d (96.2%), respectively, at a cathode potential of -0.6 V (vs Ag/AgCl). As shown in Figure 2(a), CH_4 production progressively increased, while CO_2 composition and production declined over time. This shift suggests that a portion of the CO_2 was consumed in methanogenesis or microbial electrosynthesis processes. Correspondingly, the CH_4 volume increased from 0.75 ± 0.09 mL to 14.67 ± 0.66 mL, whereas the CO_2 volume decreased from 18.91 ± 2.42 mL to 10.99 ± 0.49 mL. As illustrated in Figure 2(b), the concurrent increase in both methane and acetate concentrations indicates the successful enrichment and attachment of electroactive microorganisms on the cathode surface. This microbial colonization is crucial for efficient MES operation, as it enables direct and indirect electron transfer pathways, enhancing substrate conversion rates (Rousseau et al., 2020). Under these conditions, available substrates were predominantly metabolized by bacteria, leading to the formation of acetate and other byproducts.

Furthermore, the trends in volumetric current density were observed at 0.04 ± 0.01 A/m^3 NCC, as shown in Figure 3. A distinct peak in current density between days 6 and 8 suggests an increase in microbial electrochemical activity, which correlates with the production of CH_4,CO_2, and acetate within the system. This peak likely indicates the period when the electroactive microbial community had successfully adapted to the cathodic environment and began utilizing available substrates efficiently (Sikarwar et al., 2025). At this stage, other potential products such as alcohols and H_2 were not detected, implying that acetate and methane formation pathways were dominant in the microbial electrosynthesis process.

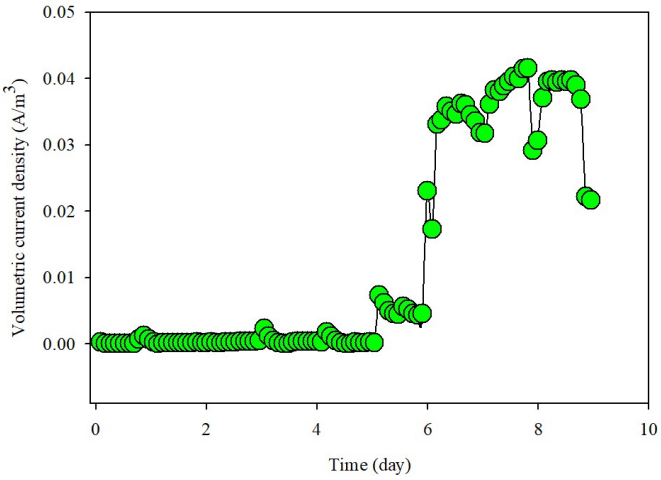

Figure 3. The Trend of The Volumetric Current Density During the Inoculation Phase

During the experimental period, a gradual increase in pH was observed, rising from 7.0 ± 0.1 to 8.2 ± 0.1, which may be attributed to the accumulation of alkaline and/or hydroxide ions (OH^-) in the catholyte. This shift in pH is common in microbial electrosynthesis systems, as the cathodic reduction of CO_2 to acetate consumes protons (H^+), leading to an increase in catholyte alkalinity. The rise in pH can influence microbial activity, particularly favouring acetogenic bacteria while potentially inhibiting the growth of some methanogens, which generally thrive under neutral to slightly acidic conditions.

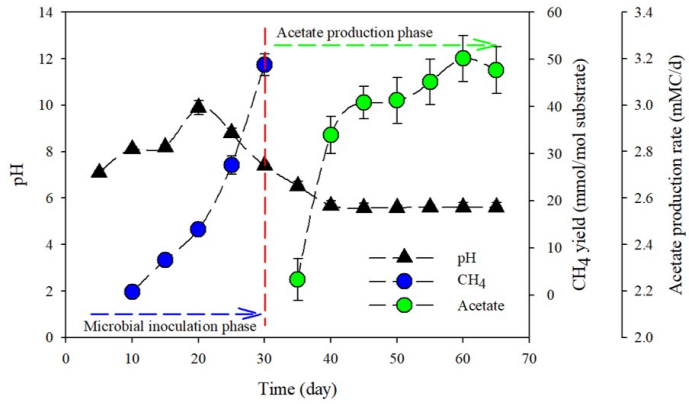

Figure 4. Typical pH Value, Methane Yield, and Acetate Production Rate from CO_2 Conversion by Using MES With NF Cathode for 70 Days

Simultaneously, a decrease in conductivity was recorded, from 7.4 mS/cm to 6.30 mS/cm, which suggests a reduction in dissolved ionic species. This decline in conductivity may be attributed to the conversion of ionic species into gaseous products, particularly CH_4 and CO_2. As dissolved inorganic carbon (DIC) in the form of bicarbonate (HCO_3^-) is utilized for microbial electrosynthesis, the release of CH_4 and CO_2 as gaseous products could result in the removal of charged species from the catholyte, thereby reducing overall conductivity.

These findings highlight the complex interplay between microbial metabolism, electrochemical reactions, and solution chemistry in MES systems. Further investigation into catholyte composition and buffering capacity could help maintain optimal conditions for enhanced acetate production while minimizing pH fluctuations that may affect microbial performance. Additionally, monitoring ionic balances and the rate of gas evolution may provide deeper insights into the mechanisms governing conductivity changes in the system.

Additionally, gas production was closely linked to the coulombic efficiency (CE), which was measured at 204.7 ± 0.1%. A CE exceeding 100% suggests that additional electron transfer reactions occurred beyond those expected from direct electrochemical reduction alone. This unusually high CE indicates that a significant portion of the gases generated (e.g., CH_4 and CO_2) originated from microbial metabolic activity, specifically from the biodegradation of residual organic substrates present in the inoculum rather than purely from electrochemical reduction of CO_2 (Batlle-Vilanova et al., 2016). The presence of fermentative and acetogenic bacteria could have contributed to this high CE by utilizing endogenous carbon sources, releasing H_2, acetate, and CO_2, which in turn influenced the gas production dynamics within the system.

Given that the primary goal of this study was to evaluate the performance of a nickel-based cathode for microbial electrosynthesis (MES) of organic products from CO_2, no chemical inhibitors such as sodium 2-bromoethanosulfonate (Na-BES)-a well-known methanogenesis inhibitor—were added to the system. The absence of Na-BES allowed methanogens to remain active, which could explain the simultaneous production of methane and acetate. To further ensure that CO_2 was the exclusive carbon source for electrosynthesis, glucose was removed from the synthetic medium after the inoculation phase, leaving sodium bicarbonate ($NaHCO_3$) as the sole available carbon substrate. This shift in substrate availability likely influenced the microbial community dynamics, favoring electroactive acetogenic bacteria over fermentative microbes, which typically rely on organic carbon sources. Under these conditions, the system promoted the reduction of CO_2 to acetate, which was evident from the steady increase in acetate concentration and decrease in CO_2 levels throughout the experiment. These findings emphasize the critical role of substrate selection and microbial community interactions in MES performance. Future studies could explore the impact of controlled inhibitor dosing (e.g., Na-BES at low concentrations) to selectively suppress methanogenesis and enhance acetate yields. Additionally, analyzing microbial community shifts via metagenomics or qPCR could provide deeper insights into the dominant electroactive species driving CO_2 conversion in this system.

3.2 Chemicals and Gas Compositions at the Production Phase

Once the inoculation phase was completed, glucose was completely removed from the synthetic medium. This transition marked a shift in microbial metabolism, favoring the electrochemical reduction of CO_2 to acetate rather than fermentation-based pathways (Ibrahim et al., 2023). It is well known that changes in the electrolyte can affect the overall performance of MES. The choice of substrate in MES plays a crucial role in system performance, particularly influencing electron transfer efficiency, product yield, microbial activity, and overall energy conversion (Gajda et al., 2021).

As shown in Figure 4, acetate production steadily increased while CO_2 concentration decreased over the 30-day experimental period. This inverse trend suggests that a substantial portion of CO_2 was converted into acetate and CH_4 by acetogenic and methanogenic bacteria colonizing the cathode. The biological activity at the cathode surface, often termed a biocathode, facilitated this transformation through MES (Dykstra et al., 2021). Parallel to earlier reports, this study confirms that the cathode surface was successfully colonized by electroactive microorganisms (Figure 5). The presence of acetate production was further supported by a significant decrease in pH, from 7.0 ± 0.1 to 5.6 ± 0.1, after 35 days of operation. The pH drop can be attributed to the accumulation of organic acids, primarily acetate (pKa = 4.4), in the system's effluent. Since acetate is a weak acid, its accumulation progressively lowered the pH, influ-

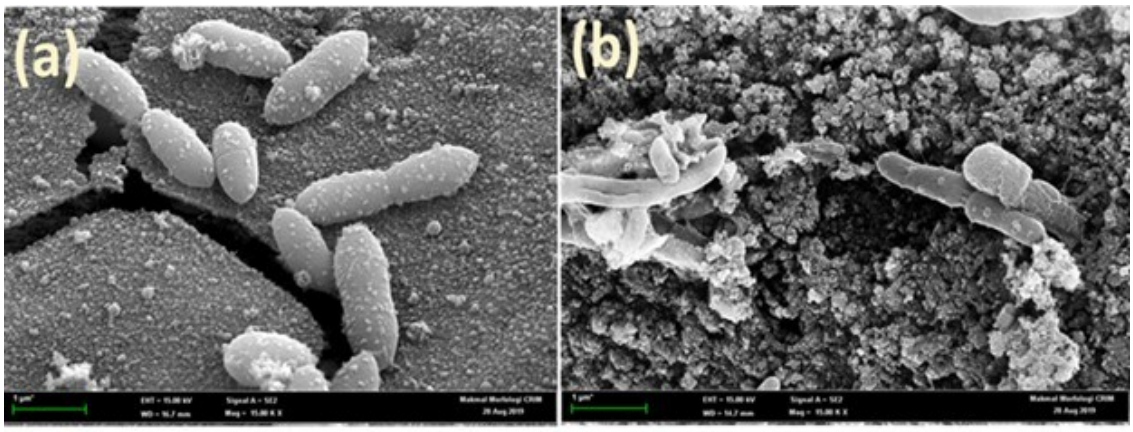

Figure 5. The Scanning Electron Micrograph Images at Different Magnifications for NF (a) and GF (b) Biocathodes after 90 Days of MES Run. The Bacterial Attachment on Both NF and GF Was Visible in The Images

encing the stability of microbial activity. Interestingly, both acetate production and pH remained relatively stable for 70 days. However, as pH dropped below 5.5, the activity of acetogenic bacteria likely declined due to unfavorable acidic conditions, leading to a decrease in acetate production (Liu et al., 2018). Acetogenic bacteria typically thrive in a pH range of 5.5-7.5, and a pH below 5.5 can inhibit key metabolic enzymes, reducing their ability to convert CO_2 into acetate efficiently. These facts highlight the importance of pH regulation in MES, as maintaining an optimal pH range is crucial for sustaining long-term microbial activity and maximizing acetate production. Future work could explore buffering strategies or periodic pH adjustments to enhance the stability of acetogenic communities and improve MES performance. Additionally, investigating the microbial community composition using metagenomic analysis could provide deeper insights into the dominant species responsible for CO_2 conversion under varying pH conditions.

Even after glucose was completely removed from the medium, methane was still detected at very low concentrations (data not shown). This persistence of CH_4 production was likely due to the absence of sodium 2-bromoethanesulfonate (Na-BES), and/or co-enzyme M, (Park et al., 2019), a well-known methanogenic inhibitor, at the cathode. Methanogens can utilize residual H_2 and CO_2 to produce methane via the hydrogenotrophic pathway, even under conditions favoring acetogenesis. Despite the lack of a methanogenic inhibitor, the acetate production rate remained comparable to those reported in previous studies (Table 2). This suggests that the dominance of acetogenic bacteria at the cathode was sufficient to drive the conversion of CO_2 to acetate, minimizing excessive methanogenesis.

Since CO_2 reduction is an electrochemically non-spontaneous process, an external voltage of 1.1 V was applied to facilitate the reaction. According to Patil et al. (2015) and Labelle et al. (2020), CO_2 reduction typically occurs within a cathode potential range of -0.59 V to -1.25 V (vs standard hydrogen electrode, SHE). This applied potential provides the necessary electron transfer to support MES, allowing acetogens to efficiently convert CO_2 into acetate. Throughout this process, the current demand and coulombic efficiency (CE) were observed at 17.4 ± 0.1 A/m^3NCC and 20.5 ± 0.3%, respectively. Interestingly, both current demand and CE remained relatively stable from day 35 to day 70, despite the continued production of acetate. This stability suggests that the electroactive biofilm at the cathode was well-established and maintained consistent electron transfer efficiency over time.

3.3 Electrode Morphology Analysis

Both NF and GF are foam materials with assumed uniform particle sizes. According to our previous research, the electrode surfaces of fresh NF and GF materials consist of 99.0% nickel (Ni) and 1.0% iron (Fe) for NF, and 100% carbon (C) for GF (Satar et al., 2018). This confirms the high homogeneity of the cathode surface's elemental composition. Furthermore, as shown in Figure 5, the surface morphology of both nickel foam (NF) and graphite felt (GF) electrodes revealed a dense colonization of bacillus-shaped microorganisms after 45 days of MES operation. The presence of these microbial communities on the cathode surface suggests the establishment of both acetogenic and methanogenic populations, which actively contributed to CO_2 conversion into acetate and methane. It is well known that anaerobic microorganisms, including acetogens and methanogens, are commonly found in the anaerobic sludge of palm oil mill effluent (POME) (Prasertsan et al., 2021). These microorganisms play a crucial role in biogeochemical carbon cycling, particularly in CO_2 reduction pathways that contribute to the global carbon cycle (Aziz et al., 2020). According to previous studies Mateos et al. (2019) several acetogenic bacterial genera, such as *Sporomusa, Sulfurospirillum*, and *Clostridium*, are frequently de-

Table 4. Summary of Acetate and Gases Productions in Microbial Electrosynthesis and A Few Selected Studies

Cathodes	Applied voltage (V)	Inoculums	Product(s)	Production rate (mM/d)	Current density (A/m^2)	CE of acetate (%)	Ref
GF	–1.1	POME sludge	Acetate	21.0 ± 2.1	1.6 ± 0.1	58.2 ± 1.2	This study
NF	–1.1	POME sludge	Acetate	24.3 ± 3.1	1.6 ± 0.1	75.0 ± 6.6	This study
Graphite rod	–0.7	*M. Maripaludis*	CH_4	NA	0.219	NA	(Mayer et al., 2019)
Activated Carbon GDE	–1.2	Anaerobic digester effluent	Acetate	1.30*	1.20	NA	(Rojas et al., 2021)
Graphene	–1.0	Mixed culture	Acetate	0.26*	2.6	NA	(Hu et al., 2021)
Carbon felt with SS	–1.1	Mixed culture WW	Acetate, CH_4 and H_2	1.3	10	26	(Bajracharya et al., 2017)
Sn-modivide electrode	–0.6	Mixed culture cow manure and urban solid waste	Acetate	0.98	5.9 ± 0.2[a]	28.9 ± 6.1	(Batlle-Vilanova et al., 2016)
Graphite plate	–0.7	Mixed culture brewery	Acetate	31.8	NA	NA	(Xiang et al., 2017)

* = unit in g/L; [a] = current demand based on the catholyte volume (A/m^3); WW = wastewater; SS = stainless-steel; GDE = gas diffusion electrode; NA = not available

tected in MES systems and are primarily responsible for the conversion of CO_2 to acetate via the Wood–Ljungdahl pathway (acetyl-CoA pathway). These homo-acetogens efficiently use electrons from the cathode to drive CO_2 fixation, making them key players in MES-driven bio-electrosynthesis. Meanwhile, methanogenic archaea, such as those belonging to the *Methanobacterium* and *Methanosarcina genera*, are responsible for methane production via two main metabolic pathways. First, the hydrogenotrophic methanogenesis pathway, where methanogens utilize H_2 and CO_2 to generate CH_4. Second, the acetoclastic methanogenesis pathway, where acetate is directly converted into methane and CO_2. The presence of both acetogenic and methanogenic microorganisms on the NF and GF cathodes indicates that MES not only facilitates electro-microbial CO_2 reduction but also supports the development of complex microbial consortia. The competition between these groups for electrons and substrates directly influences the overall efficiency of acetate production versus methane formation (Gomez Vidales et al., 2021).

In terms of acetate production, microorganisms that exclusively produce acetate are referred to as homo-acetogens. These bacteria play a key role in anaerobic carbon fixation, converting CO_2 into acetate via the Wood–Ljungdahl pathway (acetyl-CoA pathway) (Prasertsan et al., 2021). This pathway enables the synthesis of organic acids, including acetate, butyrate, and succinate, as well as ethanol, from CO_2 and reducing equivalents. Since CO_2 serves as the primary terminal electron acceptor for acetogens, its availability and concentration significantly regulate their metabolic activity and determine the types of substrates they metabolize. The efficiency of CO_2 reduction by homo-acetogens depends on several factors, including electron donor availability, pH, temperature, and competition with methanogens for resources.

In the context of CO_2 conversion to CH_4, methanogenesis occurs primarily via the hydrogenotrophic pathway, where methanogenic archaea utilize CO_2 and H_2 as substrates to produce methane (Gozan et al., 2018; Sodri and Septriana, 2022). This reaction is thermodynamically favorable under strictly anaerobic conditions and plays a significant role in biogas production and carbon recycling. The presence of methanogens in MES systems often competes with acetogens for CO_2 and H_2, potentially reducing acetate yields. Therefore, controlling cathode potential, pH, and selective inhibitors can help favor acetogenic pathways over methanogenic activity, enhancing CO_2-to-acetate conversion efficiency.

3.4 Performance of NF Compared with Selected References

As shown in Table 4, the performance of nickel foam (NF) and graphite felt (GF) cathodes in the MES system was comparable to selected references, particularly in acetate production rates. The acetate production rates achieved

with NF and GF were 24 mM/d and 21 mM/d, respectively. However, a higher acetate production rate (31.8 mM/d) was reported by Xiang et al. (2017) using a graphite plate cathode. This discrepancy may be attributed to differences in microbial community composition, as the brewery-sourced mixed culture in Xiang et al.'s study likely contained a higher density of acetogenic bacteria compared to the palm oil mill effluent (POME) sludge used in this study.

It is well established that MES performance in acetate production is strongly correlated with the density of acetogenic bacteria present in the system (Xiang et al., 2017). A more abundant and diverse acetogenic community enhances the efficiency of CO_2 conversion to acetate, leading to higher production rates. The results of this study indicate that the graphite plate cathode outperformed GF (this study), graphite granule (GG) (Batlle-Vilanova et al., 2016), graphite rod (GR) (Anwer et al., 2021; Zaybak et al., 2013), and carbon felt with SS catalyst (Shahid et al., 2021). This suggests that cathode material properties, particularly electrical conductivity and surface characteristics, significantly influence MES performance.

Furthermore, the source of the microbial inoculum plays a critical role in optimizing CO_2-to-acetate conversion efficiency. A richer acetogenic community can enhance electron uptake and carbon fixation, improving acetate yields. Therefore, selecting highly conductive cathode materials and optimizing microbial community composition are key factors for improving MES performance in CO_2 conversion applications

4. CONCLUSIONS

This study successfully evaluated the performance of nickel foam (NF) and graphite felt (GF) cathodes in a microbial electrosynthesis system (MES). Based on CO_2 conversion to acetate, the NF cathode exhibited superior performance compared to GF. In addition to acetate, biomethane and biohydrogen were also produced as byproducts during CO_2 conversion in MES. The percentage and production rate of acetate using the NF cathode were recorded at 75.0 $\pm$ 6.6% and 24.3 $\pm$ 3 mM/d, respectively. Both NF and GF cathodes provided suitable surfaces for microbial attachment. However, further experiments are necessary to identify the specific microorganisms involved in the process.

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