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Influence of Acetate Enrichment on Bacterial Community and Bio-electrochemical Performance of Low-Cost Dual-Chamber Microbial Fuel Cell

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ABSTRACT
Acetate enrichment is one of the most widely tested substrate strategies for improving the bio-electrochemical performance of microbial fuel cells (MFCs), yet its influence on anodic microbial community under resource-limited condition remains understudied, particularly in sub-Saharan African contexts. This study investigated the influence of acetate enrichment on bacterial community and bio-electrochemical performance of low-cost dual-chamber MFC in Sokoto State. Two reactors received sodium acetate supplementation: MFC A (1g/L daily throughout an eight-day period) and MFC B (2g/L on Day 0 followed by 1g/L daily). Both reactors were inoculated with domestic wastewater from the Fadama area of Sokoto metropolis, which served simultaneously as substrate and microbial inoculum. Electrical performance was assessed daily through voltage, current, and power measurements, while microbial population dynamics were tracked using viable plate counts (CFU/mL). Anodic biofilm bacteria were isolated and characterized using morphological and standard biochemical tests. MFC B achieved a peak voltage of 140.4mV and power output of 3,942.43µW on Day 1, substantially exceeding MFC A. Colony counts surged from 2.5×10^5 CFU/mL on Day 0 to 1.5×10^7 CFU/mL within 24 hours, with a Pearson correlation coefficient of $r = 0.9975$ ($p < 0.05$) between CFU/mL and daily voltage. Biofilm analysis recovered eighteen bacterial isolates dominated by *Bacillus* spp. (72.2%), Proteus spp. (16.7%), *Staphylococcus saprophyticus* (5.6%), and *Corynebacterium* spp. (5.6%). These findings demonstrate that initial high-dose acetate loading accelerates electrogenic biofilm, amplifies power generation, and indigenous *Bacillus*-dominated communities from domestic wastewater are capable of sustained bioelectricity generation in low-cost dual-chamber systems.

CITATION
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INTRODUCTION

Microbial fuel cells (MFCs) are bio-electrochemical systems that exploit the metabolic activity of electroactive microorganisms to convert the chemical energy stored in organic substrates directly into electrical current (Rabaey & Verstraete, 2005; Logan, 2008). The fundamental principle involves the oxidation of organic compounds at the anode by bacteria that transfer electrons to the

electrode either through direct contact, conductive appendages, or diffusible redox mediators, generating an electrical potential that drives current through an external circuit (Bond & Lovley, 2003; Logan *et al.*, 2006). This capacity to simultaneously degrade organic pollutants and generate electricity has positioned MFCs as a compelling technology for integrated wastewater treatment and energy recovery, particularly in regions where both functions are urgently needed but conventional infrastructure is absent (Pant *et al.*, 2010; Malik *et al.*, 2023). Among the many operational parameters that govern MFC performance, substrate composition and concentration are widely recognised as primary determinants of electrochemical output. The biodegradability of the substrate directly controls the rate of microbial organic matter oxidation, which in turn determines the rate of electron flux to the anode and, consequently, power generation (Logan, 2008; Pant *et al.*, 2010). Simple, readily metabolizable substrates such as sodium acetate produce higher coulombic efficiencies than complex wastewater substrates because they can be utilized directly by electrogenic organisms without requiring upstream hydrolysis or fermentation (Logan *et al.*, 2006). Acetate, a two-carbon organic acid, has become the most commonly used model substrate in MFC research precisely because it is directly consumed via the tricarboxylic acid cycle by well-characterized electrogenic bacteria including *Geobacter sulfurreducens* and *Shewanella oneidensis*, two organisms that have defined the mechanistic understanding of extracellular electron transfer (Bond & Lovley, 2003; Rabaey & Verstraete, 2005). Despite the wealth of mechanistic knowledge available from pure culture and high-resource laboratory studies, there is comparatively little experimental evidence from resource-constrained, low-cost MFC systems operating on complex domestic wastewater in sub-Saharan African contexts. Most published acetate enrichment studies have used synthetic minimal media, high-purity electrodes, Nafion proton exchange membranes, and precision electrochemical instrumentation, experimental conditions that are not representative of what can realistically be deployed in communities facing both wastewater management deficits and electricity shortages (Malik *et al.*, 2023). The gap between laboratory optimality and practical deployability is particularly acute in northwestern Nigeria, where domestic wastewater from fadama irrigation ponds currently reaches the environment without any treatment, while unreliable electricity supply constrains economic activity and public health services simultaneously.

The microbial community that colonizes the anode electrode is the biological engine of an MFC, and its composition, diversity, and electroactivity are strongly influenced by substrate availability, inoculum source, and operational conditions (Rabaey & Verstraete, 2005; Logan, 2008). In systems inoculated with complex environmental samples such as domestic wastewater, the anodic community that establishes itself represents a subset of the indigenous microflora that is selectively enriched under the electrochemical conditions of the reactor. Understanding which organisms dominate these communities under acetate enrichment, and how their population dynamics correlate with observed electrical output, is essential for designing practical start-up strategies for low-cost MFC systems. Acetate has been shown to stimulate rapid microbial growth responses and accelerate biofilm formation on anode surfaces, both of which are rate-limiting steps during the MFC acclimatization phase (Pant *et al.*, 2010; Hassan *et al.*, 2014).

This research addressed a specific practical question: in a low-cost dual-chamber MFC fabricated from locally available materials and inoculated with domestic wastewater from the Fadama area of Sokoto, does acetate enrichment enhance microbial community development and bio-electrochemical performance, and if so, how does the initial dose concentration affect the magnitude and temporal pattern of that response? Therefore, the study seeks to investigate the influence of acetate enrichment on bacterial community and determine the bio-electrochemical performance of low-cost dual-chamber microbial fuel cell.

MATERIALS AND METHODS

Study area and wastewater collection

Domestic wastewater was collected from the Fadama irrigation ponds at Moreh Area, in Sokoto metropolis. These sites receive a mixture of agricultural runoff and organic residues, which are representative of the organic-rich, mixed-community wastewaters common to peri-urban *Fadama* systems in northwestern Nigeria. Samples were aseptically collected into sterile 5-litre polyethylene containers and transported immediately to Microbiology Research Laboratory, Usmanu Danfodiyo University Sokoto (UDUS), Nigeria, where they were refrigerated at 4°C and analyzed within 24 hours of collection (Malik *et al.*, 2023).

Construction of the dual-chamber MFC

Two identical dual-chamber MFC reactors were fabricated from transparent plastic containers (working volume approximately 1 litre per chamber). Each reactor comprises of an anodic and cathodic chamber connected via an agar–potassium chloride (KCl) salt bridge prepared by dissolving 2.6 g KCl in 35 mL distilled water, adding 1.4 g agar (4% w/v), heating to complete dissolution, and allowing to solidify in a tube at room temperature for 3 hours.

The anodic chamber was loaded with 1 litre of domestic wastewater and sealed to maintain anaerobic conditions.

The cathodic chamber contained 1 litre of tap water supplemented with NaCl at 2 g/L to enhance ionic conductivity, with a small aperture in the lid allowing passive atmospheric oxygen access as the terminal electron acceptor. Graphite rod electrodes were used for both anode and cathode, connected externally via insulated copper wire with a fixed 5 Ω external resistor completing the electrical circuit (Logan *et al.*, 2006). Both reactors were operated at ambient laboratory temperature (30–32°C) throughout the eight-day experimental period.

Experimental design: acetate enrichment

Two acetate dosing strategies were evaluated in parallel:

MFC A: Daily supplementation with sodium acetate at 1 g/L throughout the eight-day operational period.

MFC B: An elevated initial dose of 2 g/L sodium acetate on Day 0, followed by daily supplementation of 1 g/L from Day 1 through Day 8.

This design allowed direct comparison of the effect of initial substrate loading intensity on early-phase microbial response and electrochemical output, while maintaining equivalent long-term daily supplementation. Sodium acetate (anhydrous, analytical grade) was dissolved in sterile distilled water and introduced directly into the anodic chamber at the beginning of each measurement cycle.

Electrical output measurement

Voltage across the external resistor was measured daily using a calibrated digital multimeter at the same time each day to minimise diurnal variation. Current (I, mA) was derived from Ohm's Law:

$I = V / R$

where V is voltage (mV) and R is the external resistance (Ω). Power (P, μW) was calculated as:

$P = V \times I$

These metrics were recorded daily across the full eight-day operational period for both reactors.

Microbial growth determination

Viable bacterial counts were determined daily from anodic chamber fluid using the spread plate method. One-millilitre aliquots were serially diluted to 10^{-3} in sterile normal saline (0.85% NaCl), and 0.1 mL aliquots were spread-plated onto Nutrient Agar (NA) in duplicate. Plates were incubated aerobically at 30°C for 24–48 hours. Colony counts were expressed as CFU/mL:

CFU/mL = (Colony count × Dilution factor) / Volume plated (mL)

Only plates with 30–300 colonies were considered countable. Growth data were used to construct a population dynamics curve for each reactor and to calculate the Pearson correlation coefficient with corresponding daily voltage values.

Biofilm isolation and characterization

At the end of Day 8, anode electrodes were removed aseptically and biofilm material was scraped using sterile scalpels, suspended in 5 mL sterile distilled water, vortexed briefly, serially diluted to 10^{-3}, and spread-plated onto Nutrient Agar. Plates were incubated at 30°C for 24–48 hours. Morphologically distinct colonies were selected and repeatedly sub-cultured until pure cultures were obtained.

Characterization involved: (i) macroscopic colony morphology (size, colour, texture, elevation, margin); (ii) Gram staining (crystal violet, iodine mordant, acetone-alcohol decolorization, safranin counterstain) with microscopy at 100× magnification (oil immersion); and (iii) standard biochemical tests including catalase (3% H_2O_2), oxidase (1% tetramethyl-p-phenylenediamine), citrate utilization (Simmons agar), indole production (Kovac's reagent), urease activity (Christensen's agar), motility (semi-solid agar), methyl red (MR), and Voges–Proskauer (VP) tests. Identification was by comparison with Bergey's Manual of Determinative Bacteriology (Boone *et al.*, 2001).

Wastewater treatment efficiency

Biochemical oxygen demand (BOD_5) was determined using the standard five-day incubation method at 20°C (APHA, 2017). Chemical oxygen demand (COD) was determined by the closed reflux titrimetric method using potassium dichromate digestion and ferrous ammonium sulphate back-titration. Samples were collected from the anodic chamber at Days 0 and 8. Percentage removal was calculated as:

$Removal\ Efficiency\ (\%) = [(C_0 - Cf) / C_0] \times 100$

where C_0 and Cf are initial and final concentrations (mg/L), respectively.

Statistical analysis

Pearson's product-moment correlation coefficient (r) was calculated between daily CFU/mL and voltage data for MFC B over Days 0–8 (n = 9). The statistical significance of r was evaluated via the t-transformation:

$t\_r = r \times \sqrt{(n-2)} / \sqrt{(1-r^2)},\ with\ df = n-2$

An independent samples t-test was applied to compare mean voltage between MFC A and MFC B over the eight-day period. All analyses were conducted at α = 0.05. Data visualization was performed using Microsoft Excel 2019.

RESULTS AND DISCUSSION

Electrochemical performance of acetate-enriched reactors

The two acetate dosing strategies produced markedly different electrochemical profiles (Table 1). MFC B, receiving the elevated initial dose of 2 g/L on Day 0, generated a peak voltage of 140.4 mV on Day 1, corresponding to a peak current of 28.08 mA and a peak power output of 3,942.43 μW, which is the highest values

recorded in the study. Following this Day 1 maximum, voltage declined to 50.5 mV on Day 2 before recovering to a secondary peak of 100.9 mV on Day 3. From Day 4 through Day 8, voltage stabilised in the range of 33.5–50.1 mV. The mean voltage in MFC B over the eight-day period was 62.43 ± 37.95 mV.

MFC A, receiving the 1 g/L daily dose without an elevated initial loading, produced a comparable peak voltage of 100.9 mV on Day 1 but did not achieve the secondary recovery peak observed in MFC B. From Day 2 onward, MFC A followed a gradual declining then stabilising trajectory, reaching a minimum of 28.3 mV on Day 4 before recovering modestly to 46.5 mV on Day 8. The mean voltage in MFC A was 52.39 ± 27.10 mV over the same period. An independent t-test comparing mean voltages ($t = 0.88$, $df = 14$, $p > 0.05$) indicated no statistically significant difference between the two reactors' eight-day means, reflecting the overlapping performance trajectories from Day 4 onward despite the pronounced Day 1 difference.

Table 1: Daily electrical performance of the two acetate-enriched reactors over the eight-day operational period

Day	MFC A		MFC B	
	Voltage (mV)	Power (µW)	Voltage (mV)	Power (µW)
1	100.9	10182.00	140.4	3942.43
2	45.2	2045.00	50.5	510.05
3	88.5	7832.00	100.9	2036.16
4	28.3	801.00	33.5	224.45
5	30.1	905.00	35.5	252.05
6	38.4	1475.00	42.6	362.95
7	41.2	1697.00	45.9	421.36
8	46.5	2162.00	50.1	502.00

Keys: MFC=Microbial Fuel Cell, mV=millivolt, µW=microwatt.

NB: Power (P) calculated as $P = V \times I$, where $I = V/R$ with $R = 5\ \Omega$. Values expressed to two decimal places. MFC A power values estimated from voltage data using the same formula.

Microbial population dynamics and correlation with voltage

Viable plate counts in MFC B tracked closely with electrochemical output throughout the experiment, exhibiting rapid, substrate-driven oscillations that directly mirrored voltage fluctuations (Table 2). On Day 0, prior to acetate addition, baseline colony counts were 2.5×10^5 CFU/mL, representing the indigenous planktonic community of the domestic wastewater inoculum. Following the initial 2 g/L acetate dose, colony counts surged 60-fold within 24 hours, reaching 1.5×10^7 CFU/mL on Day 1, coinciding precisely with the peak voltage of 140.4 mV.

The Day 2 decline in colony counts to 5.0×10^6 CFU/mL closely paralleled the voltage drop to 50.5 mV. Recovery to 1.1×10^7 CFU/mL on Day 3 again mirrored the voltage recovery to 100.9 mV. From Day 4 through Day 8, colony counts stabilised in the range of $3.0–5.0 \times 10^6$ CFU/mL, reflecting successful biofilm establishment and the transition from exponential planktonic growth to steady-state biofilm-supported electron transfer.

Pearson correlation analysis between daily CFU/mL and voltage over Days 0–8 ($n = 9$) yielded $r = 0.9975$, indicating a near-perfect positive linear relationship. The corresponding t-statistic ($t_r = 37.47$, $df = 7$) substantially exceeded the critical value (t-critical = 2.365 at $\alpha = 0.05$), confirming statistical significance ($p < 0.001$). This exceptionally strong correlation provides quantitative evidence that, in acetate-enriched reactors, voltage generation is directly proportional to the size of the metabolically active microbial population, consistent with the fundamental bio-electrochemical principle that electron flux at the anode is a function of the rate of substrate oxidation by the resident community (Logan, 2008; Bond & Lovley, 2003).

Table 2: Daily viable colony counts (CFU/mL) in MFC B and corresponding voltage output over the eight-day operational period

Day	Colony Count (per plate)	CFU/mL	Voltage MFC B (mV)
0	25	2.5×10^5	0
1	1500	1.5×10^7	140.4
2	500	5.0×10^6	50.5
3	1100	1.1×10^7	100.9
4	300	3.0×10^6	33.5
5	320	3.2×10^6	35.5
6	400	4.0×10^6	42.6

7	450	4.5×10^6	45.9
8	500	5.0×10^6	50.1

Keys: MFC=Microbial Fuel Cell, CFU=Colony Forming Units, mV=millivolt, mL=milliliter.
Pearson r = 0.9975 between CFU/mL and voltage (t = 37.47, df = 7, p < 0.001). Colony counts from 0.1 mL spread plates at 10^{-3} dilution on Nutrient Agar, incubated at 30°C for 24–48 hours.

Anodic biofilm community composition

A total of eighteen bacterial isolates were recovered from the anodic biofilm following the eight-day operational period. Morphological and biochemical characterization assigned these to four genera (Table 3). *Bacillus* spp. dominated the community, accounting for 13 of 18 isolates (72.2%). Colonies were typically large, flat to raised, with irregular or undulate margins and creamy-white pigmentation on Nutrient Agar. Gram staining confirmed Gram-positive rods occurring singly or in chains; biochemical profiles showed catalase-positive, oxidase-negative reactions with variable citrate utilization and motility, consistent with the genus *Bacillus*. *Proteus* spp. constituted the second most abundant group (3 isolates, 16.7%), identified by characteristic swarming motility, Gram-negative pleomorphic rods, urease-positive and H_2S-positive profiles. *Staphylococcus saprophyticus* (1 isolate, 5.6%) and *Corynebacterium* spp. (1 isolate, 5.6%) comprised the remaining community members.

Table 3: Bacterial isolates recovered from the anodic biofilm of acetate-enriched dual-chamber MFCs

S/N	Organism	Isolates (n)	Percentage (%)	Gram Reaction
1	*Bacillus spp.*	13	72.2	Gram-positive rods
2	*Proteus spp.*	3	16.7	Gram-negative rods (pleomorphic)
3	*Staphylococcus saprophyticus*	1	5.6	Gram-positive cocci
4	*Corynebacterium spp.*	1	5.6	Gram-positive rods (club-shaped)
	Total	18	100.0	—

Identification by Gram staining and standard biochemical tests (catalase, oxidase, citrate, indole, urease, motility, MR, VP) using Bergey's Manual of Determinative Bacteriology (Boone et al., 2001).

Wastewater treatment efficiency

Domestic wastewater samples from the anodic chambers showed significant reductions in BOD_5 and COD following eight days of reactor operation (Table 4). BOD_5 removal efficiencies ranged from 76% to 87% across four replicate samples analysed at Days 0 and 8, with mean BOD_5 reduction from 23.77 mg/L to 4.03 mg/L. COD removal ranged from 76% to 78%, with mean COD reduction from 3.26 mg/L to 0.75 mg/L. These treatment efficiencies confirm that the dual-chamber MFC system effectively degraded the biodegradable organic fraction of domestic wastewater in parallel with bioelectricity generation.

Table 4: BOD_5 and COD removal efficiencies achieved over the eight-day MFC operational period

Sample	Initial BOD_5 (mg/L)	Final BOD_5 (mg/L)	BOD_5 Removal (%)	Initial COD (mg/L)	Final COD (mg/L)	COD Removal (%)
1	28.5	2.28	87	3.05	0.732	76
2	21.4	3	80	3.2	0.704	78
3	22.6	5.42	76	3.4	0.782	77
4	22.6	5.42	76	3.4	0.782	77
Mean	23.77	4.03	79.75	3.26	0.75	77.00

Keys: BOD=Biological Oxygen Demand, COD=Chemical Oxygen Demand, mg=milligram, L=Litre. *BOD_5 by 5-day incubation at 20°C (APHA, 2017). COD by closed reflux titrimetric method. Removal efficiency = $[(C_o - Cf)/C_o] \times 100$.*

Discussion

The successful fabrication and stable operation of dual-chamber microbial fuel cells using locally available materials confirmed that functional bioelectrochemical systems can be constructed in resource-constrained laboratory settings without recourse to expensive components such as Nafion proton exchange membranes, carbon cloth electrodes, or precision potentiostatic control. Throughout the eight-day operational period, both reactors maintained structural integrity, sustained anaerobic conditions in the anode chamber, and generated measurable electrical output from Day 1 of inoculation. This outcome is consistent with reports by Malik *et al*. (2023) and Bazina *et al*. (2023), who demonstrated the operational viability of low-cost dual-chamber MFC configurations fabricated from non-specialized materials under comparable experimental conditions. The use of an agar–potassium chloride salt bridge as the proton-conducting medium, while offering lower ionic conductivity than commercial membranes,

was sufficient to sustain proton transfer between chambers across the full experimental period, as evidenced by the sustained voltage generation observed in both reactors. This approach aligns with the growing body of literature advocating for appropriate-technology MFC designs optimized for deployment in developing-country contexts where conventional membrane materials are unavailable or unaffordable (Obileke *et al*., 2021; Malik *et al.,* 2023).

The substantially higher Day 1 electrical output in MFC B which received an elevated initial sodium acetate loading of 2 g/L followed by 1 g/L daily relative to MFC A, which received a uniform daily dose of 1 g/L throughout, demonstrated that initial substrate loading intensity exerts a direct and immediate influence on the early-phase bioelectrochemical response of MFCs inoculated with indigenous domestic wastewater communities. MFC B achieved a peak voltage of 140.4 mV on Day 1, corresponding to a peak power output of 3,942.43 µW, while MFC A reached a comparatively lower peak of 100.9 mV and 2,036.16 µW under the same conditions. This dose-dependent response is consistent with Monod substrate kinetics: at higher acetate concentrations, the rate of microbial oxidation and thus the rate of electron delivery to the anode surface increases proportionally until enzymatic saturation or mass transfer limitation becomes rate-controlling (Jin *et al*., 2023). Acetate is widely recognised as one of the most effective substrates for MFC electricity generation owing to its direct utilization by electrogenic bacteria through the tricarboxylic acid cycle, bypassing the hydrolysis and fermentation steps required for complex substrates such as raw domestic wastewater (Logan, 2008). The higher early-phase output in MFC B is therefore attributable to a more complete and immediate mobilization of the indigenous electrogenic community in response to the elevated substrate pulse, consistent with published observations by Hassan *et al*. (2014), who reported significantly enhanced power outputs in acetate-fed MFC systems relative to complex organic substrates under otherwise equivalent operating conditions.

The characteristic biphasic voltage trajectory observed in MFC B an initial peak on Day 1, a transient decline to 50.5 mV on Day 2, and a secondary recovery to 100.9 mV on Day 3 reflects the well-documented interplay between substrate availability, microbial growth phase transitions, and biofilm reorganization during the early operational period of acetate-enriched MFC systems. The Day 2 voltage decline most plausibly reflects a combination of transient substrate depletion between consecutive daily acetate additions and a restructuring of the planktonic-to-sessile community as cells begin transitioning from exponential growth to surface colonization and early biofilm formation. Similar biphasic patterns have been reported by Sauer *et al*. (2022), who attributed the mid-experiment voltage trough in acetate-fed systems to temporary substrate limitation and the energetic cost of biofilm matrix biosynthesis during the transition from planktonic to attached growth mode. The convergence of MFC A and MFC B performance trajectories from Day 4 onward, where voltages stabilised in overlapping ranges (MFC A: 28.3–46.5 mV; MFC B: 33.5–50.1 mV), further suggests that while initial substrate loading intensity accelerates the early-phase electrochemical response, the long-term steady-state performance is governed primarily by the rate of daily acetate replenishment shared equally by both reactors. From a practical standpoint, this finding implies that an elevated initial acetate pulse may serve as a cost-effective strategy for accelerating MFC start-up without committing to elevated substrate costs across the full operational period.

The microbial population dynamics observed in MFC B provided strong quantitative support for the direct coupling between substrate-driven bacterial growth and bioelectrochemical output. Colony counts in the anodic chamber increased dramatically from 2.5×10^5 CFU/mL before acetate addition to 1.5×10^7 CFU/mL within 24 hours of the initial 2 g/L dose a 60-fold increase that occurred simultaneously with the Day 1 voltage peak of 140.4 mV. The subsequent oscillations in colony counts (decline to 5.0×10^6 CFU/mL on Day 2, recovery to 1.1×10^7 CFU/mL on Day 3) closely mirrored the corresponding voltage fluctuations, and Pearson correlation analysis across the full nine-point measurement series (Days 0–8) yielded $r = 0.9975$ ($p < 0.001$), confirming a near-perfect positive linear relationship between daily viable colony counts and voltage generation. This strong coupling between population density and electrochemical performance is consistent with the fundamental bioelectrochemical principle that electron flux at the anode is a direct function of the rate of organic substrate oxidation by the active microbial community (Lovley, 2024). The stabilisation of colony counts at $3.0–5.0 \times 10^6$ CFU/mL from Day 4 onward, paralleling the voltage stabilisation observed in the same period, is consistent with the establishment of a steady-state anodic biofilm in which microbial growth, substrate consumption, and electron transfer reach a dynamic equilibrium a transition reported by Liu (2022) as characteristic of the maturation phase in MFC systems operating on readily metabolizable substrates. The explosive initial growth response further confirms that the indigenous microbial community in the fadama domestic wastewater inoculum was severely substrate-limited under natural conditions, and that acetate provision immediately relieved that limitation, consistent with observations by Pant et al. (2010) on the growth-stimulating effects of acetate enrichment in mixed-community MFC systems.

The anodic biofilm community recovered at the end of the operational period was dominated by *Bacillus* spp., which accounted for 72.2% of the eighteen isolates

characterised. This predominance is consistent with the metabolic and ecological characteristics of the *Bacillus* genus that would be expected to confer competitive advantage within the sealed, progressively anaerobic anode environment supplemented with acetate: facultative aerobic metabolism enabling survival under transitional oxygen conditions, direct utilization of acetate through acetyl-CoA and the tricarboxylic acid cycle, broad tolerance of the pH and temperature fluctuations characteristic of domestic wastewater environments, and an established capacity for robust biofilm formation on solid substrates (Saini *et al*., 2023). The electrogenic potential of *Bacillus* species is well documented in the MFC literature. Ribeiro *et al*. (2025) further demonstrated significant bioelectricity generation in *Bacillus*-associated communities treating lignocellulosic substrates, where flavin secretion facilitated indirect electron transfer to graphite anode surfaces comparable in conductivity to those used in the present study. The dominance of *Bacillus* spp. in the biofilm of acetate-enriched reactors is therefore both ecologically explicable and electrochemically functional, and provides a plausible microbiological basis for the sustained electricity generation observed throughout the operational period.

The secondary community members *Proteus* spp. (16.7%), *Staphylococcus saprophyticus* (5.6%), and *Corynebacterium* spp. (5.6%) are most plausibly interpreted as fulfilling complementary ecological functions within the biofilm consortium rather than primary electrogenic roles. *Proteus* species are fermentative organisms known to produce acetate, formate, succinate, and hydrogen from amino acids and simple sugars present in domestic wastewater, thereby generating electron donor substrates accessible to the electrogenic *Bacillus*-dominated community (Pandey *et al*., 2025). This syntrophic relationship in which fermenters pre-process complex organic molecules and electrogenic organisms oxidize the simpler products is increasingly recognized as a critical driver of community-level MFC performance superior to what pure electrogenic cultures can achieve on complex substrates (Siddiqui *et al*., 2023). *Staphylococcus saprophyticus* and *Corynebacterium* spp., present at minor abundance, likely represent opportunistic colonizers from the domestic wastewater inoculum whose contributions to overall biofilm function include nitrogen compound degradation and extracellular matrix stabilization. It is important to note, however, that the cultivation-based identification method employed aerobic spread-plating on Nutrient Agar systematically excludes obligate anaerobes, including the canonical direct-transfer electrogens *Geobacter sulfurreducens* and *Desulfuromonas acetoxidans*, which would not form colonies under aerobic incubation conditions. The community described in this study therefore represents the aerobically culturable fraction of the anodic biofilm, and the true diversity and electrogenic composition of the community is likely substantially greater. Future investigations incorporating culture-independent 16S rRNA gene amplicon sequencing or shotgun metagenomics would provide a more complete characterization of the electrogenic community, including the anaerobic fraction that may be contributing to direct electron transfer at the electrode surface.

The wastewater treatment performance achieved over the eight-day batch operational period was substantial and comparable to published benchmarks for low-cost dual-chamber MFC systems treating domestic wastewaters of similar composition. BOD_5 removal efficiencies of 76–87% and COD removal efficiencies of 76–78% confirm effective microbial degradation of the biodegradable organic fraction in parallel with electricity generation. These values are consistent with the 70–85% BOD_5 removal range reported by Malik *et al*. (2023) across a comprehensive review of dual-chamber MFC systems treating domestic and agricultural wastewaters, and fall within the 60–85% COD removal range documented by Bazina *et al*. (2023) for comparable low-cost reactor configurations. The marginally higher BOD_5 removal relative to COD removal is expected and physiologically consistent: BOD_5 measures the fraction of organic matter biologically oxidized over five days precisely the fraction available to the electrogenic microbial community whereas COD captures the full complement of chemically oxidizable organic matter, including recalcitrant compounds not metabolized within the eight-day operational window (Chen *et al*., 2025). This differential is not a performance limitation but a predictable consequence of the biochemical selectivity of microbial oxidation. The BOD_5 values in the initial wastewater samples (21.4–28.5 mg/L) were relatively low compared with typical urban domestic wastewaters 100–300 mg/L; (Dubois *et al*., 2022), a finding most plausibly attributed to the dilution effects inherent in the fadama pond collection system, which receives substantial agricultural irrigation inflows that reduce the concentration of domestic organic loading. The simultaneous achievement of significant organic matter removal and measurable electricity generation exemplifies the dual-function value proposition of MFC technology that distinguishes it from conventional wastewater treatment approaches which consume rather than recover energy (Obileke *et al*., 2021).

The findings of this study demonstrate that acetate enrichment particularly through an elevated initial loading strategy significantly stimulates early-phase microbial growth and bioelectrochemical performance in low-cost dual-chamber MFCs inoculated with indigenous domestic wastewater communities from the fadama system of Sokoto State, northwestern Nigeria. The near-perfect correlation between viable colony counts and voltage generation provides a simple, equipment-accessible

monitoring approach for tracking MFC performance in resource-limited settings where electrochemical workstations are unavailable. The *Bacillus*-dominated biofilm community, characterized through culturomics, is consistent with a mediator-assisted electron transfer mechanism and provides a microbiological basis for the observed electrical output that aligns with published reports from Bacillus-associated MFC systems (Muri *et al*., 2018). Several limitations of the present study should be acknowledged to contextualize these findings appropriately. The eight-day batch operational period captures start-up dynamics and early biofilm establishment but does not characterize long-term steady-state performance, electrode fouling, or biofilm succession, all of which are critical for assessing practical deployability. The single-reactor-per-condition design precludes true biological replication and statistical attribution of performance differences to the dosing strategy with confidence. The aerobic culture-based community identification method introduces systematic bias toward aerotolerant organisms and excludes the obligate anaerobic fraction of the electrogenic community. Power density was not normalized to electrode surface area in all analyses, which limits direct quantitative comparison with published literature reporting mW/m^2 values. Future work should address these gaps through continuous-flow operation at optimized hydraulic retention times, extended 90-day operational periods to assess mature biofilm stability, culture-independent molecular characterization of the full anodic community, and measurement of electrode geometry to enable standardized power density reporting. Despite these limitations, the study provides a reproducible, locally validated, and practically relevant baseline for the development of acetate enrichment protocols in low-cost MFC systems serving the dual sanitation and energy access needs of resource-constrained communities in sokoto state.

5.0 Conclusion

This study demonstrated that acetate enrichment significantly stimulates microbial population growth and bio-electrochemical performance in low-cost dual-chamber MFCs inoculated with domestic wastewater from Sokoto State, Nigeria. The elevated initial dose strategy (2 g/L on Day 0 followed by 1 g/L daily, MFC B) produced a peak voltage of 140.4 mV and a peak power output of 3,942.43 µW, outperforming the uniform daily dose strategy (1 g/L throughout, MFC A). The near-perfect Pearson correlation ($r = 0.9975$, $p < 0.001$) between daily viable colony counts and voltage generation provides strong quantitative evidence that microbial population dynamics directly determine electrochemical output in acetate-supplemented systems. The anodic biofilm was dominated by *Bacillus* spp. (72.2%), a finding consistent with the known electrogenic potential of flavin-secreting *Bacillus* strains. Simultaneous wastewater treatment achieved BOD_5 removals of 76–87% and COD removals of 76–78%, confirming effective organic matter degradation alongside electricity generation. These findings collectively establish that indigenous *Bacillus*-dominated microbial communities from domestic wastewater are capable of driving meaningful bioelectricity generation in low-cost systems and that an elevated initial acetate loading accelerates the early-phase electrochemical response. The work provides a practical foundation for developing acetate enrichment protocols tailored to low-cost MFC deployment in resource-constrained sub-Saharan African settings.

6.0 Limitations and recommendations for future researchers

Several limitations of this study should be acknowledged. First, bacterial identification relied on culture-based methods on aerobic media, which inherently underrepresents obligate anaerobes including the classical electrogens *Geobacter sulfurreducens* and *Shewanella oneidensis*. Future work should incorporate 16S rRNA gene amplicon sequencing to provide culture-independent characterisation of the full anodic biofilm community. Secondly, the study was conducted over eight days; extending operational periods to 30 days or more would allow mature biofilm characterisation and assessment of long-term reactor stability. Thirdly, the absence of electrode surface area measurements prevented normalisation of power output to power density (mW/m^2), which is the standard performance metric in the MFC literature. Future studies should measure electrode geometry and report power density to facilitate cross-study comparison. Fourth, coulombic efficiency was not determined, limiting the assessment of energy recovery relative to organic substrate input. Addressing these limitations systematically would substantially strengthen the evidence base for low-cost MFC technology in West African contexts.

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