



Original Article

Performance of Cow dung as electrode source in microbial fuel cell

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ABSTRACT

Microbial fuel cells (MFCs) provide a sustainable approach to bioelectricity generation, but the cost of conventional electrode materials limits their wider application. This study evaluated the potential of carbonized cow dung as a low-cost electrode for bioelectricity generation using bacterial isolates obtained from cow dung. Five bacteria isolated from cowdung (*Pseudomonas aeruginosa*, *Geobacter sulfurreducens*, *Bacillus subtilis*, *Clostridium spp* and *Klebsiella pneumoniae*) were individually inoculated into nutrient broth in dual-chamber MFCs, while a conventional copper-wire electrode served as the control under comparable conditions. The fabricated electrode exhibited a lightweight, rigid, porous, cylindrical structure and sufficient electrical conductivity for MFC application. Measurable electricity generation commenced after approximately 24 hours, with peak voltage occurring on Day 3. The conventional electrode produced a maximum voltage of 0.24 V with *Bacillus subtilis*, whereas the cow-dung-derived electrode achieved 0.16 V with *Geobacter sulfurreducens*. Although the conventional electrode demonstrated higher electrical output, the cow-dung-derived electrode successfully supported microbial electricity generation, indicating its functional potential. The study demonstrates that cow dung can be valorized into a locally available, low-cost electrode material for MFC applications, although further optimization of electrode preparation and operating conditions is required to improve its electrical performance and durability.

Keywords: Cowdung, Microbial Fuel Cell, Biochar, Bioelectricity

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microbial metabolism. In the anode chamber, microorganisms oxidize

INTRODUCTION

Microbial fuel cells (MFCs) are bioelectrochemical systems that convert the chemical energy of biodegradable organic matter into electricity through

organic substrates and release electrons and protons. Electrons are transferred to the anode and subsequently flow through an external circuit to the cathode,

generating electrical current. Consequently, MFC performance depends strongly on electrode properties, including conductivity, surface area, porosity, chemical stability, biocompatibility, and microbial adhesion [1].

Conventional MFC electrodes, including graphite, carbon cloth, carbon felt, and activated carbon, offer desirable electrochemical properties but may be relatively expensive for large-scale or decentralised applications. This has stimulated interest in renewable and low-cost electrode materials derived from agricultural and animal wastes. Biomass can be converted through pyrolysis into carbon-rich biochar with porous structures and modifiable surface properties suitable for bioelectrochemical applications [2, 3].

Cow dung is an abundant, inexpensive, and renewable biomass containing carbonaceous organic matter that can potentially be converted into electrode material. Processing through drying, pyrolysis, particle-size reduction, and activation can enhance its carbon content, porosity, surface area, and electrochemical properties. These characteristics may promote microbial attachment and extracellular electron transfer while simultaneously converting an agricultural waste into a value-added material [2, 4].

Recent studies have demonstrated the bioelectrochemical potential of cow dung, although most have used it as a substrate or microbial inoculum rather than as an electrode. [5] reported a maximum power density of 1,465 mW/m² using cattle-dung slurry with zinc-carbon electrodes, while [6] achieved 1,480 mW/m² using a cow-dung-enriched inoculum with carbon-

mesh electrodes. Similarly, [7] demonstrated electricity generation from cow dung in 3D-printed MFCs. However, these studies did not evaluate carbonized cow dung as an electrode material.

The application of MFC technology is limited by low electrical output, high electrode costs, internal resistance, and inefficient extracellular electron transfer. Although biomass-derived materials have emerged as inexpensive alternatives, most studies have focused on conventional carbon materials or highly engineered biochar requiring complex processing. Cow dung has been extensively investigated as an MFC substrate and microbial inoculum [5, 6], yet relatively little attention has been given to its direct conversion into a functional electrode. Consequently, the conductivity, porosity, microbial adhesion, electron-transfer capacity, and electrical performance of cow-dung-derived electrodes remain insufficiently established.

This knowledge gap limits the assessment of cow dung as a locally available and sustainable alternative to conventional electrode materials. The aim of this study is to evaluate the performance of a cow-dung-derived electrode in a microbial fuel cell for bioelectricity generation using bacterial isolates obtained from cow dung.

MATERIALS AND METHODS

Biochar production by Pyrolysis

Fresh cow dung was manually cleaned to remove visible contaminants, moulded into uniform rods, and sun-dried for approximately four days with periodic turning to reduce moisture content. The dried samples were arranged on a raised wooden platform and covered with wet leaves and soil to create an oxygen-limited

environment. Controlled combustion was initiated through a small opening, and pyrolysis was allowed to proceed for approximately three days following standard biomass carbonisation procedures according to [8]. The resulting biochar was cooled, collected, crushed, and stored in airtight containers until use.

Electrode Fabrication

The biochar was pulverized using a mortar and pestle and sieved through 100-, 150-, and 200-mesh sieves to obtain a uniform particle size. Carboxymethyl cellulose binder (CMC) was prepared by dissolving 1 g of CMC in 50 mL of distilled water with continuous stirring and gentle heating. The binder was incorporated into the biochar at a 10:90 binder-to-biochar ratio (w/w) and thoroughly mixed to form a cohesive paste as commonly applied in biomass-derived electrode preparation [9]. A cylindrical paper mould measuring approximately 7 mm in internal diameter and 17 cm in length was prepared and sealed at one end. The biochar paste was introduced gradually and compacted to minimise air spaces. The filled mould was oven-dried at 80–105 °C for approximately 4 h, cooled, and carefully removed from the mould.

Electrode Activation and Electrical Connection

The fabricated electrodes were activated using 2 M KOH. The solution was prepared by dissolving 28.06 g KOH pellets in distilled water and making the volume up to 500 mL. Each electrode was immersed in the solution for 2 min, drained, air-dried for 12 h, and subsequently oven-dried at 60 °C to constant weight, following established chemical activation protocols for carbonaceous electrode materials [8, 9]. The electrodes were trimmed to 6 cm.

A 3-cm graphite pencil lead was inserted approximately 2 cm into each electrode, and a 40-cm insulated connecting wire was attached to the exposed end. The junction was secured with epoxy adhesive and allowed to cure before use.

Microbial Fuel Cell Assembly

Salt Bridge Preparation

An agar–NaCl salt bridge was prepared by dissolving 6.4 g agar-agar and 1.6 g NaCl in 160 mL distilled water with continuous stirring and gentle heating. The molten mixture was transferred into a sealed cylindrical tube and allowed to cool at room temperature until a firm gel was formed, according to standard dual-chamber MFC salt-bridge methods [10, 11].

Isolation and Identification of Bacterial Isolates from Cow Dung

Bacterial isolates were obtained from cow dung following enrichment and serial dilution. Fresh cow dung was introduced into sterile nutrient broth supplemented with glucose and sodium acetate as additional carbon sources and electron donors, and the culture was incubated under oxygen-reduced conditions at 35 °C for 15 days to favour the growth of anaerobic and facultative anaerobic microorganisms. The enriched culture was subsequently subcultured into fresh nutrient broth and incubated for a further 5 days prior to use.

Serial dilutions of the enriched culture were prepared up to 10^{-6} using normal saline, and 0.1 ml aliquots of appropriate dilutions were spread onto nutrient agar plates and incubated at 37 °C for 48 h. Distinct colonies were selected based on observable differences in colony morphology and were purified by

repeated streaking on fresh nutrient agar until pure cultures were obtained. Five morphologically distinct isolates, designated A, B, C, D, and E, were retained for characterization and subsequent inoculation into the experimental MFCs.

The purified isolates were characterized morphologically and biochemically. Gram staining was performed to determine cell shape, arrangement, and Gram reaction. Biochemical characterization included catalase testing, citrate utilization on Simmons Citrate Agar, growth on Eosin Methylene Blue (EMB) agar, and fermentation of glucose, sucrose, and lactose. Presumptive identities were assigned based on the combined morphological and biochemical profiles of each isolate, following standard identification schemes [14].

Substrate Preparation, Inoculation and Electrode Suspension

Nutrient broth (5.2 g) was dissolved in approximately 400 mL distilled water and sterilised at 121 °C for 15 min. Five dual-chamber MFCs were assembled and labelled for Isolates A–E. Equal volumes of sterile nutrient broth were aseptically introduced into the anode chambers,

followed by inoculation with the respective purified bacterial isolates obtained from cow dung. Each cathode chamber received 200 mL distilled water. The chambers were connected using the prepared agar–NaCl salt bridge, while the cow-dung-derived electrodes were suspended in the anode chambers and connected to an external circuit for electrical measurements. A control MFC was prepared under the same conditions, except that the cow-dung-derived electrode was replaced with a conventional copper-wire electrode. All experimental units were operated under identical conditions to permit comparison of electrical performance among the bacterial isolates and electrode types [5, 7].

RESULTS

Biochar production

Figure 1 shows the production of biochar from cow dung through pyrolysis. The figure presents the pyrolysis setup before (a) and after the pyrolysis process (b), as well as the resulting biochar obtained after pyrolysis (c). The colour and physical appearance of the material changed following pyrolysis, resulting in a carbon-rich biochar suitable for subsequent electrode fabrication

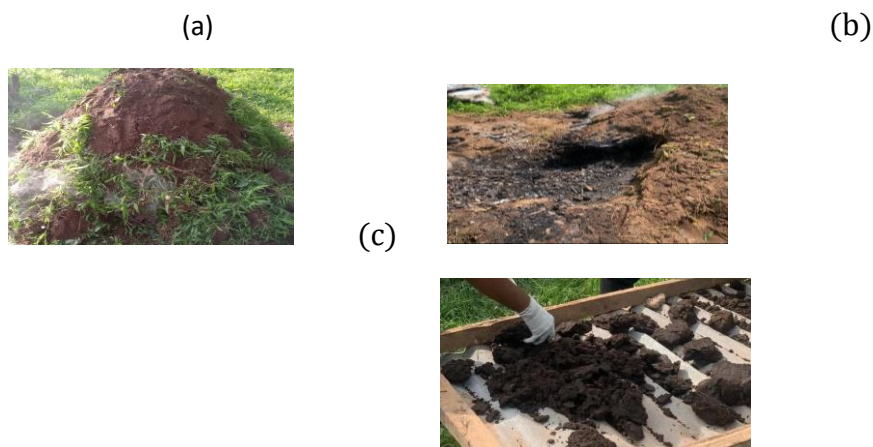


Fig 1: Biochar production from cow dung by pyrolysis.

Fabrication of electrode

Figure 2 shows the biochar paste obtained after incorporating the processed biochar

with CMC. The resulting paste had a uniform, cohesive appearance and was suitable for shaping into the electrode



Fig 2: Biochar paste obtained for electrode fabrication.

Paper mould fabrication, electrode formation

The fabricated paper mould (a) and the biochar paste incorporated within it (b)

are presented in Figure 3. The paste conformed to the shape of the mould, producing a compact structure for the electrode

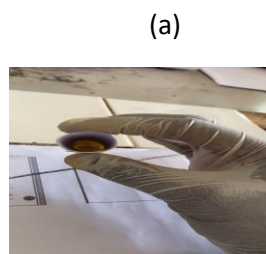


Fig 3: Fabricated paper mould and biochar paste formed into an electrode.

Activation of fabricated electrode in KOH solution

Figure 4 shows the fabricated electrode immersed in KOH solution during the

activation process. The electrode maintained its compact and well-defined structure while in the solution



Fig 4: Fabricated biochar electrode immersed in KOH solution.

Salt bridge/proton exchange membrane

A gel-like salt bridge component with a relatively uniform appearance was

obtained, as shown in Figure 5. The material was suitable for use as the proton exchange medium in the microbial fuel cell.

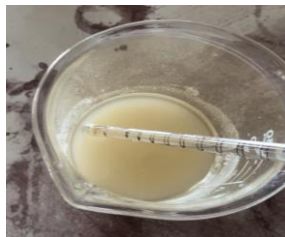


Fig 5: Prepared salt bridge component for proton exchange.

Connection of wire to electrode

Figure 6 presents the fabricated electrode with the electrical wire securely connected to it (a), alongside the

conductivity testing of the electrode (b). The testing confirmed that the fabricated electrode was electrically conductive and capable of establishing electrical contact for subsequent measurements.

(a)



(b)



Fig 6: Electrical wire connection and conductivity testing of the fabricated biochar electrode.

Physical characteristics of the fabricated cow dung electrode

The fabricated cow dung electrode exhibited distinct physical characteristics, as presented in Table 1. The electrode was

uniformly black, hard and rigid, lightweight, porous, smooth and compact, with a cylindrical shape. It was also found to be electrically conductive and considered suitable for application in microbial fuel cells.

Table 1: Physical characteristics of the fabricated cow dung electrode

PARAMETER	OBSERVATION
Colour	Uniformly black
Texture	Hard and rigid
Weight	Lightweight
Structure	Porous

Surface condition	Smooth and compact
Shape	Cylindrical
Conductivity	Conductive
Suitability for MFC	Suitable

The microbial fuel cell set up

The assembled microbial fuel cell chambers are presented in Figure 7. The chambers contained the prepared substrate before (a) and after the suspension of the fabricated electrode (b),

with the two chambers connected through the salt bridge to form the complete MFC setup.

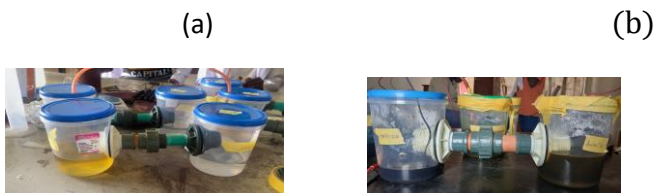


Fig 7 MFC chambers containing the substrate before and after fabricated electrode suspension

Morphological and Microscopic Characteristics of the Isolates

Table 2 presents the morphological and microscopic characteristics of the five

bacterial isolates recovered from cow dung, including colony morphology, cell shape, Gram reaction, and presumptive identity

Table 2: Morphological and microscopic characteristics of bacterial isolates

Isolate code	Colony morphology	Cell shape	Gram reaction
A	Circular, smooth colony with entire margin and convex elevation	Rod	Gram negative
B	Small circular smooth translucent colony with entire margin	Rod	Gram negative
	Large, rough, irregular creamy white colony with undulate margin		
C	Irregular, opaque, whitish colony with raised elevation	Rod	Gram positive
D	Large, mucoid, creamy colony with smooth surface and entire margin	Rod	Gram positive

E

Rod

Gram negative

Biochemical Characteristics of the Isolates

Table 3 presents the biochemical characteristics of the bacterial isolates,

including catalase activity, citrate utilization, and fermentation of glucose, sucrose, and lactose.

Table 3: Biochemical characteristics of the bacterial isolates

Isolate code	Gram reaction	catalase	Citrate	Glucose	Sucrose	Lactose	Presumptive identity
A	-	+	+	-	-	-	<i>Pseudomonas aeruginosa</i>
B	-	+	-	-	-	-	<i>Geobacter sulfurreducens</i>
C	+	+	-	+	-	-	<i>Bacillus subtilis</i>
D	+	-	+	+	+	+	<i>Clostridium spp</i>
E	-	+	+	+	+	+	<i>Klebsiella pneumoniae</i>

Electricity generation trend for the cow dung electrode and the copper wire electrode

The mean electricity generation values for the Isolates at 0, 24, 48, 72, 96 and 120 h using cow-dung electrodes and copper

wire electrodes are presented in Table 4 and 5. The values ranged from 0.00 to 0.15 V using cow dung electrode while it ranged from 0.01 to 0.22 V with copper wire electrode.

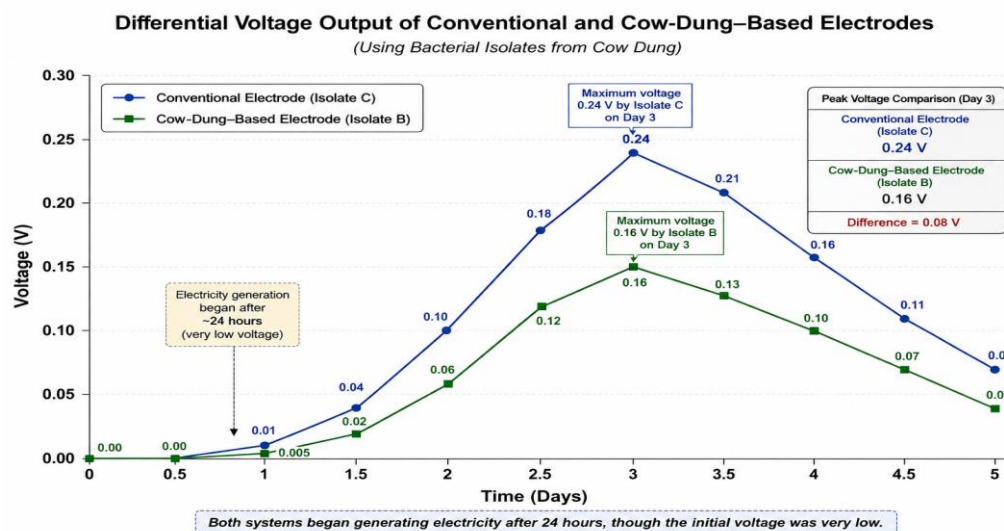
Table 4: Electricity generated by isolates using cowdung based electrodes in Volts

Hours	<i>Pseudomonas aeruginosa</i>	<i>Geobacter sulfurreducens</i>	<i>Bacillus subtilis</i>	<i>Clostridium spp</i>	<i>Klebsiella pneumoniae</i>
0	0.00	0.00	0.00	0.00	0.00
24	0.04	0.04	0.04	0.04	0.03
48	0.08	0.11	0.12	0.06	0.09
72	0.10	0.15	0.12	0.08	0.10
96	0.13	0.08	0.07	0.06	0.07
120	0.02	0.02	0.01	0.00	0.02

Table 5: Electricity generated by isolates using copper wire as electrodes in Volts.

Hours	<i>Pseudomonas aeruginosa</i>	<i>Geobacter sulfurreducens</i>	<i>Bacillus subtilis</i>	<i>Clostridium spp</i>	<i>Klebsiella pneumoniae</i>
0	0.00	0.00	0.00	0.00	0.00
24	0.03	0.03	0.02	0.03	0.03
48	0.05	0.05	0.06	0.05	0.07
72	0.10	0.14	0.22	0.17	0.10
96	0.06	0.02	0.01	0.03	0.07
120	0.04	0.01	0.00	0.01	0.05

Fig. 8 shows the differential voltage output of the conventional electrode and the cow-dung-based electrode over a 5-day period, using *Bacillus subtilis* and *Geobacter sulfurreducens* respectively. Both electrode systems began generating measurable electricity after 24 h, with initial voltage output being low. The conventional electrode (*Bacillus subtilis*) reached a peak voltage of 0.24 V on Day 3, while the cow-dung-based electrode (*Geobacter sulfurreducens*) reached a peak voltage of 0.16 V, also on Day 3, giving a difference of 0.08 V between the two systems.

**Fig 8: Graph showing the voltage output of conventional and cowdung based electrode**

DISCUSSION

The study demonstrated the successful fabrication and application of a cost-effective cow-dung-derived electrode in a laboratory-scale dual-chamber microbial fuel cell. The electrode, produced through pyrolysis, CMC binding, and KOH activation, exhibited a uniform black appearance, porous structure, lightweight nature, and sufficient electrical conductivity for bioelectricity generation.

These characteristics are consistent with the desirable properties of biomass-derived carbon electrodes reported in previous studies [5, 7, 8]. Both the conventional and cow-dung-derived electrode systems began producing measurable electricity after approximately 24 h, although the initial voltage was low. The conventional electrode achieved a maximum voltage of 0.24 V with *Bacillus subtilis* on Day 3,

whereas the cow-dung-derived electrode recorded a maximum of 0.16 V with *Geobacter sulfurreducens* on Day 3. Thus, the conventional electrode produced a higher maximum voltage, indicating superior electrical performance under the conditions of this study. Also, this result also shows that bacteria isolate/electrode material specificity determines the power output as reported in most past works. This is observed as *Bacillus subtilis* tends to produce the maximum output when copper is used as electrode while *Geobacter sulfurreducens* recorded highest output in the case of cowdung based electrode. Despite this difference, the cow-dung-derived electrode successfully supported bioelectricity generation, demonstrating its functional potential as a low-cost alternative to conventional electrode materials. Its performance may be attributed to its porous carbonaceous structure, which provides sites for microbial attachment and facilitates electron transfer. However, the lower voltage output suggests that further optimisation of pyrolysis, KOH activation, particle size, and binder composition may be required to improve conductivity and electrode-microbe interactions [8, 9]. Voltage generation increased following the initial 24-h adaptation period and generally reached its highest level on Day 3, followed by a decline. This pattern may be associated with bacterial growth and biofilm development during the initial phase, followed by depletion of readily available nutrients and reduced metabolic activity in the batch system. Although the voltage generated is insufficient to power conventional electronic devices directly, the output could potentially be accumulated and utilized for low-power applications when combined with appropriate energy-storage and power-

management circuits. Overall, the findings demonstrate the potential of cow-dung-derived electrodes for low-cost bioelectricity generation and agricultural-waste valorization.

CONCLUSION

This study demonstrated the feasibility of converting cow dung into a functional, low-cost electrode for microbial fuel cell applications. The fabricated electrode successfully supported bioelectricity generation by bacterial isolates obtained from cow dung. Both electrode systems generated measurable electricity after approximately 24 h, with peak voltage occurring on Day 3. The conventional electrode recorded a maximum voltage of 0.24 V with *Bacillus subtilis*, while the cow-dung-derived electrode achieved 0.16 V with *Geobacter sulfurreducens*. Although the conventional electrode produced a higher maximum voltage, the cow-dung-derived electrode demonstrated appreciable electrical activity under the same experimental conditions. The findings indicate that cow dung can be valorised into an inexpensive electrode material while simultaneously providing a source of potentially electrogenic bacteria. Optimisation of pyrolysis, activation, electrode structure, and operating conditions could further improve its conductivity, durability, and bioelectrochemical performance. Therefore, cow-dung-derived electrodes show promise as a sustainable and locally accessible alternative for low-cost MFC applications and agricultural-waste valorisation.

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