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Harnessing Microbes for Power: Evaluating Voltage Yield across Various Bio-waste Substrates

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Abstract

The demand for sustainable energy drives the exploration of organic waste for power generation via Microbial Fuel Cells (MFCs). This study explores the potential of microbial systems to generate electrical energy using different bio-wastes as substrates. Voltage outputs were compared across diverse organic wastes, to identify substrates that maximize energy production. Substrates used for the study include banana peel, sugarcane bagasse, rhizosphere soil and cattle manure. A dual-chambered MFC setup consistently utilized aluminum anodes, while cathode configurations varied viz., water with an aluminum electrode for banana peel, potassium ferricyanide with copper electrodes for sugarcane bagasse and rhizosphere soil and potassium permanganate with a copper electrode for cattle manure. Voltage and pH monitoring revealed significant variations in performance with cattle manure yielding the highest output of 1255 mV and banana peel the lowest at 252 mV. The identification of Rhizobium in rhizosphere soil emphasizes the critical role of specific microbial communities in driving the electrochemical process. The findings indicate that MFCs can effectively utilize organic residues for electricity generation while also helping waste utilization and renewable energy production. It also highlights the potential of MFC technology for environmental challenges and energy self-sufficiency, while offering insights into effective waste-to-energy conversion strategies.

Keywords: Microbial fuel cells (MFC), Bio-electricity generation, Organic waste, Waste management.

Introduction

The search for alternative energy technologies has intensified with increasing energy requirements and environmental concerns along with conventional fossil fuel usage. Industrial development, urban expansion and population growth continue to raise global energy demand, while fossil fuel reserves are finite and contribute substantially to greenhouse gas emissions and environmental deterioration. Consequently, there has been ample attention has been directed toward renewable and cleaner energy resources which can ensure sustainable development and long-term energy security[1]. In this regard, the utilization of organic wastes for bioelectricity generation represents an attractive alternative because it combines renewable energy production with effective waste management practices. Microbial fuel cells (MFCs) are bioelectrochemical systems in which microorganisms oxidize organic substrates and transfer electrons to electrodes, thereby enabling the generation of electrical energy from various waste materials[2].

Microbial fuel cells: A conventional microbial fuel cell is composed of two compartments *viz* an anode and a cathode chamber, which are separated by a proton-conducting membrane. Microorganisms in the anode chamber metabolize organic compounds and release electrons and protons. The electrons move through an external circuit to the cathode, where reduction reactions occur in the presence of suitable electron acceptors. Several factors such as membrane type, electrode materials, substrate composition, pH, temperature and the nature of the microbial community involved in electron transfer processes may influence the performance of MFCs.

Substrates for MFCs: Bioelectricity can be generated in MFCs using a variety of organic substrates, including rhizosphere soil, fruit wastes, cow dung, and sugarcane bagasse[3]. Rhizosphere soil, the region surrounding plant roots, is a highly dynamic and nutrient-rich environment enriched with organic compounds released by plants. These root exudates serve as substrates for diverse microbial communities, which metabolize the organic matter and release electrons that can be harnessed in MFC systems. Plant-microbial fuel cells (p-MFCs) utilize the synergistic interaction between plants and microorganisms, where plant roots continuously supply organic carbon sources that are oxidized by rhizospheric bacteria, leading to sustained bioelectricity generation[4].

Fruit wastes and sugarcane bagasse which are agro-industrial residues have also been widely studied as effective MFC substrates[2]. Fruit wastes are rich in readily degradable carbohydrates and organic compounds that are easily metabolized by microorganisms into simpler molecules, facilitating electron release during degradation. Similarly, sugarcane bagasse, the fibrous by-product obtained after sugar extraction, contains lignocellulosic materials and residual sugars that can be utilized by microbial consortia. Pretreatment methods, including thermal and chemical processes, have been shown to enhance the biodegradability of sugarcane bagasse and improve its energy recovery potential[5].

Animal-derived wastes such as cow dung further enhance MFC performance by introducing a diverse microbial population along with essential nutrients that support microbial growth and electron transfer processes. Studies have also identified animal dung and leaf litter as promising substrates for bioelectricity generation. Additionally, optimized combinations of substrates, such as sugarcane bagasse and cow dung at specific food-to-microorganism ratios, have been reported to improve biogas and energy yields[5].

The primary objective of this study was to evaluate the potential of different organic waste substrates—namely rhizosphere soil, fruit wastes, sugarcane bagasse, and cow dung—for bioelectricity generation using MFC technology. The study further aims to isolate and analyse the microbial communities that transfer electrons and are involved in electricity production and to compare the voltage output efficiency of the various substrates. Additionally, this work seeks to assess the probability of utilizing these organic wastes as sustainable resources for simultaneous waste management and renewable energy production, thereby contributing to circular bioeconomy applications.

Materials and Methods

Substrate preparation: Banana peels were collected from the local fruit vendor and stored in a plastic zip lock cover to prevent over drying or rotting. After 48 hours, a slurry was made by blending 750 g of banana peel and 250 ml of distilled water[6].Rhizosphere soil sample was collected from a cultivated bean field and root nodules were harvested from *Mimosa pudica*, both of which were transported in sterile plastic bags. Rhizosphere soil (200g) was mixed with, coco peat (200g) and charcoal powder (100 g) which acted as the carrier material. For the sludge mixture, a slurry was prepared with crushed root nodules (10-20g), glucose (0.5 g) and calcium carbonate (0.5 g) added to the carrier mixture along with 200 ml of distilled water[7,3]. Sugarcane bagasse waste was aseptically collected from a local sugarcane juice vendor and transported in a sterile container.

The stalks (100 g) were cut into small pieces was combined with 200 ml of distilled water. This mixture was sterilized to minimize the risk of cross-contamination. This high-temperature treatment also initiates the breakdown of lignocellulosic components, primarily targeting the hemicellulose fraction and converting it into simpler sugars[8]. Cow dung and cow urine were collected from a domestic setting with 6-7 cows, which were fed a diet of jowar (*Sorghum bicolor*) and ragi (*Eleusine coracana*). The organic waste was collected in a sterile container. Fresh cow dung and cow urine was mixed in the ratio of 1:2 to form a slurry.

Working principle of microbial fuel cell: The standard design of a dual-compartment microbial fuel cell includes two chambers—anode and cathode—linked by an external circuit and separated by a proton exchange membrane (PEM). In MFC's, microorganisms catalyze the decomposition of organic matter, generating electrons that can be captured as electrical energy. Specifically, electroactive bacteria, known as exoelectrogens, help the transfer of electrons to the anode surface, enabling electricity production[9]. Under anaerobic condition microorganisms oxidize substrates in the anode chamber thereby generating carbon dioxide as a byproduct with free electrons and protons. In the cathode chamber, reduction reactions occur as electrons from the anode combine with protons and terminal electron acceptors, such as oxygen from the atmosphere, resulting in the formation of water[10].

As microbial degradation progresses, organic compounds are eventually transformed into carbon dioxide and water, and the released electrons contribute to electricity generation. The design of the two-chamber microbial fuel cell is depicted in Figure-1.

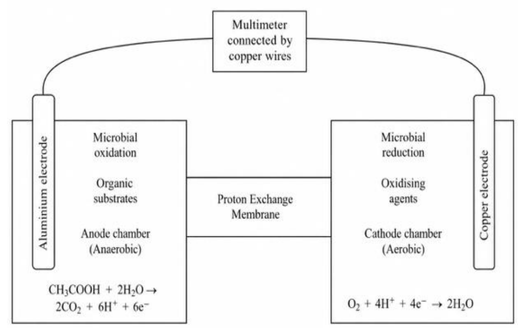

Figure-1: Diagrammatic illustration of a two-chamber MFC.

Fabrication of MFC: Two 1500mL BPA-free plastic containers served as anode and cathode chambers. A PVC pipe, fitted to the diameter of the proton exchange membrane (PEM), was attached to the chambers using hot glue to ensure a leak-proof seal. Two holes were drilled into the container caps to insert copper conductors (70-80cm length), which held the electrodes. A digital multimeter, connected to the wire ends, measured the generated voltage every 24 hours for 10 days. The fabricated dual-chambered microbial fuel cell design is illustrated in (Figure-2).

The anode chamber contained the respective substrates i.e. banana peel slurry, rhizosphere soil slurry, pretreated sugarcane bagasse mixture and cattle manure slurry which were mixed with distilled water to make upto1000ml. Oxidizing agents and water (1000 ml) was added in the cathode chamber (Table-1).

Salt bridge prepared using a PVC pipe (10 cm length x 2.5cm diameter x 0.4cm thickness) was used. 1g of agar and 3.5g KCl/NaCl in 50mL distilled water was boiled, cooled, and poured into the pipe to set. Aluminum rods served as anode while copper rods served as cathode for all substrates in the experimental setup.

Each of the electrode size measuring 7 mm diameter x 150 mm length was rinsed with ethanol to maintain aseptic conditions and one end of the electrode was immersed in its respective chamber, while the other end was connected to insulated copper wires (70-80 cm long) that linked to a multimeter for voltage measurement (Figure-2). The MFC was allowed to operate for 10 continuous days, during which the initial and final open-circuit voltage (OCV) was measured at 24-hour intervals using a digital multimeter.

Isolation of electroactive microorganisms: To increase the efficiency of bioelectricity generation in the MFC, the electroactive microorganisms that are efficient in transferring the electrons were screened from organic materials used for the experiment. The MFC setup utilizing rhizosphere soil slurry as the substrate successfully generated bioelectricity, achieving a measurable maximum voltage. Based on this performance, the rhizosphere soil substrate was selected for the isolation of microorganisms potentially responsible for bioelectricity generation. The isolation was done by serial dilution and plating on Yeast extract Mannitol Agar, Ashby's Mannitol agar and Nutrient Agar.

Mass Culturing and Voltage Assessment: Dominant colonies observed on the initial isolation plates were selected for mass culturing. Three separate 1000 mL fresh broth media—Nutrient Broth, Ashby's Mannitol Broth, and Yeast Extract Mannitol Broth (YEMA)—were prepared and inoculated with the selected isolates. Each broth was transferred into the anode chamber of the MFC equipped with an aluminium electrode. The cathode chamber contained potassium ferricyanide solution and a copper electrode, and both chambers were connected via a salt bridge (Figure-3). The MFC setup was used to measure voltage output using a digital multimeter[3,11]. Two sets of voltage measurements were recorded: one without inoculum (control) and one with inoculum. In the control setup (without inoculum), the initial voltage and the voltage after 25 minutes were measured. In the experimental setup (with inoculum), voltage readings were recorded at regular 25-minute intervals for 5–6 consecutive observations to evaluate changes in electrical output following microbial activity.

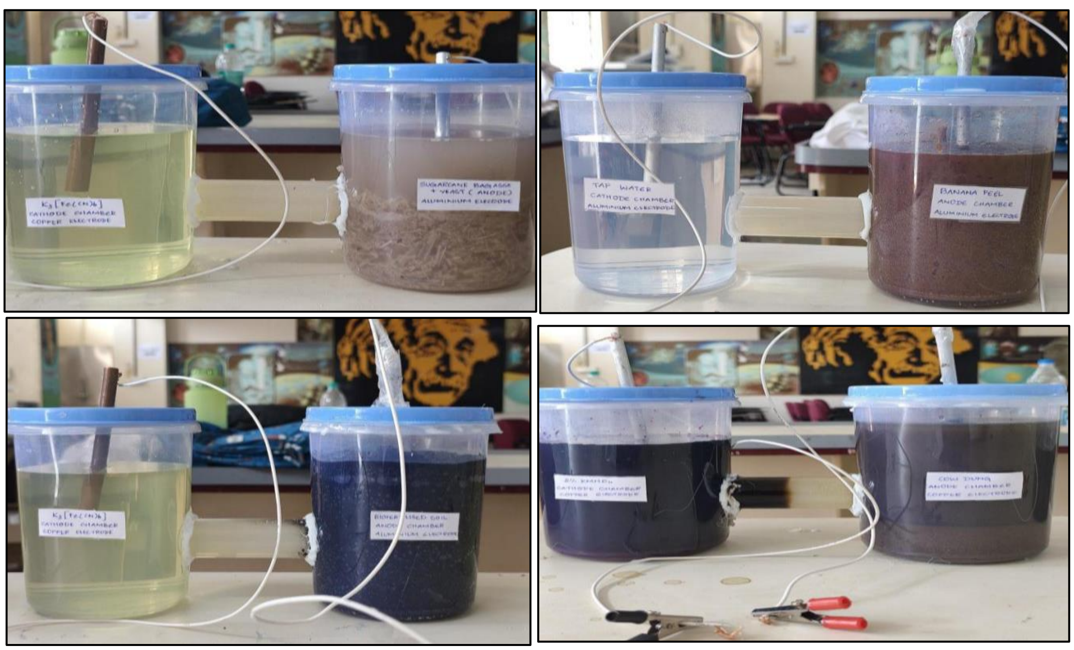

Figure-2: Dual chamber microbial fuel cell set-up containing different organic substrates.

Table-1: List of components and proportions in anode and cathode chamber.

Anode chamber	Cathode chamber	Microorganism
1000ml of prepared banana slurry	1000ml tap water	Normal flora
800ml of prepared rhizosphere soil slurry in 200ml of distilled water	1M $K_3[Fe(CN)_6]$ (32g in 1000 ml distilled water)	Root nodules and normal soil microflora
300ml of pre-treated sugarcane bagasse mixture in 700ml of distilled water	1M $K_3[Fe(CN)_6]$ (32g in 1000 ml distilled water)	*Saccharomyces cerevisiae* (commercial baker's yeast)
300 ml of prepared cow manure slurry in 700 ml of distilled water	5g of $KMnO_4$ in 1000 ml distilled water	Normal flora

Results and Discussion

The efficiency of MFC using various substrates was assessed by monitoring changes in voltage output and pH variations throughout the experiment. The electrical output of the MFC system is affected by several factors, including microbial metabolic activity and substrate characteristics[12]. The use of organic waste materials offers an effective approach for integrating waste disposal with sustainable energy generation[2].

Among the tested substrates, banana peel exhibited a maximum voltage output of 252 mV on the third day of operation, with the cathode chamber maintaining a pH of 8. After reaching this peak, the voltage gradually declined to 155 mV by the end of the experimental cycle (10 days). Over the same period, the cathode pH increased from an initial value of 7 to 10, indicating increasing alkalinity, while the anode pH decreased from 6 to 4, reflecting acidification within the anodic chamber (Table-2). The MFC utilizing sugarcane bagasse as the substrate exhibited a maximum voltage output of 680 mV on the third day, at which point the cathode pH was 8. Following this peak, the voltage steadily declined to 250 mV by the end of 10 days. Over the same period, the cathode pH increased significantly from an initial value of 7 to 12, indicating strong alkalinization, while the anode pH showed a decrease from 5 to 4, suggesting continued acidification within the anodic chamber (Table-2).

Figure-3: Mass culturing and voltage assessment (Nutrient broth, Ashby's mannitol broth and YEMA broth).

Table-2: MFC setup with banana peel and sugarcane bagasse as substrate.

	Banana peel			Sugarcane bagasse		
No of Days	Voltage in mV	pH Readings		Voltage in mV	pH Readings	
		Anode	cathode		Anode	cathode
1	102	6	7	560	5	7
2	209	ANEROBIC	8	680	ANEROBIC	8
3	252		8	514		8
4	140		8.5	470		8
5	138		8.5	468		9
6	144		9	390		10
7	158		9	266		10
8	143		9.5	270		11
9	149		10	283		11
10	155	4	10	250	4	12

With rhizosphere soil as substrate, MFC produced a maximum voltage of 550 mV on the second day, at which point the cathode pH was 8. Following this peak, the voltage gradually decreased to 410 mV on day10. During this period, the cathode pH increased markedly from an initial value of 7 to 12, indicating strong alkalinization, while the anode pH showed a slight decline from 8 to 7 (Table-3). The MFC with cattle manure demonstrated the highest overall performance, achieving a maximum voltage of 1255 mV on the ninth day, with a corresponding cathode pH of 10. After reaching this peak, the voltage decreased slightly to 1195 mV by the end of the cycle. Over the course of the experiment, the cathode pH rose from 7 to 10, while the anode pH decreased from 8 to 6, indicating moderate acidification in the anodic chamber (Table-3).

A comparative analysis of the different organic substrates used in the MFC setups revealed significant variations in bioelectricity generation, as shown in Figure-4. Among all substrates tested, cattle manure emerged as the most effective, producing the highest open-circuit voltage (OCV) of 1255 mV, with peak performance observed on the ninth day. This high output can be attributed to its rich organic composition and the presence of diverse electroactive microbial communities, which enhance electron transfer efficiency in MFC systems[13,14]. Similar studies have shown it to be mostly a mixed community of exoelectrogenic bacteria (species of *Geobacter sulfurreducens* and *Shewanella oneidensis*) along with methanogenic archaea like Methanobrevibacter smithii[15-17]. Additionally, the use of copper electrodes and potassium permanganate as a cathodic oxidizing agent likely contributed to the elevated voltage output. However, the toxicity and environmental risks associated with strong oxidants such as potassium permanganate must be carefully considered when evaluating scalability and sustainability[1,14]. The substantial power generated was sufficient to illuminate a light-emitting diode (LED), demonstrating its potential for practical, small-scale energy applications. The final integration of cattle manure, rhizosphere soil, sugarcane bagasse, and banana peel MFCs into a serial circuit successfully converted chemical energy into a functional electrical output. This concludes that while individual organic substrates vary significantly in electrogenic potential, their collective integration overcomes the inherent voltage limitations of single-cell systems. This model provides a robust framework for utilizing diverse agricultural residues as a combined, sustainable power source.

In comparison, rhizosphere soil and sugarcane bagasse exhibited moderate performance, with peak voltages recorded earlier in the cycle, around the second day (Figure-4). Rhizosphere soil generated approximately 550 mV, while sugarcane bagasse reached about 680 mV. These results indicate their capability to support bioelectricity production, likely due to the presence of active microbial populations in soil and the fermentable organic matter in lignocellulosic biomass. However, their comparatively lower outputs suggest limitations in substrate biodegradability or microbial efficiency, highlighting the need for optimization[1,15]. The use of the yeast Saccharomyces cerevisiae in the bagasse system underscores the importance of introducing specific microbial strains to enhance bioelectrochemical performance. In the case of rhizosphere soil, the identification of Rhizobium suggests that plant-associated microbial communities contribute to electron transfer processes. However, both systems employed potassium ferricyanide as a cathodic electron acceptor, which, although highly effective in improving power output, presents toxicity and environmental concerns. The use of copper electrodes similarly enhances conductivity but raises issues related to long-term sustainability and potential metal leaching. Furthermore, large-scale application could be hindered by the reliance on rhizosphere soil and the need for manual preprocessing which would introduce practical and operational limitations[15,18].

Table-3: MFC setup with Rhizosphere soil and Cattle manure as substrate.

No of Days	Rhizosphere soil			Cattle manure		
	Voltage in mV	pH Readings		Voltage in mV	pH Readings	
		Anode	cathode		Anode	cathode
1	549	8	7	1031	8	7
2	550	ANEROBIC	8	1130	ANEROBIC	7
3	505		8	1080		7.5
4	530		8.5	1174		8
5	485		9	1180		8
6	390		9.5	1101		8.5
7	416		10	1215		9
8	405		11	1234		9
9	400		12	1255		10
10	410	7	12	1195	6	10

Despite its abundance as an agricultural waste, banana peel produced the lowest voltage (252 mV) among the tested substrates (Figure-4). While it contains fermentable carbohydrates, its lignocellulosic structure can limit microbial accessibility and degradation efficiency. Furthermore, the likely absence of a well-adapted electroactive microbial community reduces its performance in MFC applications. Although banana peel is an inexpensive and readily accessible organic substrate, its comparatively low electrical yield and limited scalability reduce its suitability for use as a sole substrate in MFC applications. Earlier investigations showed that *S. cerevisiae*-treated banana slurry generated a peak power density of 86.9 ± 0.4 mW m^{-2}, while banana peel slurry without microbial inoculation produced only 44.6 ± 0.8 mW m^{-2} output[19]. An optimal power density of 910 mW m^{-2} using bacterial consortia and banana peel residues as the substrate was also reported[20].

Isolation and identification of microorganisms from rhizosphere soil: Following serial dilution and plating, substantial microbial growth was observed on YEMA, Ashby's Mannitol Agar, and Nutrient Agar. Similar observations have been reported in rhizosphere-based studies, where diverse microbial populations readily grow on both selective and non-selective media[21]. Across all media, the dominant colonies displayed uniform morphological characteristics—small, white, glistening, translucent, mucoid, and convex—suggesting the possible prevalence of specific functional groups such as diazotrophs or exopolysaccharide-producing bacteria. Among the media tested, YEMA plates exhibited the highest abundance of dominant colonies compared to Ashby's Mannitol Agar and Nutrient Agar plates. This observation is consistent with earlier reports indicating that YEMA is particularly effective for isolating members of the genus *Rhizobium*, which are commonly found in rhizosphere environments and are known for their mucoid colony morphology due to exopolysaccharide production. The predominance of similar colony morphologies across all media suggests that a specific group of microorganisms may play a significant role in the observed bioelectricity generation, warranting further biochemical and molecular characterization to confirm their electroactive potential.

Voltage Readings from Mass Cultures: The voltage outputs obtained from mass-cultured broths provided significant insights into the electrogenic potential of the isolated microorganisms. The YEMA broth culture inoculated with the isolates exhibited consistent and stable voltage generation, closely comparable to that observed with the original rhizosphere soil substrate (Table-4) in the MFC system. This suggests that the dominant microbial population enriched in YEMA possesses effective electroactive capabilities, likely mediated through Extracellular Electron Transfer mechanisms. In contrast, cultures grown in Nutrient Broth and Ashby's Broth did not exhibit consistent or stable voltage outputs. Recent studies emphasize that efficient bioelectricity generation in MFCs depends strongly on the enrichment of specific electroactive microbial communities capable of direct or mediated electron transfer to the anode. Furthermore, stable voltage production is often associated with the formation of robust anodic biofilms and optimized microbial interactions, which are influenced by growth media and substrate availability. *Rhizobium anhuiense*-based MFCs showed improved power output, particularly with redox mediators like menadione, which enhanced electron transfer[21]. Studies have also identified *Klebsiella pneumonia* and *Escherichia coli* as the primary bacteria responsible for electricity production, with gutter sludge and mixed fruit producing the highest voltage of 650 mV and 400 mV respectively[22].

Recent advancements highlight that MFC performance, including voltage stability, is closely linked to microbial diversity, electron transfer efficiency, and metabolic activity within the system[23,24]. The superior performance observed in YEMA broth may therefore be due to its ability to preferentially support metabolically active and possibly exopolysaccharide-producing microorganisms that facilitate biofilm formation and sustained electron transfer.

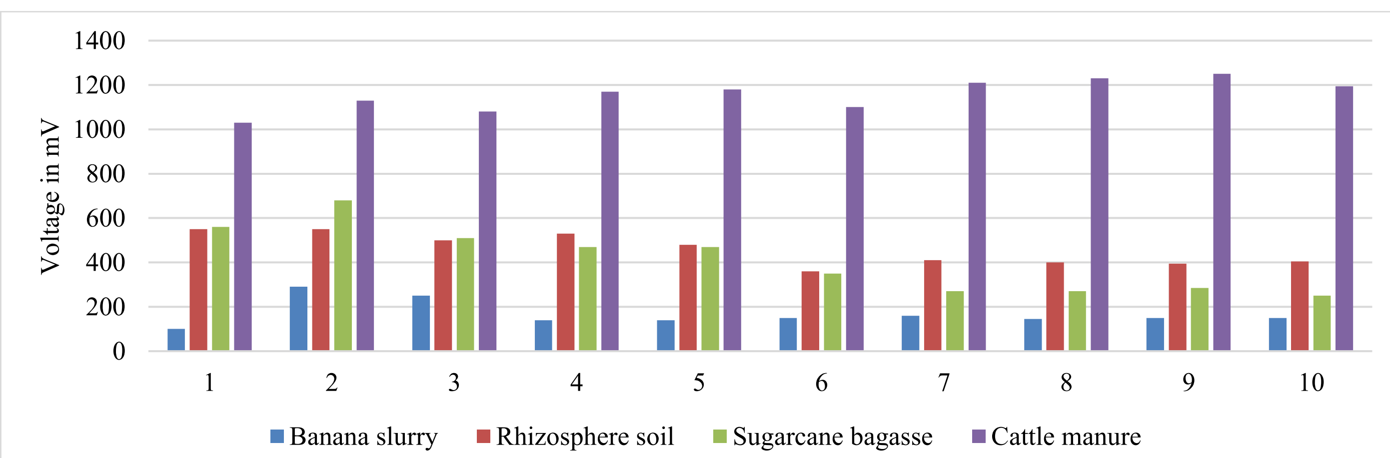

Figure-4: Comparative analysis of different substrates with respect to voltage at various days.

Table-4: Voltage readings with inoculum from mass cultures at an interval of 25mins.

Inoculum	Time	Nutrient broth (mV)	Ashby's broth (mV)	YEMA broth (mV)
Without inoculum	Initial voltage readings	609	512	444
	After 25 mins of interval	609	512	444
With inoculum Time interval of 25 mins each	1	615	520	535
	2	612	500	520
	3	628	550	551
	4	623	545	535
	5	620	531	527

Re-Isolation and confirmation: Based on the voltage assessment results, the organism from the most promising broth culture (YEMA broth) was re-isolated by plating onto fresh YEMA agar media and incubated at 25-28^{o}C to confirm its purity and dominance. To identify microorganisms involved in electricity generation, biochemical tests and gram staining was performed for the colonies from the re-isolated YEMA plate including the study of colony characteristics. The bacterial isolates were identified as Gram-negative rods on gram staining and were found producing small, circular, convex, mucoid and glistening colonies with whitish-pink pigmentation and smooth entire margins. Confirmation of *Rhizobium* was achieved using a series of biochemical assays. The isolates tested positive for Indole production, Citrate utilization and Methyl Red reaction, whereas Voges–Proskauer, Nitrate reduction and Urease tests yielded negative results.

Conclusion

The present study demonstrated the potential for MFC's for generating bioelectricity from a range of organic waste substrates, including banana peel, sugarcane bagasse, rhizosphere soil, and cattle manure, MFCs. Among these, cattle manure produced the highest voltage output, indicating its strong potential as an efficient substrate owing to its rich organic content and diverse, metabolically active microbial community. Measurements of pH and microbial isolation further underscored the importance of microbial activity in electricity generation, with the presence of *Rhizobium* identified in rhizosphere soil and other unidentified isolates contributing to electrogenic performance in cattle manure. Upon completion of the experimental cycle, a distinct ethanolic odour was detected during the disposal of the sugarcane bagasse substrate. This byproduct suggests that anaerobic fermentation occurred alongside the electrogenic process, indicating a complex metabolic environment where complex polysaccharides were converted into alcohols and organic acids.

The findings of this work emphasize the potential contribution of MFC's to both sustainable waste management and renewable energy generation. By utilizing abundantly available agricultural and organic wastes, this method offers a dual advantage of reducing environmental pollution while producing bioelectricity. Additionally, the potential reuse of post-MFC residues as biofertilizers supports the concept of a circular economy, enhancing soil fertility and agricultural sustainability.

Despite these promising outcomes, certain limitations remain, including the use of non-sustainable electrode materials and toxic catholytes, which may hinder large-scale application. Addressing these challenges will be essential for advancing MFC technology. Future studies should concentrate on the designing of eco-friendly cathode systems, improved electrode materials, and a deeper understanding of electroactive microbial communities and their interactions, particularly those involving Extracellular Electron Transfer. Integrating these advancements into scalable waste-to-resource systems could significantly increase the practical viability of MFCs for sustainable energy production and environmental management.

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