Investigating the ohmic behavior of mediator-less microbial fuel cells using sewerage water as the bio-anode

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Abstract: There is a great challenge in ratification of electrochemical principles to energy-storage devices utilizing natural systems such as microbial fuel cells (MFCs). The purpose of this study is to investigate how increasing impedance affects key cell dynamics of MFCs at ambient conditions using untreated sewage as the bio-anode. A H-type mediator-less MFC of capacity 4,556.25 cm³ using porous graphite electrodes and raw sewage as the bio-anode was studied over one 10-day retention period. The results indicated an exponential increase in OCV up to the sixth day followed by its gradual reduction. Ohmic behavior was observed in the current and power densities with the highest power density being 0.173 mW/cm³ for the 1,000 Ω resistor. The charge/discharge times ranged between 11.15 and 11.40 days with daily discharge rates of 8.77–8.96%. Ohmic behavior was also observed as the energy balance, capacity and density of the MFCs decreased with applied impedance. The highest values were obtained in the 1,000 Ω MFC (energy balance, 336.1 J/s; energy capacity, 4.227 Wh/cm³ and energy density, 17.237 Wh/kg, respectively). The MFCs ohmic behavior when using raw sewage proved to be quite novel and an inspiring finding to enhance applicability of MFCs using raw industrial effluents.

Subjects: Environmental Management; Physics; Power & Energy

Keywords: Microbial fuel cell; power; sewage water; ohmic behavior

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The authors of this paper have diverse experience in fabrication and development of energy storage devices. In particular, Dr Aloys Osano is a senior lecturer of Chemistry in Maasai Mara University where he has done numerous researches in renewable energy systems and energy storage devices. Mr Bakari Chaka is a doctoral student of Chemistry in the same University with avid research interests in the same areas. On the other hand, Mr Thiong'o Mbarire is a Master’s student of chemistry in the same University. His research interests are on different fuel systems for use in tough environs.

PUBLIC INTEREST STATEMENT
This paper describes how some crucial electric disciples have been proven to work in a microbial fuel cell utilizing untreated sewage water. The sewage water contains numerous confounding variables that on a normal basis would make it impossible for Faradaic principles to be observed. The findings are quite essential and will go a long way in diversifying the use of these cells.
1. Background of study

Microbial fuel cells (MFCs) are bio-electrochemical systems that are used to generate low currents for small electrical appliances. Despite increased populace of MFCs and their appliances over the years, there are still few MFC gadgets in the market. This can be partially attributed to fast growth of other energy-saving devices such as the lithium-ion cell, aluminium cell among others (Leisegang et al., 2019). However, adaptability of MFCs to diverse bio-electrodes and their bio-electrical implications to the overall MFC performance is largely unstudied and wanting (Choudhury et al., 2017). Different bio-electrodes, especially those that are naturally sourced, are known to have numerous compounding variables (Griesshaber et al., 2008). It is thus worthwhile to investigate how pure scientific laws such as ohmic behavior would perform in such natural bio-electrodes. Positive findings of the same would strongly influence more uptake and revolutionize MFC appliances vis-à-vis contemporary energy-saving devices.

Raw sewage is quite abundant and a public nuisance that comprises a myriad of archa (Eissa et al., 2022; McLellan et al., 2010)—some with bio-electrical significances. Majority of MFCs have their bio-electrodes derived from strains of redox bacteria (Conley et al., 2018). It is however worthwhile to exploit use of raw sewages as a bio-electrode and the consequent adherence of the MFC to pure scientific laws such as ohmic behavior. In the contemporary society, MFCs utilize the redox potential of several strains of oxo-electrogen bacteria, e.g., Shavella putrificiens and Aeromonas hydrophila (Conley et al., 2018). Depending on the nature of these microbes, they can be placed on either electrode to create a bio-anode or bio-cathode. The microbes can be supplied from pure commercial strains or wastewaters with a biological background such as sewer (Eissa et al., 2022). The other electrode compartment is filled with an oxidizing or reducing salt, e.g., NaCl solution. Electron transfer in MFCs is spontaneous, assuming galvanic-state conditions. However, there is need for mediators to facilitate movement of electrons if meaningful open currents are to be realized in MFCs (Babauta et al., 2012; Bretschger, Osterstock, Pinchak, Ishii, Nelson et al., 2010b).

The current generated by MFCs is very low, primarily because of use of biological substances (oxoelectrogen bacteria) whose redox potential is quite small (Kracke et al., 2015; Ucar et al., 2017). The average open current generated by any bacterium do not exceed 0.1 A (Bond & Lovley, 2003; Luo et al., 2005). The corresponding voltage is thus too little and does not meet the threshold voltage required to be stepped up by a transformer. It is for this reason that MFCs are largely reserved for small electrical appliances such as sensors. Most of the bacteria strains are also quite expensive to acquire further worsening popularity of MFCs. Nevertheless, MFCs have energy efficiencies >50% (Gajda et al., 2018; Santoro et al., 2017)), closer to the proverbial Carnot engine cycle. Optimization of this phenomenon can be used to minimize energy losses for more current production by MFCs.

Energy (electrical) losses in MFCs result from many sources. The most common origins are permeability of the membrane or salt bridge used, mediators, nature of bio-electrode used and the electrical appliances employed. While it is almost impossible to cheaply optimize on these sources of energy loss, a keener insight into the conductors used can help minimize the losses. It is therefore helpful to evaluate the nature of material used as a conductor carefully. An integral part of this evaluation is the ohmic behavior of the material, which relates the current output to the resistance of the material (Schweiger et al., 2010). While many researches have studied on the ohmic behaviors of different materials at different working conditions, very few have focused on the ohmic behavior of MFCs. Of more importance is the adherence of Ohm’s law to MFCs utilizing untreated effluents as the source of redox strains in their natural niches.

Different materials are known to have different resistance levels. For any ohmic current, the output current density is a function of the nature of material (resistivity factor), surface area, length and time. The baseline of this is in Ohm’s law in equation 1:
$V = IR$ (1)

where $V$ is the voltage output, $I$ is the current and $R$ is the resistance of material.

It is of great interest to explore the applicability of ohmic behavior in MFCs utilizing untreated sewage as the bio-anode. In such systems, all the current generated is not fully Faradaic, yet assume galvanic-state and depends not only on chemical systems but also biological ones. This study aimed at exploring the ohmic behavior of mediator-less MFCs utilizing raw sewage as bio-anodes at ambient temperature conditions. The findings are key in scaling up the current generated by MFCs for higher electrical appliances.

The article is structured to provide the Introduction (with gaps, justification and literature) followed by intervention measures used to try counter the existing challenge, the findings and discussions therein.

2. Materials and methods
The study was carried out as illustrated in the flowchart (Figure 1).

2.1. Design of study
The study was carried out in Maasai Mara university, Narok, Kenya (coordinates 1.0918° S, 35.8498° E). A H-type MFC of capacity 4,556.25 cm$^3$ was fabricated from locally purchased materials. No mediator was used. The MFC was filled with lab-grade NaCl as the anode and raw sewer water was used as the bio-anode. The salt bridge was filled with commercial agar to facilitate movement of ions. A multimeter was used to monitor voltage output in presence of several resistors, in series with the multimeter. Open-circuit voltage, current, power densities, discharge times and energy balance were then monitored over a duration of 10 days with varying ohmic behavior.

2.2. Assembly of the MFC
A H-type MFC was adopted for its ease in fabrication and conceptualization. This was a key factor (conceptualization) due to the nature of the study to be undertaken, i.e. identifying ohmic behavior in movement of the ions generated from the semi-Faradaic bio-anode of MFC. The MFC was fabricated from clear polyvinylchloride (PVC) polymeric materials. Each of the electrode chambers had the dimensions 135 mm by 135 mm base by 125 mm height. The specific surface area of these chambers was calculated to be 0.0376 mm$^2$/mm$^3$.

Both electrodes were made from graphite (surface area 5127 mm$^2$ each). Porous graphite (SFG6L) was preferred for their bio-compatibility with biological environment in concern as well as chemical stability in varying chemical electrolytes and concentrations. The porous graphite also provided more surface area for biofilm attachment. Copper wire was connected to the graphite electrodes on the grooves made. For the anode, the connecting wire was passed through a hole in the bottle lid and sealed with epoxy adhesive. Electrodes were then tested with a multimeter (Model: UT33B DC voltage (V): Sensitivities of 200 mV, 2000 mV, 20 V, 200 V ±(0.5%+2)/500 V ±(0.8%+2). AC voltage (V): 200 V/
500 V ±(1.2%+10). DC current (A)). There should be a small amount of resistance (1–3 Ω) between a point on the graphite electrodes and the end of the connecting wire.

The salt bridge was fabricated from a PVC pipe (diameter 50 mm, length 120 mm). The salt bridge was filled with 30% agar solution (Sigma-Aldrich Co.) spiked with 2% NaCl solution at 45°C. The salt bridge was connected to the two cathodic chambers at this temperature and allowed to gradually anneal while enjoining the chambers.

The MFC was developed according to Yang et al. (2019) and Li et al. (2018). The MFC was then tested for porosity using water at varying temperatures between 20°C and 50°C to avoid any leakages. Thereafter, the cathodic compartment was filled with 1 M NaCl solution, before pumping in more oxygen. An air pump was connected for this purpose. The anodic compartment was then filled with raw sewage water spiked with 10% agar solution to nourish the microbes present. The bio-waste was characterized as follows: pH = 6.5, temperature = 23.5°C, total solids = 11%, dissolved oxygen, DO = 4.45% and greenish-blue in color due to the algae colonies present. The DO was then reduced to below detectable limits by suctioning with an air-pump. The bio-anode mixture was also tightly shut and a biofilm of bacteria let to develop at the anode surface. The external circuit was then completed to facilitate movement of ions. The assumption is that the sewage water was devoid of heavy metals attributed to affecting the conductivity and thrive of microbes (Buaisha et al., 2020). The study also assumes constant oxygen uptake rate in the system—which would otherwise affect the entire substrate load including essential microbes present (Mirra et al., 2020).

Data collection was done for a period of 10 days varying the amount of resistance in the circuit as follows: 1000, 2500, 5000 and 16,000 Ω. The parameters monitored were based on the scientific relevance in regard to the study question—conformity of MFCs using untreated sewage to electrical principles such as Ohm’s law. These parameters were obtained experimentally and through appropriate calculations. The said parameters sensitivity ranges are those of Multimeter (Model: UT33B DC voltage (V) as illustrated above. The open circuit was determined when no current passed through the resistor (resistor muted). The current density and power densities values were obtained from the multimeter, while the estimate discharge time and energy balance were calculated from the graphs above as illustrated by Jorge et al. (2021). The estimated discharge times were calculated by equating the power vs time polynomials with zero (0). The energy balance was found by calculating the area under graph of power against time. The area was calculated by integrating the polynomial equations of the various resistors. The energy capacity was calculated by dividing the energy balance (in J/s) with the MFC volume (equation 2). The energy density was calculated by dividing the energy capacity by the weight of the MFC (equation 3). The specific gravity of 1 M NaCl solution and sewer water used were the theoretical values of 1.069 and 0.721 g/ml, respectively.

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\text{Energy capacity} = \frac{\text{Energy balance}}{\text{MFC Volume}} \tag{2}
\]

\[
\text{Energy density} = \frac{\text{Energy capacity}}{\text{MFC gravimetric weight}} \tag{3}
\]

Data analysis was done using Ms Excel (2016) and MathGraph App.
3. Results and discussions

3.1. Open-circuit voltage of the MFC
The open-circuit voltage (OCV) of the MFC increased exponentially until the sixth retention day then began reducing. The OCV of the MFC gave a quadratic behavior, adopting a quadratic mathematical function with a maxima value on the sixth day. The increment of the OCV values can be attributed to increasing current generated by the bacteria over time. At the onset of the experiment, the bacteria population are few and yet to acclimatize to their new environment. The level of biofilm attachment is also less as most microbes are evenly dispersed in the chamber. Similar occurrences and behavior in potential differences are reported by Greenman et al. (2021). With time, the zeta potential toward the anode surface gradually draws them there. The anodic surface is slowly covered and the distance taken for the protons generated to reach the anodic surface is smaller. This phenomenon reduces the time taken for Faradaic current to be generated, increasing the effective current. The more the anodic surface is covered by the biofilm, the more the current generated. Marcus et al. (2007) made similar observations when developing anodic biofilms used to investigate how electrons transfer from bacteria to the anode. On the other hand, the bacteria microbes start to multiply as they acclimatize and have enough nutrients. This increase their population leading to exponential production of protons and thus Faradaic current. An optimal OCV value of 1.02 V is achieved on the sixth retention day. This value closely resembles that obtained by Patel et al. (2019) during catalysis of a two-chamber MFC using cow dung as inoculum and Saccharomyces cerevisiae as biocatalyst. Such biocatalysts have a potential to multiply their coliform units within a short period as long as there is sufficient a conducive environment (present in sewer; Olaoye et al., 2022). In the experiments with a frit membrane separator, an OCV maxima value of 1.143 V for 8 h was attained while that with a nafion separator a maxima OCV value of 1.128 V for 32 h was attained. Several other experiments have cited OCV values of these margins (Badea et al., 2019; Nguyen et al., 2019).

After the sixth day, there was a gradual and exponential fall in the OCV values. This is because the nutrients available for bacteria might be depleted. Other factors favoring growth of bacteria might as well be saturated by this point leading to stiff competition for the same and thus death of the microbes. The anode surface might also be poisoned by by-products (e.g., excreta products of the bacteria) of bacteria metabolism. The average lifetime of most bacteria species is 24 h (Lodish et al., 2000) and therefore after 6 days a significant fraction of them are dead by this time. The NaCl electrolyte was used in batch and therefore most of the ions are used up at this point. There is also a flux of NaCl ions toward the bio-anode compartment via the salt bridge as time progresses. These ions increase the salinity levels in this chamber intoxicating the bacteria present. Figure 2 illustrates the trend in OCV of the MFC in the retention period used. The instantaneous OCV of the MFC can be calculated from the derived quadratic function in Figure 2. The ohmic behavior for OCV could not be monitored since there was no resistor connected to the system in this mode.

3.2. Effects of ohmic behavior on voltage, current and power generated from MFC
Ohmic behavior (increase in voltage with current density) of the MFC was linearly observed up to the sixth day. Thereafter, there was a decrease in voltage and current density toward the end of the retention period. The magnitude of the resistor was inversely proportional to these changes, with the highest resistor (16,000 Ω) having the lowest and 1,000 Ω resistor having the greatest voltages. The trend in voltage increment was similar to that of OCV. The rate of increase in voltage and current can be attributed to increasing microbial population in the anodic chamber (Hodgson et al., 2016). A higher bacteria population leads to more generation of H⁺ as a by-product of their metabolic activities. This can be explained by the growth of the microbial ecology as observed during hydrogen evolution during methanogenesis in the rumen (Bretscher, Osterstock, Pinchak, Ishii, Nelson et al., 2010b). These ions are used in current generation leading to Faradaic current.
After the sixth day, the number of bacteria and their requirements decline leading to less Faradaic current. The salt bridge, whose role is to replenish used up ions in the system also increase the salinity level of the bio-anode compartment. This increase the stress on the bacteria limiting their production potential (Hanin et al., 2016; Shrivastava & Kumar, 2015). The general rate of voltage increment with current density followed a linear equation, similar to Ohm’s law. The slope of this behavior was positive between the first and sixth day, then negative thereafter. The magnitude of the gradient was 16.3 mV/A/m² for the increment and 18.9 mV/A/m² for the decrease after the sixth day. Koffi and Okabe (2020) reported power values of a similar range using air-cathode MFCs utilizing wastewater equipped with a low booster voltage multiplier. These changes are illustrated in Figure 3a.

Power is a function of current and voltage (Fish & Geddes, 2009). The rate of change in power over time was therefore directly proportional to the voltage generated in the MFC. Power exponentially increased up to the sixth day, due to increase in bacteria population and attraction toward the anode then reduced as the bacteria population fell and anodic surface was poisoned. The ratio of power between the various MFC resistors was equal to the magnitude of the resistance (Figure 3b). The power density values obtained were in agreement with those highlighted by Kamau et al. (2017) who found out that MFC produce power densities in the range of 10–100 mW/m² for 10,000 Ω resistors at ambient temperature conditions. The optimal power densities (on the sixth day) obtained by each of the resistors were 0.173, 0.075, 0.038 and 0.016 mW/cm² for the 1,000, 2,500, 5,000 and 16,000 Ω MFC resistors respectively further illustrating ohmic behavior of the MFCs.

3.3. The estimate discharge times of the MFC

The estimate discharge time of MFCs denotes the time taken to fully charge or discharge it. A 100% discharge/charge is practically impossible due to the threshold cell depth of discharge (DOD) value. A high discharge time implies more cell capacity or small discharge rate. From section 3.2, the MFC power capacity was found to range between 0.016 and 0.173 mW/cm³ (in order of decreasing resistor magnitude). Most MFC appliances do not require intense power discharge therefore the discharge time of MFCs are quite longer. This observation concurred with that of Santoro et al. (2019) who observed similarly elongated discharge times were also observed in self-stratified MFCs fed with
human urine. This reduces the charge/discharge cycles of the MFC which are responsible for the cell damage (Kim et al., 2014; D. Y. Wang et al., 2017). The discharge times of the MFC increased with the resistor magnitudes as observed in Table 1. This can be attributed to the increase in the MFC capacities. A higher discharge time increase the operating lifetime of the MFC.

From Table 1, the daily DOD/depth of charge (calculated from the full MFC capacity divided by the discharge time) ratios were 8.96%, 8.81%, 8.83% and 8.77% in order of decreasing MFC resistors. This implies that the 16,000 Ω MFC would require topping up the bacteria colonies or ionic solution (cathode) by a rate of 8.96% of its capacity every day. The 1,000 Ω MFC would require a daily top up of 8.77%, which is actually lesser than the 16,000 Ω cell. The daily DOD/depth of charge is a very critical parameter as it defines the organic loading rate range of the MFC. MFCs with a higher daily depth of charge/DOD are susceptible to more charge/discharge cycles thus straining their working
regimes (Naha et al., 2020). The discharge times calculated are theoretical and denotes a 0% MFC state of charge (SOC). However, in real-life situation, these values are not practically possible due to the MFC DOD and environmental regimes (Wang et al., 2018). The environment of MFC comprise of several biotic and chemical media that influence the available SOC (Santoro et al., 2017). From the findings, ohmic behavior proportionately affected the discharge time of the MFCs.

### 3.4. Energy balance of the MFCs

The energy balance of MFCs illustrates the difference in charge and discharge power at any instant of the MFC. The energy balance of the MFC is a function of its Coulombic and voltage efficiency. Coulombic efficiency denotes the ratio of input charges to the output ones during discharging (Wang et al., 2018). The energy balance of the MFCs was found to increase linearly with magnitude of the impedance of the cell. On the other hand, voltage efficiency denotes the voltage balance during charging and discharging the cell (Nakata, 2019). The cells' SOC have a direct impact on their voltage efficiency. A battery whose voltage varies linearly with the SOC (such as these MFCs) is likely to have less voltage efficiency. From Table 2, the MFC connected to a larger resistor (16,000 Ω) had less energy balance (28.2 J/s) implying less Coulombic efficiency. The ratio of charge input/output was lower than the rest indicating more stable regimes. The MFC connected to the smallest impedance (1,000 Ω) had the most energy balance (336.1 J/s) implying more deviation in input and output power. The stability of the regime in this MFC was thus affected. This deviation can be attributed to more secondary reactions resulting from a higher power capacity in this MFC. Some of the reactions include electrolysis of water, weak acids and other side-reactions contributing to Faradaic and non-Faradaic current in this MFC. In a research trying to recover energy during wastewater treatment using MFCs, Capodaglio et al. (2013) observed a Coulombic efficiency ranging from 0.8% to 1.9%. This value is by far less than those of conventional battery systems which enjoy Coulombic efficiencies. MFC systems with higher Coulombic efficiencies are affiliated with more unstable regimes. This phenomenon is good for wastewater purification but bad for the cell health.

### Table 1. The discharge times and daily discharge rates of the MFCs

| Resistor (Ω) | Polynomial equation | Discharge time (days) | Daily charge/discharge rate (%) |
|-------------|---------------------|-----------------------|---------------------------------|
| 16,000      | \( y = -26.05x^2 + 326.01x - 397.09 \) | 11.15                | 8.96                            |
| 5,000       | \( y = -11.531x^2 + 145.98x - 170.89 \) | 11.35                | 8.81                            |
| 2,500       | \( y = -5.6061x^2 + 70.92x - 84.247 \) | 11.32                | 8.83                            |
| 1,000       | \( y = -1.9481x^2 + 24.649x - 27.225 \) | 11.40                | 8.77                            |

*Discharge time and rates of the MFCs.

### Table 2. The energy balance, capacity and density of the MFCs with various resistor values

| Resistor (Ω) | Energy balance (J/s) | Energy capacity (Wh/cm³) | Energy density (Wh/kg) |
|-------------|----------------------|--------------------------|------------------------|
| 16,000      | 28.2                 | 0.355                    | 0.087                  |
| 5,000       | 76.5                 | 0.962                    | 3.923                  |
| 2,500       | 159                  | 1.999                    | 8.152                  |
| 1,000       | 336.1                | 4.227                    | 17.237                 |

*Variation of energy balance, capacity and density with impedance in MFCs.
The energy capacity and energy densities increased exponentially with the resister, further affirming ohmic behavior of the MFCs. The energy capacities in Table 2 imply that as more impedance is applied onto the MFCs, the lower its capacity to carry energy. Energy density is crucial in determining the portability of the cells. This is an essential parameter to consider for mobile sensors using MFCs for power generation. From Table 2, the more the impedance applied, the lesser the energy density. Therefore, one would require several 16,000 Ω cells to yield the same amount of power that the 5,000 Ω would. The same logic applies to the MFCs with lower impedance. The 1,000 Ω MFC is thus the lightest and most portable cell.

4. Conclusions
The study proves linear impedance (Ohm’s law) can be followed in MFCs utilizing natural and untreated bio-electrodes such as raw sewer despite numerous confounding parameters in such systems. Consequently, MFC parameters such as open and closed current voltage, power density, energy balance and charge/discharge times were found to obey Ohm’s law. Relatively high energy capacities and densities (336.1 J/s, 4.227 Wh/cm² and 17.237 Wh/kg, respectively) were recorded.

The study shows great potential and conformity to electricity principles by MFCs utilizing naturally occurring and untreated bio-electrodes and thus exhibiting more applicability of MFCs. Further studies using other “wastes” with redox bacteria strains are expected.

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Data availability statement
All the data used are within the manuscript and any supplementary sheets attached.

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