







## ORIGINAL RESEARCH ARTICLE

## Bioelectricity Generation from Microbial Fuel Cell utilising Sewage Wastewater and Cow Urine from Dutse Metropolis Jigawa State.

Aisha Salisu Buhari<sup>1,2</sup> , Hindatu Yusuf<sup>2\*</sup> , Peter Gimba Shiaka<sup>2</sup> , Haris Nura Garba<sup>3</sup> , Sadiq Haruna<sup>2</sup>  and Zainab Jamilu Abdulsalam<sup>4</sup> 

<sup>1</sup>Department of Science Laboratory Technology, College of Science and Technology, Jigawa State Polytechnic Dutse. P.M.B 7040-Nigeria.

<sup>2</sup>Department of Microbiology and Biotechnology, Faculty of Science, Federal University Dutse, 7156 Dutse Jigawa State, Nigeria.

<sup>3</sup>Department of Biological Science, Federal University, Dutse. P.M.B 7156-Nigeria.

<sup>4</sup>Department of Nutrition and Dietetics, College of Health Science, Jigawa State Polytechnic Dutse. P.M.B 7040-Nigeria.

### ABSTRACT

Microbial fuel cells (MFCs) are technologies that directly transform chemical energy into electrical energy by oxidizing organic matter using bacteria as biocatalysts. MFCs offer a potential technology for converting wastewater into useful energy source and at the same time serve as wastewater treatment facilities. This makes it superior to other wastewater treatment methods. This study focused on the utilization of MFCs to generate bioelectricity from sewage wastewater using cow urine as inoculum and identify the bacteria colonizing the anode electrode. The experiment were conducted using two-chambered MFC constructed using locally sourced materials. Wastewater was characterized using standard methods. The characteristics of the sewage wastewater are: 680 mg/L Chemical oxygen Demand (COD), 457 mg/L Biochemical oxygen Demand (BOD) and pH of 7.4. The maximum voltage, power and current density obtained were 196 mV, 18.26 mW/m<sup>2</sup> and 97 mA/m<sup>2</sup> respectively. The MFC shows a reduction in COD value by 82 % (680mg/L initial and 120 mg/L final). The identification of the anodic biofilms showed the presence of *Bacillus* spp and *klebsiella* spp based on their microscopic and biochemical characterization. The results of this study can contribute to improve understanding and optimizing electricity generation in MFC, Further study would be conducted in order to identify the microorganisms at molecular level.

### ARTICLE HISTORY

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### KEYWORDS

MFC, Bioelectricity, Power density, cow urine, wastewater.

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## INTRODUCTION

Energy infrastructure, which was formally taken over by coal, oil, and natural gas, is now observing a pattern to step back and reduce net CO<sub>2</sub> emissions from these energy conversion processes and to focus on renewable and clean energy sources. Over the coming 30 years, replacing 70% of energy sources would bring between 170 \$ and 200 \$ billion annually (Nor *et al.*, 2015). Although complex, these profitable numbers aren't veritably delicate, given the length of time involved, new technologies may potentially arise and prop in reshaping the profitable assessment. Energy cost, or the quantum of electricity demanded to run these installations along with the cost of the pricy chemicals and the cost of system maintenance, is a pivotal consideration when examining wastewater treatment structure. For instance, sewage from homes is reused physically, chemically, and biologically to exclude poisons before it's released into the environment at a position that is respectable to the ecosystem (Xu *et al.*, 2018). It's pivotal to treat this wastewater for used and

offer suitable water given that the earth is 70 % water and that only 2.5 % of it is fresh water (Bose *et al.*, 2018).

Using MFCs could be one method to ground the energy structure and the wastewater treatment facilities. Given the global consensus to focus on renewable and emission-free technologies, MFC technology offers the potential to treat wastewater and generate power at the same time (Logan *et al.*, 2006).

Microbial fuel cells (MFCs) are technologies that directly transform chemical energy into electrical energy by oxidizing organic matter using bacteria as biocatalysts (Yusuf *et al.*, 2019). Typical MFCs consist of two chambers separated by a proton exchange membrane (PEM), an active biocatalyst oxidizes organic substrates to produce electrons and protons (Hindatu *et al.*, 2017). The PEM carries the protons to the cathode chamber, whereas the external circuit carries the electrons. In the cathode

**Correspondence:** Hindatu Yusuf. Department of Microbiology and Biotechnology, Faculty of Science, Federal University Dutse, 7156 Dutse Jigawa State, Nigeria. ✉ [hindatu.y@fud.edu.ng](mailto:hindatu.y@fud.edu.ng). Phone Number: +234 806 959 4199.

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chamber, protons and electrons react and oxygen is simultaneously reduced to water (Hindatu *et al.*, 2017). MFCs technology is powered by bacteria because of their innate capacity to break down organic matter and produce electricity. It is crucial to comprehend the impact of the inoculum nature on the anodic bacterial diversity and establish its relationship with the power performance of the system because the development of an effective anodic biofilm has a significant impact on the power performance of this technology (Choudhury *et al.*, 2017). One of the most important advantages of MFC technology is that it is environment friendly in nature as compared to other energy production technologies which involve, fossil fuels and methanogenic anaerobic digestion, which result in the emission of greenhouse gases like carbon dioxide, thus contributing to global warming (Du *et al.*, 2007). The various applications of MFC technology include the production of bioelectricity from various organic sources such as solid waste biomass, food waste, domestic and other wastewater, etc. Thus, the inclusion of these waste products as substrates in MFC technology makes it a more potent means of sustainable energy generation.

Due to its availability, buffering ability and high chemical oxygen demand, clean urine has recently attracted a lot of attention as a feed stock for many types of bioelectrochemical systems (Addi *et al.*, 2018). The bacterial community's enrichment in the anodic biofilm is a powerful indicator of process functionality. Nevertheless, despite the abundance of bacteria found in MFCs, only those with an electroactive metabolism will directly contribute to the production of energy (Heidrich *et al.*, 2018).

This work was aim in utilizing sewage wastewater and cow urine to generate bioelectricity and identified the bacteria present in the anodic electrode within dutse metropolis. Similarly Jigawa state has no any documented literature similar to this article.

## MATERIALS AND METHOD

### Materials

Fresh cow urine was collected from the Federal University Dutse research and teaching fam, sewage wastewater (WW) was collected from gida dubu area Dutse Jigawa state. Stainless steel sieve, Stainless steel mesh with a diameter of 4.4 cm and surface area of 38 cm<sup>2</sup> and rubber gasket were obtained from Dutse ultra modern market, Jigawa state, Distilled water, 3% sodium hypochlorite solution, 70% ethanol solution, Phosphate buffer solution (PBS), glass wares, Media and biochemical test reagents were obtained from Sabon gari market Kano, Nigeria. Poly Vinyl Chloride (PVC) plastic bottles with 250 ml capacity used as MFC container, PVC pipe with 1.99 cm diameter, Deionized water, 0.1 M H<sub>2</sub>SO<sub>4</sub> Solution, 0.1 M, H<sub>2</sub>O<sub>2</sub> solution, copper wires, external resistors with the resistance of 510 - 15000 Ω, Digital multimeter (DT-9205A) and other reagents used for WW characterization

were all obtained from main market Azare, Bauchi state, Nigeria. The infrared thermometer (BTG06) was obtained from Guangdong Bioall Medical Technology Co., Ltd, China. Nafion 117 membrane was obtained from Dupont, Delaware, USA.

### Sample Collection

#### *Sewage Wastewater*

A sewage sample was collected around Gida Dubu Area in Dutse, Jigawa State. Prior to sample collection, the collection bottle was thoroughly washed and sterilised in the laboratory before going for sample collection. About 500 mL of the Sewage wastewater was collected in a dark sample bottle of 1 L capacity and placed in an ice cooler packed before transporting it to the laboratory. The physicochemical characteristics were determined immediately. The remaining sample was stored in the refrigerator at 4°C until it is needed for subsequent experiments.

#### *Source of Inoculum and Preparation*

Cow urine was used as inoculum for electricity generation, and it was collected from the university research and teaching farm.

The inoculum was prepared by serial dilution method, and 2 ml from the dilution tube was transferred into 20 ml of sterile nutrient broth and then incubated for 24 h. This was utilised as the biocatalyst in the MFC anode.

### Chemical Analysis

The pH, Temperature, Total suspended Solid (TSS), Biochemical Oxygen Demand (BOD<sub>5</sub>) and Chemical Oxygen Demand (COD) were determined before and after the operation of MFCs according to the standard methods for the examination of water and wastewater by the American Public Health Association (APHA, 2012).

### MFC Construction

The construction of the MFC was based on a previously reported MFC set-up used by Hindatu *et al.*, (2017). However, the materials utilised for constructing the MFC used for this research were locally sourced. The MFC was constructed using PVC plastic bottles of 250 ml capacity as cathode and anode chamber, respectively. A PVC P/T socket (PPS) was used as the membrane holder. A hole equal in diameter to the PPS (1.99 cm) was made on the sides of the PVC plastic bottles, and the PPS was inserted into the hole and glued with the help of a four (4) minutes gum (Epoxy Steel Gum). A rubber gasket was fitted to the open end of the PPS, and the proton exchange membrane (PEM) was sandwiched between the PPS and held together with the help of a clamp with nut and bolt. Openings were made on top of the lid of the PCV bottles for inserting the copper wire that holds the electrodes, and

extra opening was made on the cathode lid to allow aeration in order to maintain aerobic condition. Stainless steel Mesh (SSM) were cut using scissors to make a total surface area of 38 cm<sup>2</sup> with a point allowing attachments for the copper wire. Copper wire was soldered to the SSM and passed through the holes on the lid of both chambers. The 2 lids holding hanged electrode (SSM) were placed in both chambers and closed. The same gum was placed around the wire on anode lid to make it anaerobic during operation. The two suspended wires on both chambers served as attachment point to resistors and multimeter.

### MFC Operation

Throughout the study, two-chambered plastic MFC bottles with a capacity of 250 mL each and a working volume of 230 mL were used. The PEM (Nafion 117) was positioned between the two chambers. The PEM was fitted with a rubber gasket to the PPS assembly in order to prevent leaking. A 1.99 cm diameter plastic pipes was used to connect the chambers. Stainless steel mesh with a diameter of 4.4 cm and surface area of 38 cm<sup>2</sup> was used as the anode and cathode electrode material respectively. The two electrodes were connected by clamp with copper wire, and a resistor (1000 Ω) was connected across the circuit. The anode chamber was filled with sewage wastewater of (210ml sterile substrate) and 20 ml broth culture of cow urine as inoculum, and the cathode chamber was filled with 230 ml phosphate buffer and kept open to air to maintain an aerobic condition (Moqsud *et al.*, 2011). The MFC was operated via fed-batch mode at room temperature and pH within the range of 6-7 throughout the experiment. Daily readings were recorded over 36 days of operation (Hindatu *et al.*, 2018).

### Measurement of the Bioelectricity generated

With the use of a digital multimeter (DT-9205A), the voltage was measured across a 1000Ω external resistor every 12 h. When the voltage dropped below 30 mV, the anode medium was replaced. The polarization data was gathered by monitoring the voltage throughout a broad range of resistance (510 - 15000 Ω) when the voltage reached plateau as reported by Logan (2008). The current (I) was calculated using the voltage by Resistor (Eq 1) and current density was calculated using the current and then normalized by the anode surface area (Equation 2). The polarization curve was obtained by plotting the graph of the obtained voltage against the current density (mA/m<sup>2</sup>). The power density (PD) in (mW/m<sup>2</sup>) was calculated using Equation 3

$$I = \frac{V}{R} \quad \text{Eq (1)}$$

where

I is the current (amperes), V is voltage (volts) and R (ohms) is the resistance.

$$CD = \frac{I}{A} \quad \text{Eq (2)}$$

where

CD is current density (mA/m<sup>2</sup>), I is the current (amperes), is the anode surface area (cm<sup>2</sup>)

$$PD = \frac{IV}{ASA} \quad \text{Eq (3)}$$

where

PD is the power density and ASA is the anode surface area (cm<sup>2</sup>)



Figure 1: Two Chamber MFC utilized for this work

### Isolation of Bacteria Colonizing the Anode Electrode

In order to determine the different electrogenic bacterial population present in the anodic MFCs, the electrode surface was scraped aseptically using sterile scalpel in 50 ml sterile distilled water, and then shaken for 5 minutes at maximal speed to obtain biofilm suspension followed by serial dilution. Bacteria was isolated by the spread plate method, about 0.1 ml from the dilution factor was inoculated on the surface of sterile Nutrient agar, Blood Agar, MacConkey agar, Eosin Methylene Blue (EMB) agar, and Mannitol salt agar, the inoculated plates were incubated at 37 °C for 24 h. The isolates were sub culture to obtained a pure culture (Cheesbrough, 2004).

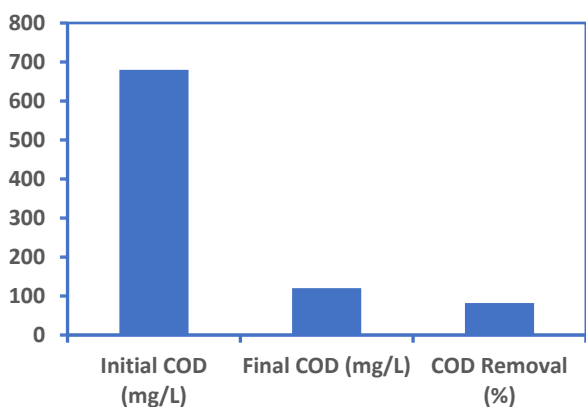
### Microscopic and Biochemical Identification of the Anodic Bacteria

The bacterial isolates were characterized on the basis of cellular/microscopic morphology through Gram staining and biochemical characteristics such as catalase, Indole production, Motility, oxidase, Methyl red, citrate utilization, urease and voges proskauer test according to Cheesbrough (2004).

**RESULT AND DISCUSSION**

**Wastewater Characterization**

Wastewater was characterized in this experiment using standard methods for the examination of water and wastewater by the American Public Health Association (APHA, 2012). During the operation period, the pH was stable at 7.4 to support organism existence, the BOD value was 457 mg/L, the total suspended solids were found to be 40 mg/L and the Temperature of 33.6°C.



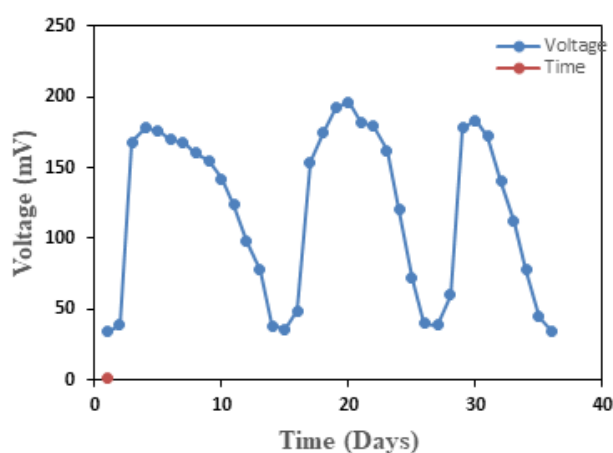
**Figure 2:** Percentage of COD removal

Figure 2 shows the percentage of COD removal. Within 36 days of operation, COD removal efficiency dropped from an initial 680 mg/L to 120 mg/L, resulting in an overall removal efficiency of 82%. Faster COD consumption rates, less loss to background operations, shorter time requirements, and higher COD removal rates are all facilitated by the system's power generation. The COD consumption of a batch process that continuously generates current while fluctuating during operation is referred to as current density (Bose *et al.*, 2018). The current density and rate of COD elimination drastically decline after about a week of operation, insufficient current is being produced by the system if the current density drops off quickly. According to Liu *et al.*, (2004), power production should continue until all COD has been utilized.

As can be seen from figure 2, the electricity generation completely stops when the COD value hits 120–110 mg/L. This is because the COD levels are insufficient to produce bioelectricity. Therefore, an anaerobic digester would be required for commercial purposes to finish the process and dispose of the water at levels that are acceptable to the environment (Logan, 2008).

**MFC Analysis**

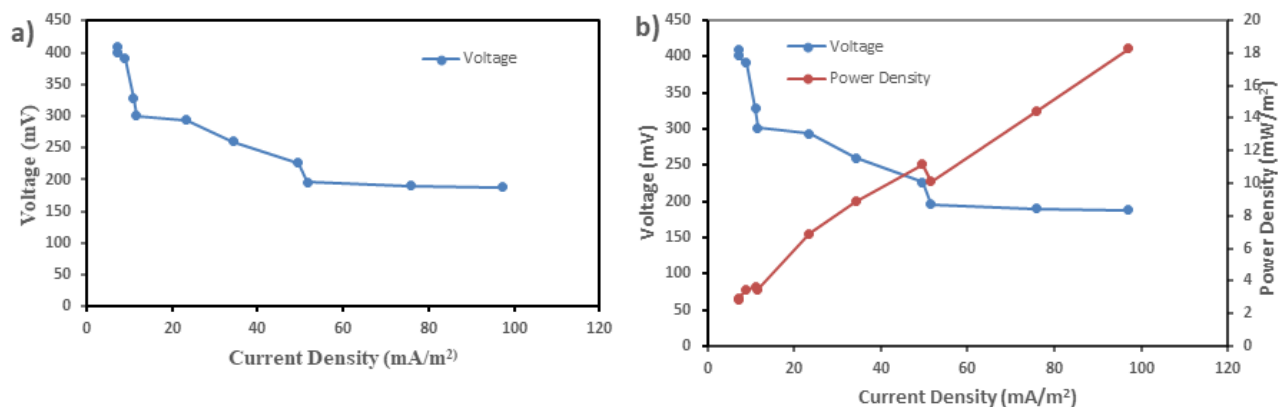
The MFC performance was assessed in terms of voltage recorded over time (Fig. 3), as well as polarization and Power Density (Fig 4a ad b) over 36 days of operation. The power generation capacity of the MFC in fed-batch mode was analyzed and was found to be capable of generating power from concentrated microbial community wastewater. After an acclimation period of 3–4 days, a steady increase in both voltage and current was observed. The microbes acting as biocatalysts are responsible for the rapid increase in voltage, which is probably caused by the presence of some bacterial redox chemicals that boost the activity of the microbes in the substrate. With the addition of a new substrate, the cell potential can be further restored once it drops (Kundu *et al.*, 2015)



**Figure 3:** Voltage generated over 36 days of operation

As shown in Figure 3, The maximum voltage was 178 mV from 1 - 14 days (measured against external resistance of 1000 Ω), a decline in the voltage was observed and a fresh medium was replaced and reading was recorded from the following days for a period of 12 days and has the highest voltage of 196 mV. Fresh substrate was also replaced after the second cycle and reading was recorded for another 10 days with a maximum voltage of 183 mV. This means that the currents decreased once a peak value was reached; however, greater sustained values were recovered with the addition of fresh substrate. These results showed that the microbes in the MFCs start to function effectively as soon as their nutrient source is replenished (Sevda *et al.*, 2013). The MFC has a feature that is very similar to that of secondary storage batteries in that it can be operated for at least 30 days under well-maintained conditions, minimizing the acclimation period and making the MFCs quickly chargeable (Chaudhuri *et al.* 2003).

The control MFC was also operated without the broth culture of cow urine, all operational parameters were the same with the experimental MFC, however, voltage reading was zero throughout the period of the experiment.



**Figure (4a and b) :** Polarization and and power density curves formed over the total days of operation

Figure (4a and b) show the polarization and power density curves obtained following the achievement of a stable voltage. The polarization curve was produced by varying the external resistance from 510 to 15000 Ω. The current measurement and power density at a given resistance were calculated and normalized by the anode surface area. The polarization curves indicated the features of the voltage drop. The sudden voltage drop that was seen at increased external resistance can be used to explain the energy loss for initiating the oxidation-reduction reaction, that is, for transporting electrons from the protein or enzyme of the cell to the anode surface (Logan 2008).

It was found that the voltage drop was greater at lower resistances than it was at higher resistances, which allowed electrons to easily pass across the circuit and gradually degrade the substrate (Liu *et al.*, 2005). It has been established that the development of biofilm and the oxidation of organic molecules raise the MFC's internal resistance, which affects the function of the cell as a whole (Bose *et al.*, 2018).

According to the polarization curve (Fig 4a and b), the greatest power density value was 18.26 mW/m<sup>2</sup>, which corresponds to current density of 97.11 mA/m<sup>2</sup>. The power output found in this work was significantly higher as compared to the power obtained by (Jadhav *et al.*, 2016)

using sludge and raw cow urine to generate electricity in MFC. This may be attributed to the different in the concentration of the inoculum. It was reported that higher organic load as it is in raw urine results in organic overloading which leads to system imbalance and decreased the anolyte pH thereby ceasing the microbial activities of electrochemically active bacteria. Sharma *et al.* (2010) reported that when the substrate concentration is high, more molecule of the substrate bind together to the active sites of enzyme carrying electrons which results in inhibitory effect for enzyme and thus, reduces the electron transfer activity toward the anode which influenced bacterial metabolic activity and in turn affected the electron and proton generation process. The power generated in MFC is still low and has limits the practical applications of MFC.

The main practical challenges and limitations associated with bioelectricity generation using MFCs are; low power output, current instability, high internal resistance, expensive materials, nature of inoculum type, variation in the concentration of the substrate, regular cleaning and maintenance of MFC container. Also, operational conditions such as pH and temperature affects the microbial activities in the MFC especially at temperature below 20°C (Shantaram *et al.*, 2005).

**Table 1; Microscopic and Biochemical Characteristics of the Anodic Bacteria**

Isolates Id	Microscopic Characteristics	Biochemical Characteristics								Suspected Organisms
		Oxidase	Indole	Urease	Catalase	Mortality	VP	MR	CU	
Plate 1	Gram positive Rod shape	-	-	-	+	+	+	-	+	<i>Bacillus spp</i>
Plate 2	Gram positive Rod shape	-	-	-	+	+	+	-	+	<i>Bacillus spp</i>
Plate 3	Gram negative Rod shape	-	-	+	+	-	+	-	+	<i>Klebsiella spp</i>
Plate 4	Gram negative Rod shape	-	-	+	+	-	+	-	+	<i>Klebsiella spp</i>
Plate 5	No growth									

**Key;** VP = Voges proskauer test, MR = Methyl Red, CU = Citrate utilization, - = negative and + = positive.

As shown in Table 1, The anodic bacterial isolates were identified by microscopic and biochemical characteristics. The microscopic and biochemical characteristics for plates 1 and 2 showed that the bacteria were *Bacillus*, gram positive, rod shape and motile. Catalase, motility, voges proskauer, and citrate utilization test were found to be positive, and negative for indole, urease, oxidase and methyl red test. Plates 3 and 4 were identified as gram negative, Rod shape, catalase, citrate, VP and urease positive and negative for indole, motility, methyl red and oxidase test which revealed the typical characteristics of *Klebsiella* spp. Plate 5 shows no growth on cetrimide Agar plate. The biofilms formation of these organisms has been reported in previous literature and are found to exhibit electrogenic properties, which are responsible for the direct electron transfer from the cell to electrode during electricity generation (Islam *et al.*, 2020)

## CONCLUSION

This study focused on using MFCs to generate electricity from sewage wastewater using cow urine as inoculum. The bioelectricity experiment was run under facultative anaerobic environment in a fed batch mode for 36 days with the used of two chamber MFC. The study found that

the maximum voltage of 196 mV, current density of 97.11 mA/m<sup>2</sup>, and power density of 18.26 mW/m<sup>2</sup> were achieved. The study also assessed parameters such as pH, Temperature, Chemical oxygen demand (COD), and biochemical oxygen demand (BOD) and Total suspended solid (TSS) to optimize bioelectricity generation. The results of this study can contribute to improve understanding and optimizing electricity generation in microbial fuel cells. Although the identification of the anodic biofilms showed the presence of *Bacillus* spp and *klebsiella* spp based on their morphological and biochemical characterization, future research will focus on the molecular profiling of the electrogenic bacteria present in the anodic biofilm.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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