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Performance Evaluation of Different Electrode Materials and Substrate Modifications on Bioelectricity Generation from Bacteria Fuel Cells

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Abstract

The global energy crisis is caused by high energy demand and insufficient resources. Non-renewable energy sources are diminishing, while renewable energy sources are underutilized. An urgent search for alternative energy generation routes is necessary. A microbial fuel cell is a process that makes use of microorganisms like bacteria or fungi as biocatalysts that oxidize waste organic matter to release electrons which in turn are used to produce electricity. An MFC reactor is made of a cathode, an anode, and a substrate onto which microorganisms are fed so that electrons are released for bioelectricity generation .A two-chamber cathode was fabricated in this study. The chamber has a total volume of 120ml and a working volume of 100ml. The chamber was used to investigate the influence of substrate enrichment and type of electrode on electricity production by some selected bacteria (*Pseudomonas Tawanensis* (PT), *Myroides Odoratimimus* (MO), *Sphingobacterium Mizutaii* (SM). The substrate used is locust beans wastewater. The substrate was enriched with either sucrose or acetate. The electrodes include copper, aluminum, aluminum-zinc alloy, soft zinc, and zinc. To determine the most suitable enrichment sources (sucrose and acetate) a mixed culture of the three bacteria was inoculated in the substrate (locust bean wastewater) with a standard graphite electrode. Cellulose acetate was used as the membrane for the chamber in place of the cation exchange membrane. The setup was operated for 20 days. The effect of substrate enrichment and electrode use on bioelectricity and stability was later analyzed. The results from the mixed culture showed that the substrate enriched with sucrose generated a higher voltage ($2.15x10^{-3}$ mA) when compared with an acetate-enriched substrate (this generated a voltage of $1.62x10^{-3}$ mA) with graphite as the electrode. Following this result, we selected sucrose as the enrichment source in the remaining experiment. Each bacterium used in this study generated electricity in the chamber containing sucrose-enriched substrate with each of the electrodes used. This implies that all the adopted electrodes are sufficient site for the formation of biofilm through which bioelectricity can be generated. However, the highest voltage (1.72mA) was recorded in the chamber containing *Pseudomonas taiwanensis* with zinc as the electrode in the chamber. We noted that in all the bacteria used in this study, bioeletricity generation was more stable and consistent with copper as the electrode of choice.

Keywords: Bioelectricity, Cellulose acetate, Electrode Microbial fuel cell; Salt Bridge;

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Introduction

The rapid depletion of fossil fuels and their environmental impacts necessitate a shift toward sustainable energy sources. Microbial fuel cells (MFCs) offer a promising alternative by harnessing microorganisms to convert organic waste into electricity. In an MFC, microorganisms act as biocatalysts, oxidizing organic matter and releasing electrons, which generate electricity as they transfer from anode to cathode (Li et al., 2018; Thulasinathan et al., 2022). This process not only produces electricity but also treats wastewater, making MFCs environmentally beneficial and space-efficient.

The performance of MFCs depends on various operational parameters, including design type (Du et al., 2007), mediator type (Ichihashi, 2010), separator Ali et al., 2021).), electrode materials (Merina, 2015), and substrate type (Pant et al., 2009). For instance, materials like carbon felt and graphite brushes have been tested to improve electrode efficiency (Min et al., 2005). Similarly, the choice of ion exchange membrane, a critical component for ion transport, significantly affects MFC efficiency and power output (Rozendal et al., 2006; Asensio et al., 2018). Substrates, which serve as the fuel for microbial activity, influence bacterial growth and the overall energy yield (Wu et al., 2014; Liu et al., 2009). Acetate, known for its high Coulombic efficiency, is commonly used due to its stability and high power output (Pant, 2010).

However, high costs and limited availability of some MFC components present challenges, particularly in developing regions. In this study, locally sourced alternatives was explored to enhance MFC performance and affordability (Ullah et al., 2019) Specifically, substrates used was locust bean wastewater which is a food processing byproduct known for its abundance and potential as a nutrient source for bacteria hoping to simultaneously reduce waste and generate electricity. Additionally, a cellulose acetate (photographic film) as an affordable membrane substitute (Palanisamy et al., 2023and introduced a novel bacterial strain to test its bioelectricity generation capabilities. Through these modifications, locally sourced components like locust bean wastewater as substrate and cellulose acetate membrane was evaluated, alongside a new bacterial strain for their effect on MFC performance, cost-efficiency, and environmental impact.

The findings of this study aim to demonstrate the feasibility of using cost-effective, locally available materials in MFCs, potentially making bioelectricity generation more accessible and sustainable.

Materials and Methods

Substrate Collection

A sterile ten (10)-liter container was used for the collection of the substrate sample (locust bean wastewater LBWW) from the residential area at Idigba, Ogbomoso (80 08′, 40 15′E) in, Oyo State. Nigeria.

Inoculum Preparation

In this study, three species of bacteria isolated from cowshed wastewater were utilized. The bacteria were isolated and identified in a prior study conducted by one of the authors (Oluyide et al., 2020). The identification and characterization of the bacteria were achieved through molecular identification utilizing the amplification of 16S rRNA. The identified bacteria include Pseudomonas taiwanensis (PT), Myroides odoratimimus (MO), and Sphingobacterium mizutaii (SM).

Microbial fuel cell (MFC) setup and operation

The two-chambered MFC was designed in triplicate, for both experimental set-up and control (Figure 1). The anode and cathode chamber of the MFC reactor made of clear polyacrylic jar waste were used in this study. Each MFC chamber has a total volume of 120 ml and a working volume of 100 ml. The Anode chamber was filled with 100ml of sterilized locust beans wastewater as substrate and a loopful of Inoculum while 100ml of 0.1 M Potassium permanganate was charged into the cathode chamber. All the components of MFC were connected via cellulose acetate membrane internally, and externally with wires from the electrode to the multimeter. A leakage test was carried out to check if the parts were properly coupled for the possibility of leakage and eventual loss of electrolytic fluids, which can affect the successful operation. The MFC reactor setups were sterilized with 70% alcohol and dried under UV light for 20 minutes (Kassongo and Togo, 2010) kept in static conditions, and voltage generation was recorded five (5) times daily for twenty (20) days.

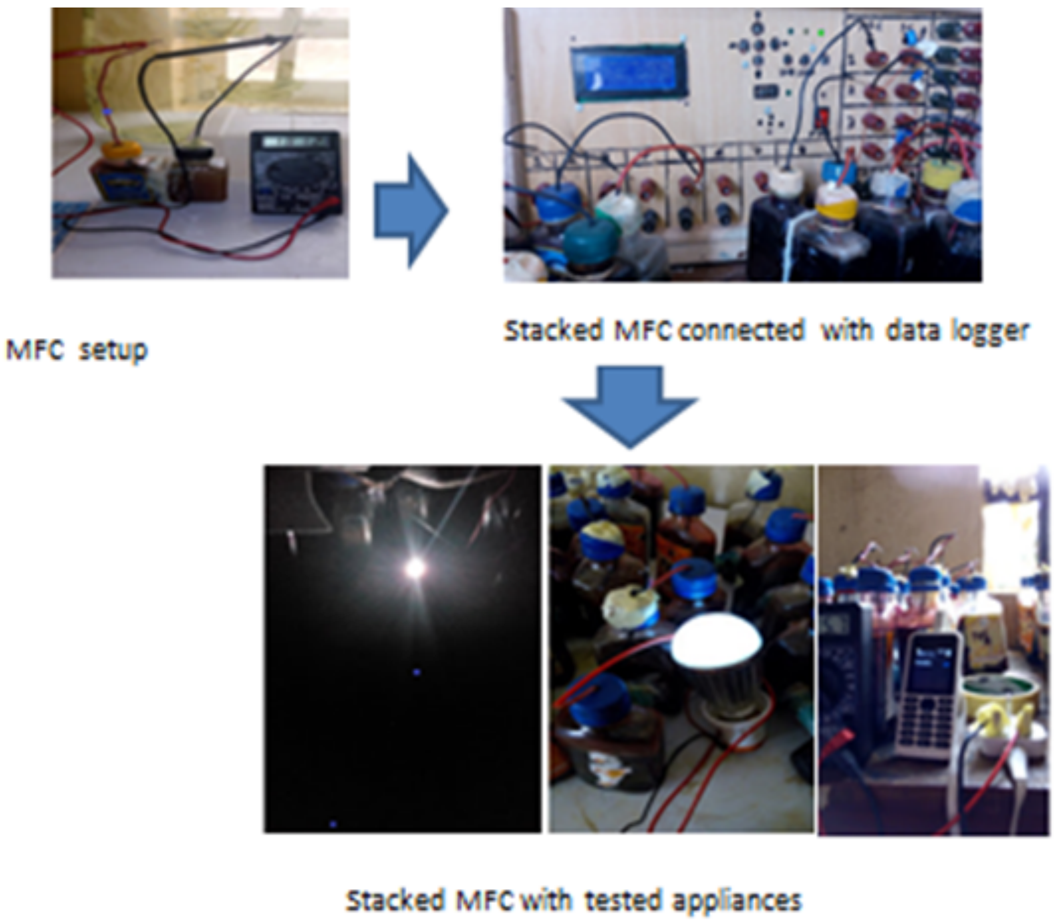

Fig. 1: Scheme of the experimental setup

Electrode Preparation

Electrodes were prepared before usage in the MFC chambers. Five different electrodes were tested in different MFC chambers to determine their influence on bioelectricity generation by the activities of the bacteria. All the bacteria were individually tested with each electrode used in the assay. These electrodes include copper (C), aluminum (Al), Alloy (A), Soft Zinc (SZ), and Zinc (Z), each having a total surface area (S.A) (s.a) of 10.44m^2. The ends of each cathode and anode were fixed with the wired containers containing anolyte and catholyte. Inorganic contaminants and metals were removed and neutralized, respectively. Ethanol (100%) was used to soak the electrodes for 30min. followed by washing in 1 M Hydrochloric acid and 1 M Sodium hydroxide, for 1 hour each and stored inside distilled water until they are used (Ghanapriya et al., 2012).

Modified anodic medium for bioelectricity generation

Locust beans wastewater was used as a substrate for the MFC chamber and was enriched with acetate (CH_3COOH) and sucrose ($C_{12}H_{22}O_{11}$) as carbon sources. Acetate and sucrose were used separately in the chamber to determine the most suitable carbon source substrate enricher for the MFC chamber. The concentration of acetate medium as described by Zhang et al., 2017 and sucrose as described by Behera and Ghangrekar, 2011 were used for enrichment. The anodic medium was modified by adjusting the pH to neutral (7.0) with the addition of casein as an aerobic inhibitor. The MFC setup was operated using both pure and mixed cultures as biocatalysts individually. The biocatalysts were used in separate chambers at a time with each carbon source (sucrose or acetate) in an individual chamber, the control, however, does not contain any carbon source. At this time cation exchange membrane (CEM) was used as the proton exchange membrane. However, to evaluate the influence of the electrodes in optimum conditions, sucrose was used as the carbon with cellulose acetate film membrane (CAFM) as the proton exchange membrane for the pure culture biocatalysts (*Pseudomonas taiwanensis* (PT), *Myroides odoratimimus* (MO), and *Sphingobacterium mizutaii* (SM)). The electrodes used are Copper, Aluminum, Alloy, Soft Zinc, and Zinc. Each has a total surface area(S.A) of 10.44m^2.

A total volume of 120 ml and a working volume of 100 ml of both electrolytes (locust bean wastewater and potassium permanganate) were charged into the double chamber. The anode chamber of two different MFCs was filled with 100ml of sterilized locust beans wastewater medium with acetate and sucrose as substrate enrichers in each chamber respectively. The substrate was then inoculated with 0.1ml of each of the 24hr old cultures of the three bacteria to form a mix-culture in the chamber. A 100ml of 1 Molar Potassium permanganate was charged into the cathode chamber of each MFC chamber. The external resistance connecting the anode with the cathode was 1 kΩ for each of the MFC. The voltage (V) across the external resistance (R) for each of the MFC was recorded five (5) times daily using Multimeter for twenty (20) days. Current (I) was calculated as $I = V/R$. The observed values were used to evaluate and compare the performance of the two carbon sources (substrate enrichers) used in the chambers. A similar procedure was carried out with the pure culture using sucrose as the carbon source enricher and CAFM as the membrane separator as earlier stated.

Influence of electrode surface area and anode cavity volume on the performance of microbial fuel cell using mixed culture

To evaluate the influence of electrode surface area and anode cavity on the performance of the MFC with a mixed culture biocatalyst, we set up four chambers of MFC in triplicate. The anode and the cathode of each chamber were separated by a cellulose acetate membrane. The working volume of each chamber is equal to 80 ml, 60 ml, 40 ml, and 20 ml respectively. The copper electrode was used in all the MFC chambers and the surface area of each chamber at the end of the setup is 10.34 m^2, 7.83 m^2, 5.22 m^2, and 2.61 m^2 respectively. An external resistance of 1k Ω was connected to the wires on the MFCs chambers. The electrodes were sterilized, attached to the wire, and inserted into the chamber for the attachment of microorganisms. Voltage measurement was monitored using a locally made Agilex machine for 40 days.

Assessment of the efficiency of cellulose acetate film membrane compared with cation exchange membrane

SEM (scanning electron microscopy) and EDS/EDX (Energy Dispersed X-ray Spectroscopy) analysis were carried out on the membrane to compare the efficiency of the cellulose acetate film membrane and the cation exchange membrane used in this study.

Result and Discussion

Bacteria isolates used for the study

The bacteria isolates employed for this study were identified as *Sphingobacterium mizutaii* strain AV5 KX436993.1, *Pseudomonas taiwanensis* strain AV2 with accession number KX436991.1 Myroides *odoratimimus strain* AV1 with accession number KX436990.1.

Modified substrate for bioelectricity generation

The performance evaluation of the two different carbon sources (acetate and sucrose) used for the enrichment of the substrates in the MFC chambers using different electrodes showed that the substrate enricher induced the generation of bioelectricity from the 1st day of the MFC chamber setup till the day the 20th day of the experiment. However, the intensity of the bioelectricity generated by the two MFC chambers differs significantly from each other. The MFC chamber enriched with sucrose generated the highest amount of electricity from the second day of the experiment, reaching a maximum voltage of $2.15x10^{-3}$ mA, but this later dropped gradually, and they maintain a steady state current until the last day of the experiment (Figure 2). The MFC chamber enriched with acetate generated a maximal current of 1.62 x 10^{-3-} mA on the first day but the current generated was not stable as it continued to drop gradually till the last day of the experiment. These observations imply that sucrose is a more efficient substrate enricher than acetate. This observation corroborates the findings of Zafar et al, (2018) who reported a better performance for an MFC chamber enriched with sucrose. Zhang et al., 2011 have also demonstrated that the substrate used in enriching the MFC cell has an important role in the amount of electricity that can be generated by the MFC. In their study, they reported that the variation in the amount or quantity of electricity generated could be associated with bacterial acclimatization (Zhang et al., 2011).

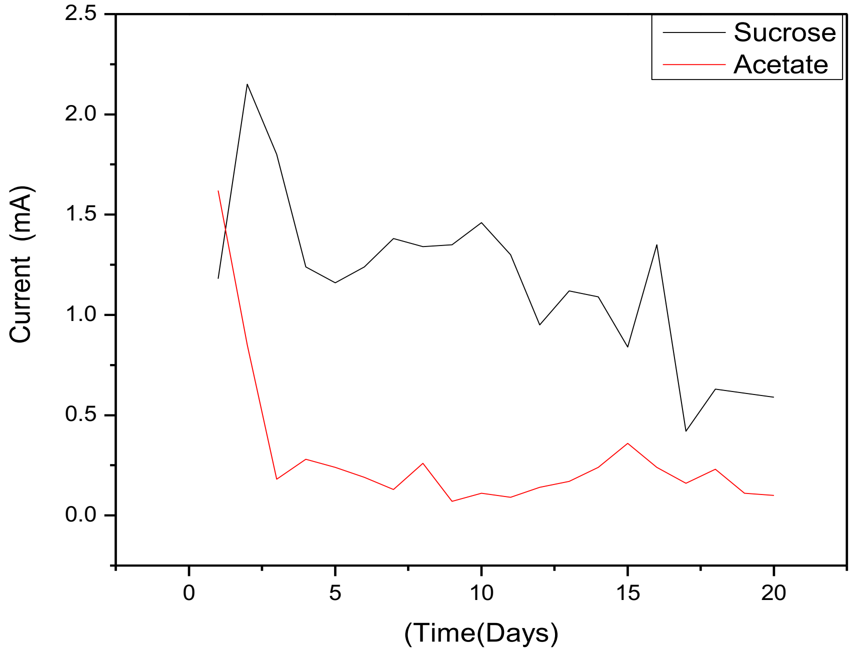

Fig. 2: Performance of bioelectricity generation using mixed culture in both sucrose and acetate carbon source enriched locust beans wastewater as substrate

Performance of different electrode materials in microbial fuel cell

We tested five different electrodes with individual bacterial cultures as biocatalysts to determine the influence of electrodes on the generation of electricity. The cellulose acetate membrane was used as a membrane separator in each MFC chamber where the electrodes were tested. The working potential of these electrodes was checked and compared in our study. The working potential was determined by the amount of electricity generated by each chamber with distinct electrodes. The current generated in the MFC reactor inoculated with *Pseudomonas taiwanensis* using sucrose as carbon source, with Aluminum (IA) as electrode had the highest current generation of 0.98mA on day 1, followed by Zinc electrode (IZ) which achieved its optimal electricity generation (1.72mA) on day 3. With copper as the electrode (IC) an average current of 1.27mA was obtained on day 1, Alloy electrode (IAL) yielded 0.51mA on day 5, while soft zinc (ISZ) yielded a current of 1.31mA on day 1, (Figure 3). For the chamber inoculated with *Sphingobacterium mizutai,* the current generation is as follows, Aluminum (IA) yielded 1.18mA on day 11, followed by Zinc electrode (IZ) 1.24mA on day 6, copper electrode (IC) with an average current of 1.19mA on day 10, Alloy electrode (IAL) with 1.22mA on day 9, Soft zinc (ISZ) with 1.22mA on day 8. (Figure 4). While *Myroides odoratimimu*s with Aluminum electrode yielded (IA) 1.05mA on day 3, Myroides in zinc electrode (IZ) MFC generated 1.64mA on day 4, with the copper electrode (IC), *M. odoratimimus* generated an average current of 1.26mA on day 1 while using Alloy electrode (IAL) an electricity value of 0.72mA was generated by *M. odoratimimus* on day 16, For soft zinc (ISZ) electrode used in MFC chamber on which *M. odoratimimus* was cultured resulted in an electric current with a value of 1.27mA on day 1(Figure 5).

The maximum voltages reached by all the electrode materials were not sustained, however, after the first drop in current, copper electrode plates maintained a steady current for some days during the study with all biocatalysts used. This implies that copper is the best electrode among those tested for the generation of electricity in the MFC chambers. This observation is like the report of Jeetendra and Ramesh (2017), who compared the effect of zinc and copper as electrodes on the generation of electricity by the activities of a biocatalyst in an MFC chamber. They reported a steadier current and better stability for copper electrodes compared to the zinc electrode used in their study (Jeetendra and Ramesh, 2017). In a related study, the effect of electrode metal on power generation was evaluated by replacing copper with a carbon electrode, this resulted in the generation of a higher voltage with decreased resistance in contrast to the copper electrode (Manohar et al., 2017).

In summary, the best bioelectricity output in this study was achieved with *Pseudomonas taiwanensis* as a biocatalyst as the electrode material (Figure 3). Aluzinc electrode with *Pseudomonas taiwanensis* yielded the next level of electricity after the copper electrode in this study (Figure 5). Also, the performance of the other electrodes is not consistent and stable when compared with the copper electrode. They, however, generated a substantial amount of electricity (Figure 3,4,5). The material used in electrode preparation always has a remarkable effect on MFCs efficiency. Better performing electrode materials usage will always improve the performance of MFC because different anode materials result in different activation polarization losses (Zhao et al., 2016)Experimental data obtained from this study showed that different electrodes could be used in bioelectricity as a site for biofilm formation, but copper was more stable and consistent in the current generation.

The optimal generation of electricity by the microbial fuel cell has been linked with the type of electrode used in the MFC chamber. Previous studies have reported an increase in power generation when one electrode type is substituted for another in an MFC chamber (Nandy et al., 2015). Sangeetha et al., 2012 reported an increase in power generation from graphite electrode-MFC when compared with stainless aluminum electrodes (Sangeetha and Muthukumar, 2013). As observed in this study electrode type significantly influence the amount and stability of the electricity generated with copper being the most stable electrode for electricity production. This observation about copper is in congruency with previous studies where copper has either been used as an electrode or as an electrode coating to enhance an optimal generation of electricity in an MFC cell (Li and Zhou, 2019). Although our observation contrast with that of Nandy et al., (2015) electricity generation using copper electrode will be sustainable. Another benefit of all the electrodes tested in this study is that they are cheap and accessible.

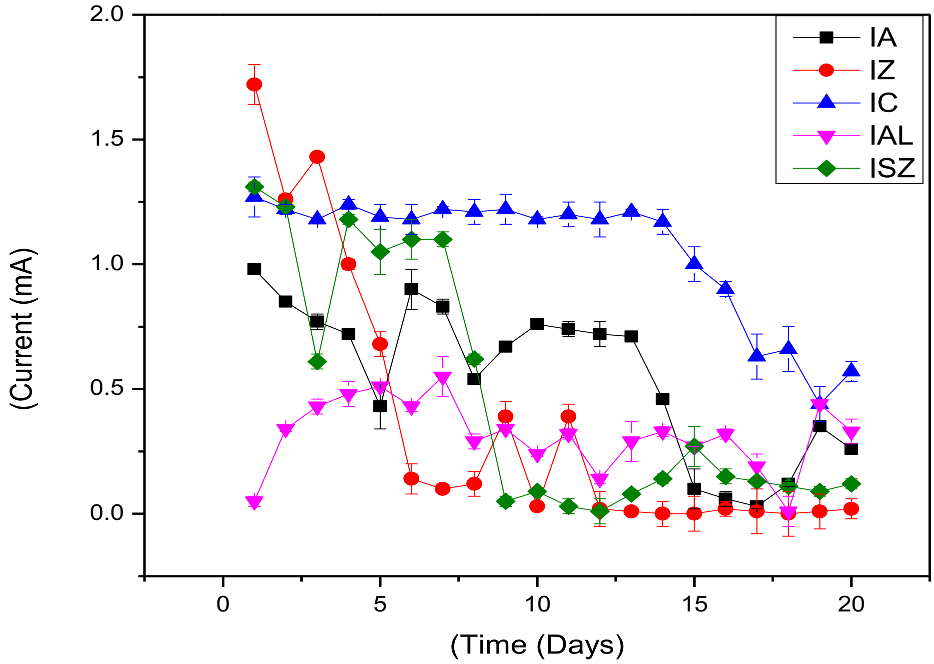

Figure 3: Graph showing the performance trend of different electrode material with *Pseudomonas taiwanensis* (PT)

KEY

IA- Aluminum electrode

IZ- Zinc electrode

IC- Copper electrode

IAL- Alloy electrode

ISZ-Soft zinc electrode

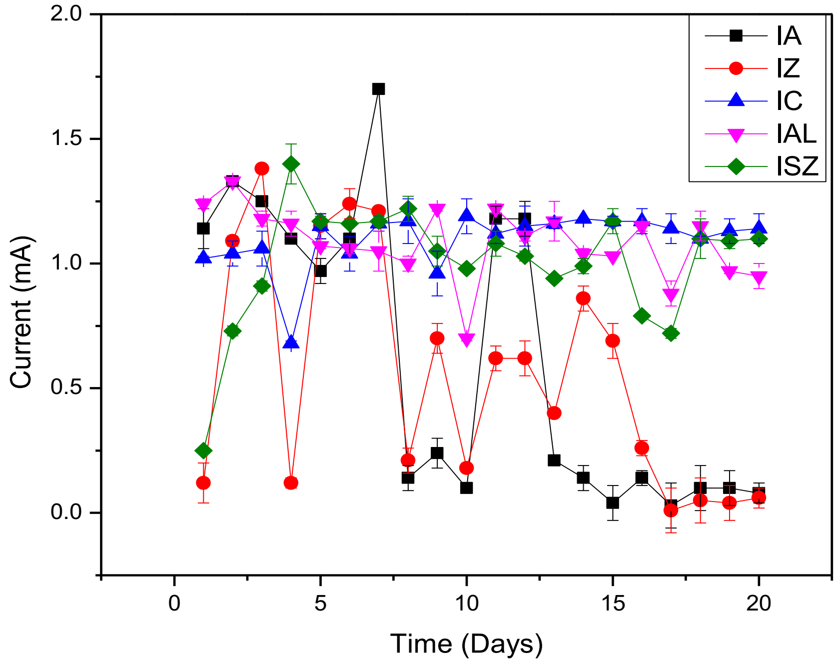

Fig. 4: Graph showing the performance trend of different electrode material with Sphingobacterium mizutaii (SM)

KEY

IA- Aluminum electrode

IZ- Zinc electrode

IC- Copper electrode

IAZ- Alloy electrode

ISZ-Soft zinc electrode

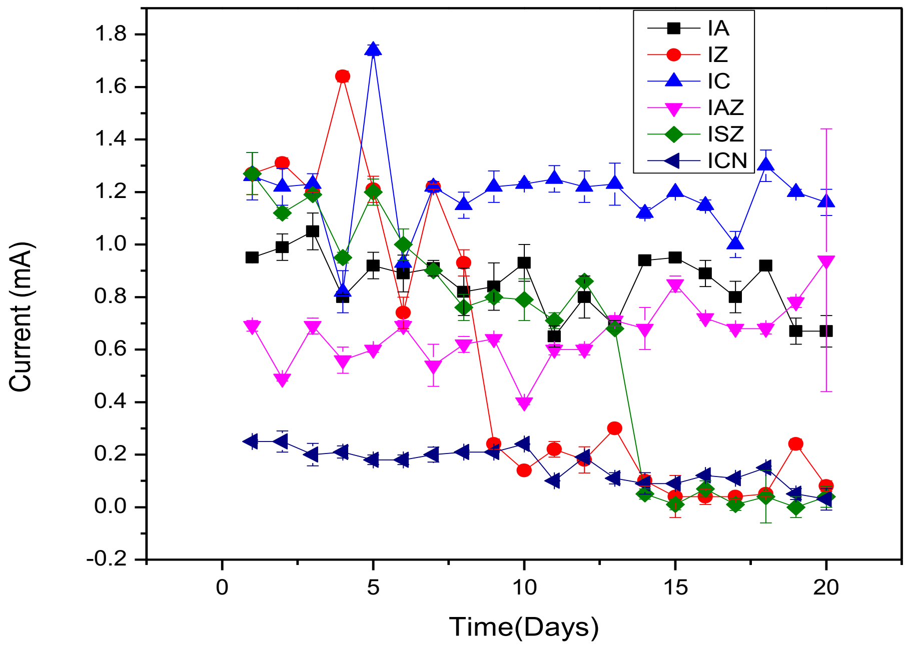

Figure 5: Graph showing the performance trend of different electrode material with *Myroides odoratimimus* (MO)

KEY

IA- Aluminum electrode

IZ- Zinc electrode

IC- Copper electrode

IAZ- Alloy electrode

ISZ-Soft zinc electrode

Influence of electrode surface area against different anode cavity volumes on the performance of microbial fuel cell performance.

The highest current and power density 9.38mA/m^2 and 9.1038mW/m^2 were obtained when 80ml was used. However, the 60ml, 40ml, and 20ml anode cavities had current density of 7.28 mA/m^2, 5.36 mA/m^2, 4.21 mA/m^2, and power density of 4.15 mW/m^2,1.5mW/m^2,4.64 mW/m^2 respectively (Table 1). This observation is congruent with the findings of Eduardo et al., 2018 that reported that electrode surface area/anode volume ratio has a significant influence on the performance of a microbial fuel cell. They also reported that it also influenced the COD consumption rate. They noted an increase in the current intensities from 583 to 2416 mA m-2 when the ESAVR (electrode surface area volume- ratio) increased from 0.15 to 0.75 cm^2 /cm^3. Similarly, Ghangrekar and Shinde, 2006 showed that anode surface area can influence the amount of electricity generated in a MFC chamber thus corroborating our observation that anodic surface area can influence the amount of electricity generated by MFC.

Table 1: Current density, power, and power density of different electrode surface areas against anode cavity volume on the performance of microbial fuel cell using mixed culture.

Anode cavity volume	**Electrode surface area (m^2)**	**Current (mA)**	**Current density (mA/m^3)**	**Power (W)**	**Power Density(mW/m^3)**
20ml	2.61	0.11	4.21	1.21	4.64
40ml	5.22	0.28	5.36	7.84	1.5
60ml	7.83	0.57	7.28	3.25	4.15
80ml	10.34	0.97	9.38	9.41	9.10

Assessment of the effectiveness of cellulose acetate as a membrane separator based on the morphology of the membranes

The SEM images of both membranes (Cation exchange membrane and Cellulose acetate film) surfaces at a magnification of 9000x, 10000x, and 11000x respectively showed differences in their surface structural bodies (Figures 6 and 7). The surface of the Cation exchange membrane (CEM) is more homogeneous than the Cellulose acetate membrane. The diameters of a single sphere less than 0.2 µm are present on the CEM-type membrane surface and similar microstructures cover the entire surface of the Cellulose acetate type membrane. Differences in the structure of the body layer of the membranes suggested that their manufacturing technologies vary widely. The cellulose acetate membrane support layer is porous while the CEM membrane has a cellular structure. As a result, the CEM membrane is a thinner "body" layer than the cellulose acetate membrane indicating a higher coefficient of the filtration of the CEM membrane than does the cellulose acetate membrane. After the experiment investigation showed that the support layers of both membranes have a lesser thickness, but a similar structure with fewer porosity sizes.

The Energy Dispersed Spectroscopy (EDS) revealed four main elements: carbon, oxygen, nitrogen, and sulfur are present in both membranes. There were also small amounts detected of sodium, chlorine, and calcium. Quantitative analysis shows that the mass and atomic shares of these elements are less than 1%. The presence of chlorine may be interpreted as the result of incomplete condensation of the monomers forming the body of the membrane or incomplete hydrolysis of its free chlorocarboxylic groups. The presence of sodium may be the result of the specific nature of the manufacturing process of the membranes (Figures 8 and 9)

The effectiveness of cellulose acetate as a separator was tested for the movement of ions in and out of the cell. Most of the MFC designs require a separator in between the anode, and the cathode compartments, and cellulose acetate membrane separator showed more or less equal chemical energy conversion when compared to other membranes used (Min et al., 2005). Naturally separated systems such as sediment MFCs or specially designed single-compartment MFCs are exempted. When a membrane is used in an MFC, it is very important to note that it may be permeable to chemicals such as oxygen, ferricyanide, other ions, or organic matter used as the substrate. The membrane used in this work allowed the movement of ions, thereby producing current.

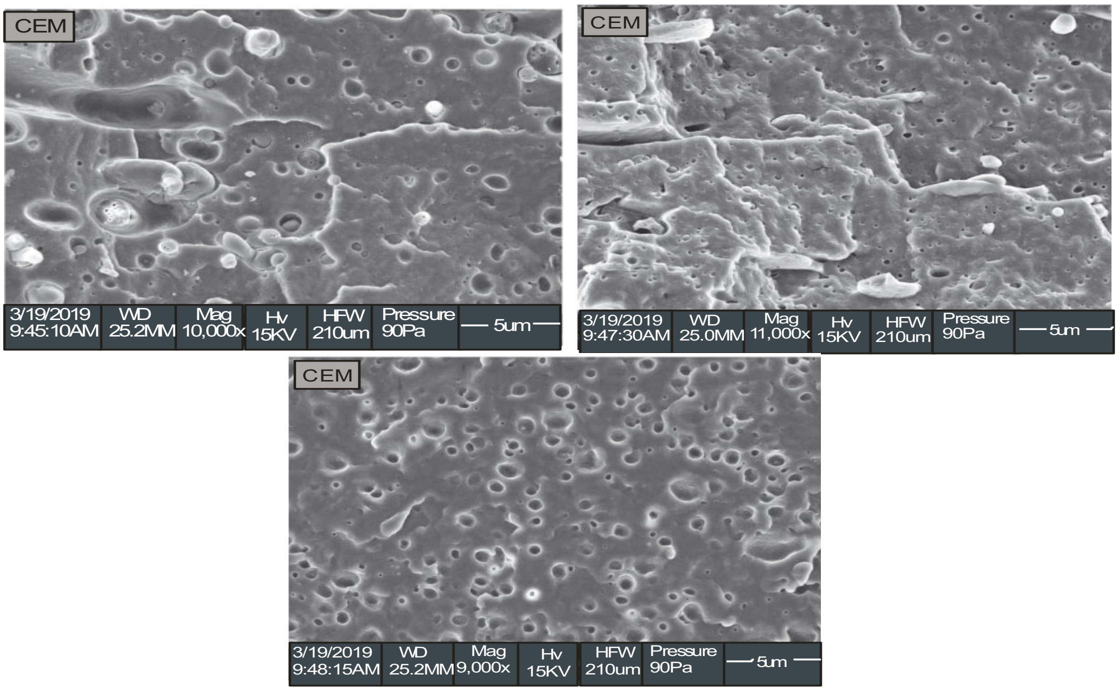

Fig. 6: Scanning electron microscope (SEM) image of cation exchange membrane at 9000x, 10000x and 11000x magnification

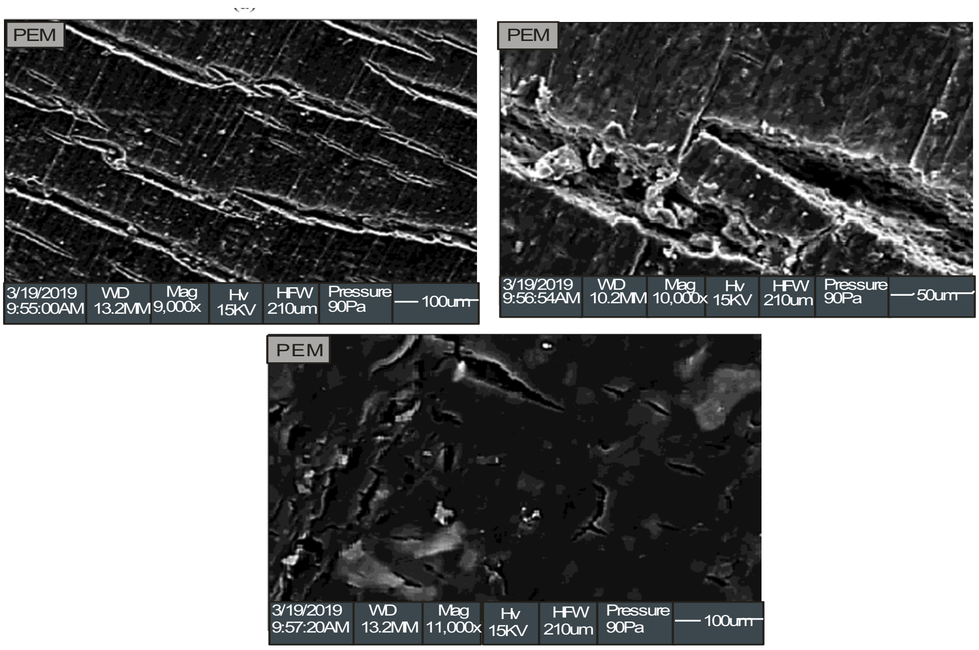

Figure 7 - Scanning electron microscope (SEM) image of cellulose acetate film membrane at 9000x, 10000x, and 11000x magnification

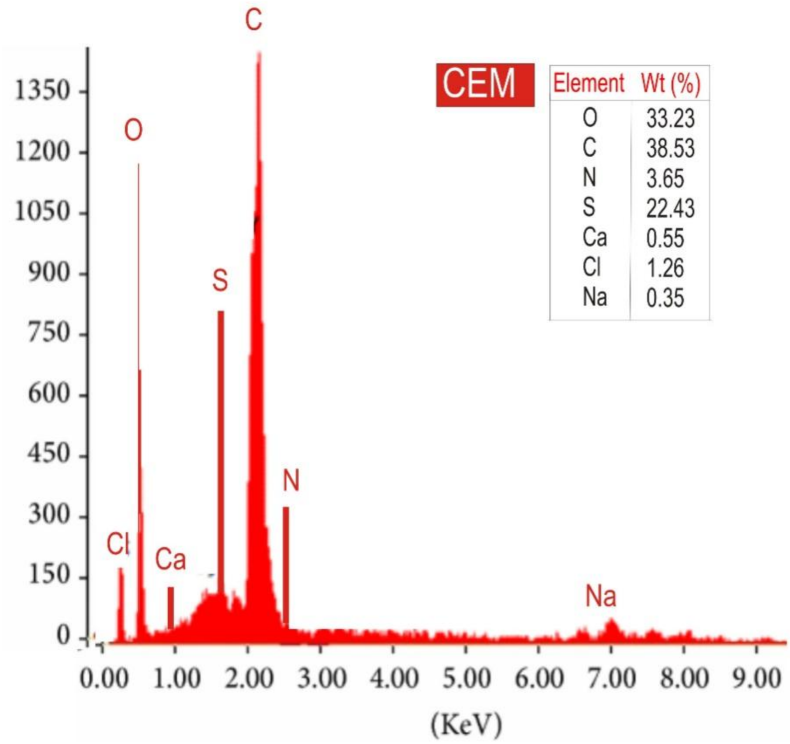

Fig. 8: The Energy Dispersed Spectroscopy (EDS) of Cation exchanged membrane

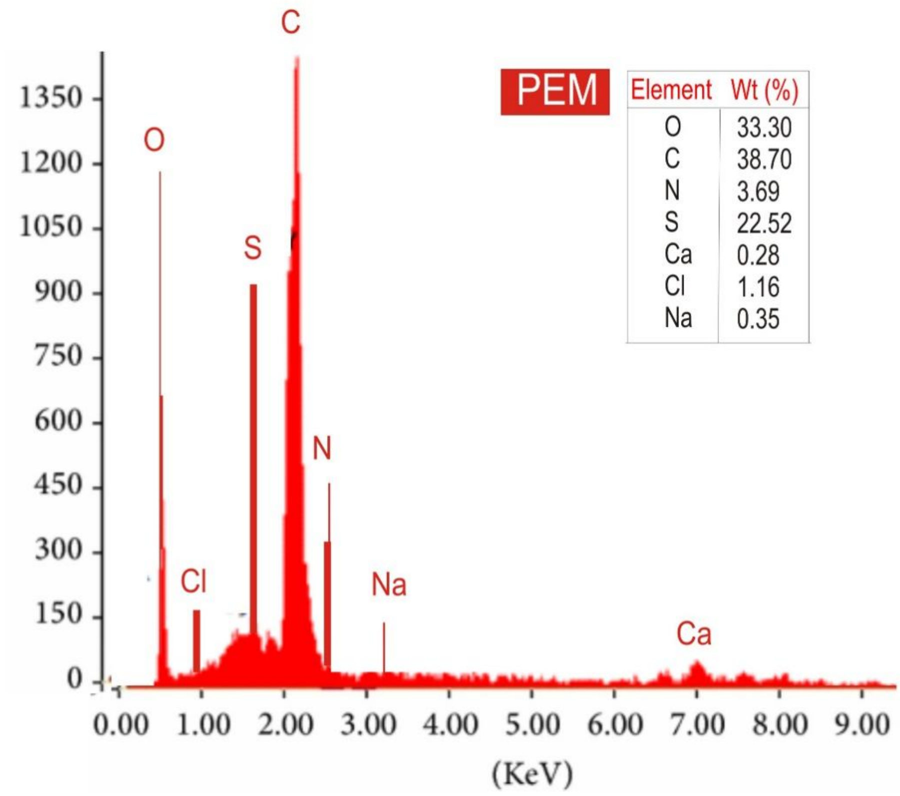

Fig. 9: The Energy Dispersed Spectroscopy (EDS) of Cation exchanged membrane

Conclusion

Locust beans wastewater with a sucrose carbon source was suitable in the bacterial-catalyzed fuel cell with copper electrode plate and cellulose acetate employed to achieve maximum bioelectricity generated. The effect of substrate modification investigated in a double-chamber microbial fuel cell revealed that bioelectricity production by MFC can be enhanced by using different electrodes. However, the copper electrode was more stable and consistent to produce bioelectricity in this study. Thus, the copper electrode could be regarded as the best electrode for the bacteria used in this study. Similarly, cellulose acetate can serve as an alternative to the cation exchange membrane in the design of MFC. A higher energy output was generated at optimum OD of 0.6 with a peak current generation of 2.15 mA when MFC was fuelled with sucrose-enriched locust bean wastewater. Copper plate was used as electrode material at both the anode and cathode and cellulose acetate material as a separator, which has been rarely reported to be used singly in place of another separator in a microbial fuel cell. Electrode surface area exhibited a high influence on the performance of microbial fuel cells. it was found that current densities increased from 4.21 to 9.38 mA m^{-2} when the electrode surface area increased from 2.61 to 10.34 cm2 cm^{-3}.

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