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Poultry Residual Biomass as Substrate to Generate Bioelectricity using a dual Chamber Microbial Fuel Cell "MFC" with Graphite and Copper Electrodes

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Microbial fuel cells (MFC) are electrochemical systems through which sustainable energy can be produced due to the degradation of organic matter using substrates with a varied chemical composition. The bioprocess that takes place inside the MFC takes advantage of the oxidation of organic matter. This process releases protons and electrons extracellularly, and the latter are transferred from the anode to the cathode generating bioelectricity. The MFC operating system produces energy due to the bacterial metabolism, through an electron transfer phenomenon that reflects into a bio energy conversion with minimal impacts on the environment. With the MFC system, it is possible to investigate the use of new residual substrates for energy production, the types of native microbial communities that develop during the degradation of specific compounds and the design of more efficient cells. In this research, copper and graphite were evaluated as low-cost electrodes using batch microbial fuel cells for 208 hours of operation, a data logger was used, and physicochemical parameters were taken during this period. The maximum power density presented was 14 mW/m^2 with the graphite electrode and 6.7 mW/m^2 with the copper electrode. Electrogenic bacteria were identified through biochemical and molecular tests such as bacterial culture, strain purification, DNA extraction and sequencing of microorganisms. The bacteria were uploaded to the NCBI gene data bank and the identity of these strains was identified: avian graphite 1 "Av_A1" (*Pseudomonas aureginosa*), avian graphite 2 "Av_A2" (*Bacillus cereus*) and avian copper 1 "AV_C1" (*Bacillus tropicus*). A dual chamber MFC was assembled, so each microbial cell can contain the residual substrate and the corresponding electron acceptor, both for the anodic and cathodic cell. These cells were separated by a Nafion® or Ultrex® membrane cation exchange membrane. The results showed us that optimal conditions for the generation of bioelectricity can be established in MFC cells, adding information to the literature on the behavior of bacteria that thrive in stressful environments such as copper and simple materials such as the graphite.

Keywords: electrogenic, electrochemistry, MFC, poultry biomass, electrode.

Introduction

Currently there is a worldwide need for renewable energy. This need is due to the excessive use of fossil fuels and the decrease in energy production. This need is generating production trends of sustainable ecological technologies (International Energy Agency, 2022; Du et al., 2007). This need has produced research on energy generation. In this context, microbial fuel cells (MFC) have been developed as an alternative to traditional energy production technologies, which aims to apply microorganisms as bio catalyzers using organic complexes (Ruscalleda Beylier et al., 2019).

Microbial fuel cells are an emerging technology, which can help achieve one of the objectives faced by industrial activities with their wastewater. The goal is "contamination free" in the treatment of wastewater that includes textiles, oils, heavy metals and biodegradable organic matter (Kaur et al., 2017; El-Nagar et al., 2017; Pacheco et al., 2022; Pacheco, Elguera, Ancco, et al., 2023; Pacheco, Elguera, Mamani, et al., 2023).

The biomass is used as a primary energy source and is metabolized by redox reactions to breakdown organic complexes to interchange protons and release electrons completing a circuit to produce bioelectricity (Harnisch, 2011; Ho et al., 2011; Li et al., 2010). There are different kinds of biomass that could be used as a substrate such as grass, tree leaves, rice bran and even animal manure; organic sources as bamboo and food waste in general are also used as a substrate, demonstrating that MFCs are an efficient solution to provide green electricity (Moqsud et al., 2014; Moqsud et al., 2013). For this reason, poultry residual biomass represents an organic source rich in carbon, nitrogen, and phosphorous complexes, useful for bacterial activity; however, this organic compound is a high-risk pollutant for environment and human health due to the increasing activity on poultry farming (Li et al., 2011; Ramasamy et al., 2008; Gržinić et al., 2023).

Besides substrates management that focuses on the search of new sources of renewable energy (Du et al., 2007), microorganisms are decisive in MFC performance, due to their main function as biocatalizers and the community network that makes possible bioelectricity production (Jaeel and Salah Lafta Farhan, 2015). Inoculum can be developed as indigenous bacteria or a transferred one from a different substrate (Tatar, 2018), to produce bioelectricity and to determine the electroactive microorganisms (Patel et al., 2021). Due to this fact, exoelectrogenic bacteria must be isolated and identified with the purpose to screen bacterial strains prepared to work as electroactives capable to generate biofilms or mediators to adapt its metabolism and produce electricity (Greenman et al., 2021). Some bacteria like *Geobacter sulfurreducens* or *Shewanella onoidensis* have been proved as perfect candidates for bioelectricity production; furthermore, the electron transfer process and respiration into the anode electrode have been extensively studied on these microorganisms (Choudhury et al., 2017).

One of the very useful tools to broaden the understanding of communities developed in MFCs is DNA sequencing, which provides us molecular information of exoelectrogenic microorganisms (Vandecandelaere et al., 2010; Zhou et al., 2013).

The MFCs generate new opportunities in bioenergy and biodegradable compounds oxidation and promote bacterial strains management for industrial activities, bioremediation, and bioelectricity production (Du et al., 2007). Bacterial attachment is one bacterial adaptation in MFC besides to the working electrode (Angelaalincy et al., 2018). The electrode effect in MFCs operation conditions determines the efficiency in MFC design, and works as an important variable in bioelectricity generation; thus, the material, surface area and pre-treatment methods are important details in electrode selection for MFC operation (Feng et al., 2010). Platinum, gold, stainless steel and graphite are the most studied electrodes. Platinum (Pt) electrodes are the best catalysts, and graphite electrodes and its variants (plates, brush, etc.) are the most suitable for microorganisms in MFCs. However, platinum, due to its huge demand in electronics area, needs further development with optimal characteristics (Erable et al., 2017; Yang, 2009). In this context, copper as an electrode in an anode chamber is an interesting proposal in this search to find new material to replace Pt, and it is interesting to know how microorganisms could behave with stressful materials due to the bactericide capacity of copper plates. Finally, this research aims to evaluate different types of materials as copper and graphite, find new options for electrodes, evaluate bioelectricity production and provide new information about adaptation processes in bacterial strains with stressful conditions.

Methods

Biomass procedure and MFC set up

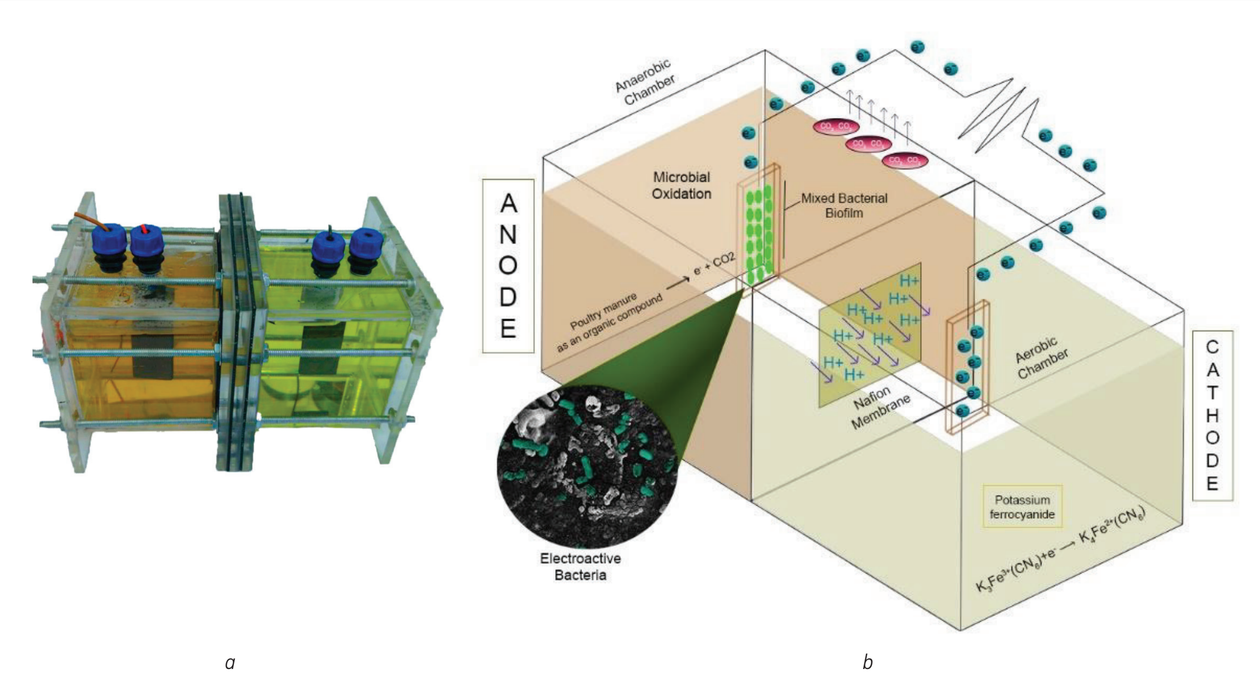
Collection and preparation samples of poultry residual biomass were 5 kg. In this experimentation, chicken manure was used as the main substrate. It was collected from the poultry farm of the company Rico Pollo S.A.C. in the city of Arequipa, Peru. Impurities and residues were cleaned from chicken manure, sieved, filtered, and homogenized. The residues were placed into clean and dry PVC containers, which were separated: one group for the MFC with graphite electrode and the other group for the MFC with copper electrodes. Chicken manure was dried at room temperature, then granulometric separation was carried out with meshes No. 60, 100 and 200 of 215, 150 and 74 microns, respectively; after this procedure, the samples were pulverized and finally sieved with No. 400 mesh of 36 microns. This preparation was diluted in 10% weight/volume phosphate buffer and deposited in the anodic cell. For the cathode, a 50 mM $K_3Fe(CN)_6$ potassium ferrocyanide solution was prepared, and for the proton exchange, a Nafion® N-117 or Ultrex® membrane with a 33.29 cm^2 working surface area was used and located in the middle of the dual-chamber MFC.

The chambers specifications were 18 cm x 11.4 cm, and 2 L volume. They had permeably connected each other with Nafion proton exchange membrane. The prepared substrate was set on a volume of 1.7 L (see *Fig. 1*). This MFC system consists of two electrodes, an anode connected through a load resistance (R) to the cathode through an external electrical circuit where the electrons complete de circuit. The electrodes used were high-density graphite (Tennry; Shandong, China) and copper plates acquired in a local ironmongery with a 28.08 cm^2 surface area each one.

MFC operation

Electrons travel from the anode to the cathode due to the difference in redox potential between the anodic and cathodic solutions. The literature reports the fact that the direct transfer of electrons from microbial cells to the electrodes occurs with very low efficiency, except for bacteria

Fig. 1. *(A) Dual chamber, (B) Microbial fuel cell construction and illustrative scheme*



from some special groups, such as *Geobacter sp.*, among others (Ramasamy et al., 2008). The operating principle of MFC is based on the electron transfer from microbial metabolism (electron transport chain) (Wang et al., 2011; Lepage et al., 2012). A growth substrate feeding solution was placed into the anodic cell, and potassium phosphate buffer containing 50 mM Potassium Ferricyanide - $K_3Fe(CN)_6$ was placed into the cathodic cell, both with 1.7 L solution. Previously, the anode was purged with nitrogen to maintain anaerobic conditions (Aelterman et al., 2008). The voltage output produced in the cell was recorded continuously every 60 seconds using a Keysight Multimeter 34461 multiparameter. The system was then tested with external resistances from 10 Ω to 1 MΩ, which were connected for 30 minutes, and once the voltage was stabilized, they were removed. The intensity (I) was calculated from the voltage obtained (E) using the power (W) and the Ohm Law. Physicochemical parameters such as pH, dissolved oxygen, conductivity, and total dissolved solids were evaluated with a Hanna HI multiparameter 5522.

Sequences and phylogenetic analysis

16s RNA analysis of Av_A1, Av_A2 and Av_C1 was performed using the BLAST tool available at the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). Sequences obtained were processed with online tools for bioinformatic analysis as ClustalOmega and JalView software. The phylogenetic and evolutionary analysis was performed using MEGA 11 using the bootstrap method and the modelling assistant to determine the variables to find the best model that fits with our sequences (Chen et al., 2014).

Results and Discussion

Molecular and bioinformatic analysis of microorganisms

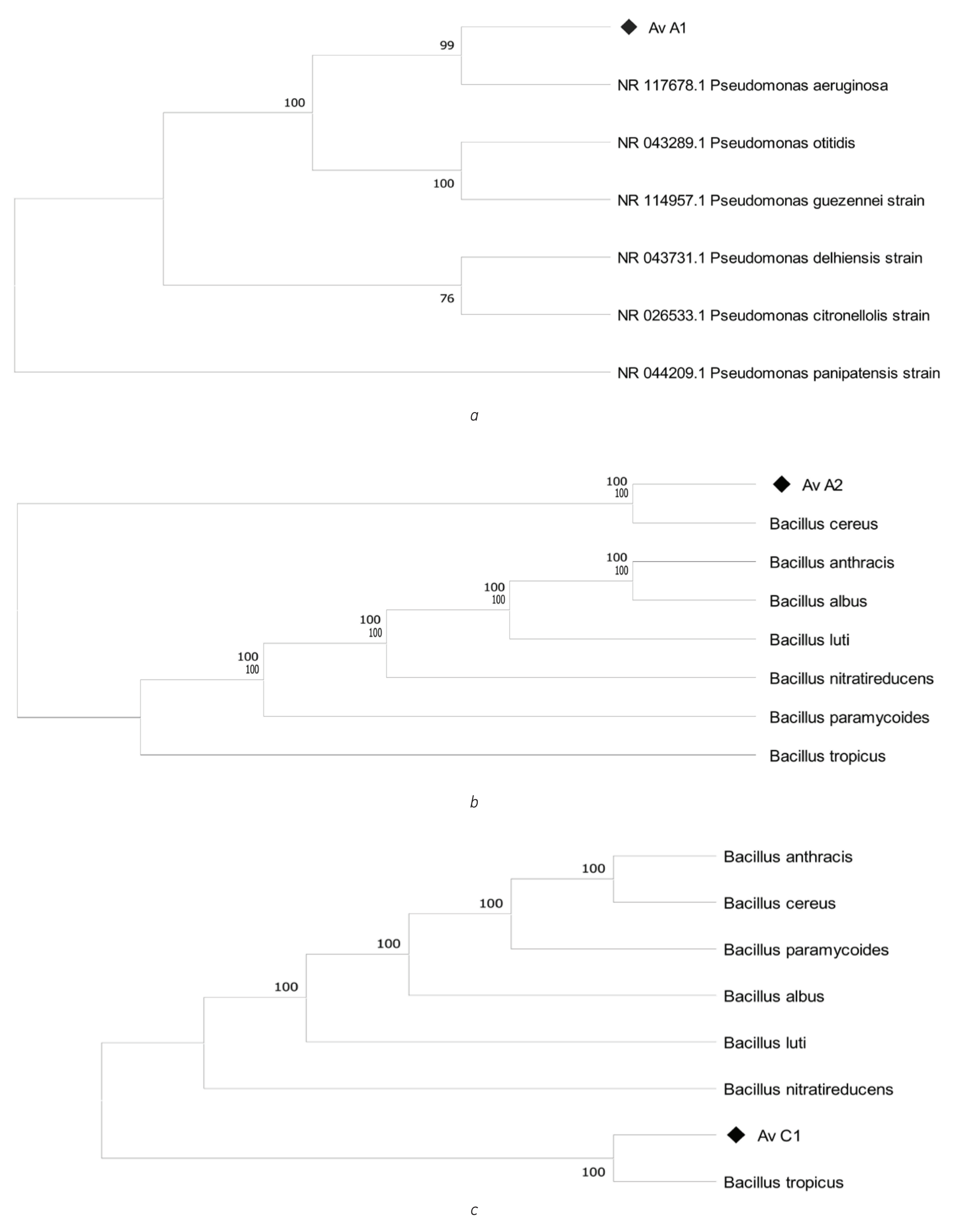
The biofilm formed on the copper and graphite electrodes were cultivated in nutrient agar. Five replicates were made to purify, finding 3 types of bacteria, which were cultivated in blood agar for 24 hours at 37 degrees. Then we proceeded to prepare the pure strains that were sent to DNA Sequencing Functional Biosciences, Inc. for DNA extraction and sequencing. These sequences were uploaded to the NCBI database with the accession names OQ144910, OQ144899 and OQ144901 to finally perform the phylogenetic inference of the bacteria sequences (see *Fig. 2*).

Sequences were aligned in ClustalOmega (McWilliam et al., 2013) and translated into DNA in Jalview (Clamp et al., 2004). Phylogenetic inference was performed using the Bootstrap method with 1000 replicates (Felsenstein, 1985); the Hasegawa-Kishino-Yano (HKY) substitution model and the maximum-likelihood statistical test were used in MEGA 11 (Tamura et al., 2021; Hasegawa et al., 1985). We use the MEGA 11 model wizard to get the best possible result. The Av_A1 strain had six matches with the BLAST tool with 100%, but the model resulted in *P. aeruginosa* as the most similar strain with very robust nodes reaching 100. This indicates that, like the NCBI search, the strain observed is likely from the genus Pseudomonas. This was analyzed in the same way with the Av_A2 and Av_C1 strains that have robust trees and make clusters with *B. cereus* and *B. tropicus*, respectively. *Table 1* explains the search for sequences like our strains. The result of this search resulted in the "bacteria description" part, thus resulting in a 100% match with the strains highlighted in black.

Table 1. *Identity of the rRNA gene sequences with the NCBI BLAST tool*

N°	Strain	Accession Number	Phylum	Bacteria description	Similarity
1	Av_A1	NR_117678.1	Gamma Proteobacteria	***Pseudomonas aeruginosa*** strain DSM 50071 16S ribosomal RNA, partial sequence	100%
2	Av_A2	NR_114582.1	Firmicutes	***Bscillus cereus*** ATCC 14579 16S ribosomal RNA, partial sequence ***Select seq NR_157736.1***	100%
3	Av_C1	NR_114582.1	Firmicutes	***Bscillus tropicus*** Strain MCCC 1A01406 16S ribosomal RNA, partial sequence	100%

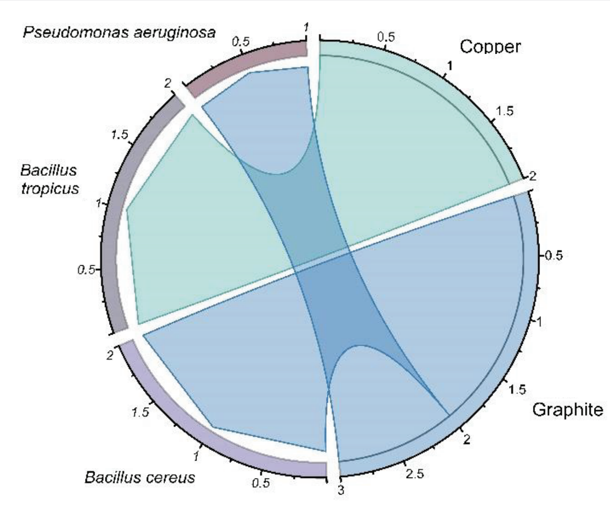
Fig. 2. *Phylogenetic analysis of bacteria found on copper and graphite electrodes from poultry manure: a) Av_A1 strain sharing a clade with P. aeruginosa, b) Av_A2 strain sharing a clade with B. cereus, and c) Av_C1 strain sharing a clade with B. tropicus*



The Av_A1, Av_A2 and Av_C1 strains had a percentage of similarity of 100% with the search in the database. For Av_A1, 6 16s rRNA gene sequences from bacteria belonging to the Gamma proteobacteria, which contain Pseudomonas Azomonas, Azotobacter, Cellvibrio, Mesophilobacter, Rhizobacter and Rugamonas, were used. The genus Pseudomonas is facultative, and anaerobically uses nitrate as an electron acceptor instead of oxygen. This metabolism allowed the bacteria to thrive in the poultry substrate, which is rich in nitrogenous components. In the case of the Av_A2 and Av_C1 strains, these belong to the phylum Firmicutes, within which is the order Bacilliales with the families Bacilliaceae, Alicyclobacillaceae, Listeriaceae, Paenibacil-laceae, Pasteuriaceae, Planococcaceae, Sporolactobacillaceae, Staphylococcaceae and Thermoactinomycetaceae. The found strains are in the genus Bacillus, which have a facultative anaerobic metabolism, and use carbon sources for their development. Poultry waste is characterized by being a very concentrated carbon source, which allowed the strains of the bacillus genus to prosper, in addition to also containing nitrogenous substances, which allowed the development of these strains (see *Fig. 3*).

The chord diagram in *Fig. 3* suggests the relationship of the bacteria with the electrode used. The blue color is related to graphite and the green color to copper. For example, *Bacillus tropicus* is related to the copper electrode.

Fig. 3. *Interrelation between the bacterial strains B. Cereus, P. Aeruginosa, T. Tropicus and the material of the electrodes used in bioelectricity generation (copper and graphite)*



From the experimental data, it is assumed that the operation inside MFCs mimics various unit wastewater treatments, such as biological (anaerobic) process (Mohan et al., 2009), electrochemical decomposition of salts and symbiotic electrochemical oxidation. In view of this great inherent advantage that MFCs present, the research that has been carried out regarding them assumes great importance since it can be applied in the wastewater treatment industry. *Tables 2* and *3* show the values obtained for the parameters of pH, DO, conductivity and TDS up to 208.5 hours of operation. *Table 2* shows the values with the graphite electrode and *Table 3* shows the values with the copper electrode.

Table 2. *Physicochemical parameters evaluated in MFC with graphite electrode*

GRAPHITE ELECTRODE				
Hours (h)	pH	OD ppm	Conductivity µS/cm	TDS ppm
0.00	6.84	1.34	9044	4523
24.70	6.72	1.57	8731	4369
42.57	6.66	1.7	7612	3810
74.12	6.57	2.17	9083	4547
95.20	6.61	2.04	4712	2356
119.00	6.52	2.4	7418	3706
143.50	6.54	3.69	6174	3087
164.50	6.58	3.78	6382	3193
196.75	6.61	3.85	6586	3249
208.50	6.62	4.12	6933	3469

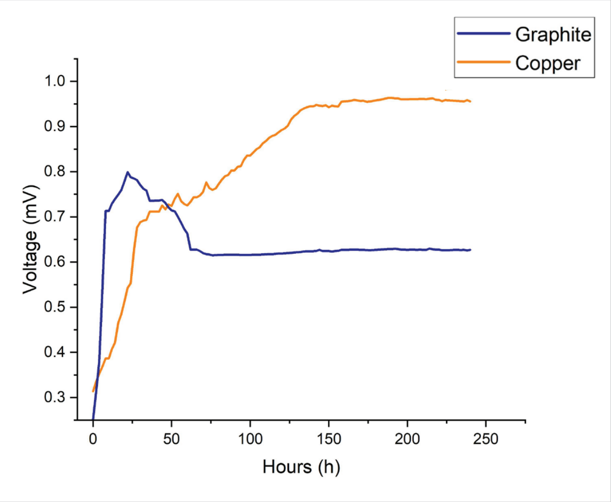
Table 3. *Physicochemical parameters evaluated in MFC with copper electrode*

COPPER ELECTRODE				
Hours (h)	pH	OD ppm	Conductivity µS/cm	TDS ppm
0.00	6.84	1.34	9044	4523
24.70	6.89	2.03	6309	3156
42.57	7.06	1.94	7615	3806
74.12	7.04	3.57	8173	4088
95.20	7.18	2.76	17230	8646
119.00	6.64	3.01	15350	7680
143.50	6.63	2.44	12470	6236
164.50	6.61	6.18	7149	6386
196.75	6.57	5.89	6917	4283
208.50	6.59	5.28	6480	3243

The residual substrate used (biomass) showed better yield, higher power density and higher substrate degradation (Reiche and Kirkwood, 2012). Power density, electrode potential, Coulomb efficiency, and energy recovery in MFCs were examined as a function of solution ionic strength, electrode gap, and composition.

Fig. 4 shows the behavior of the voltage of both electrodes as a function of time; the voltage with the graphite electrode increases considerably after approximately 25 hours and from 75 hours it remains constant; on the other hand, the copper electrode remains constant from approximately 150 hours.

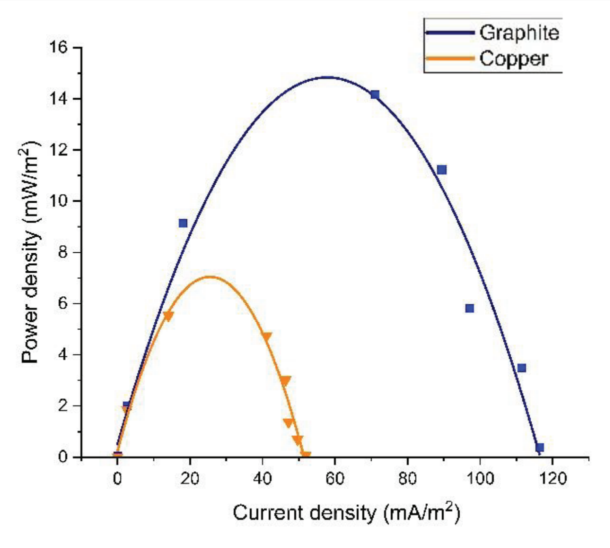
Fig. 4. *Power production based on graphite (blue) and copper (orange) electrodes*



From the stabilization of the energy production, the power density produced and the current density with external resistances of different values of 10 Ω – 100 KΩ began to be evaluated for intervals of approximately 30 min. A maximum power density of 14 mW/m^2 was obtained for the cell with a graphite electrode for a surface area of 28.08 cm^2 and of 6.7 mW/m^2 for the cell with a copper electrode for a surface area of 28.08 cm^2 (see *Fig. 5*).

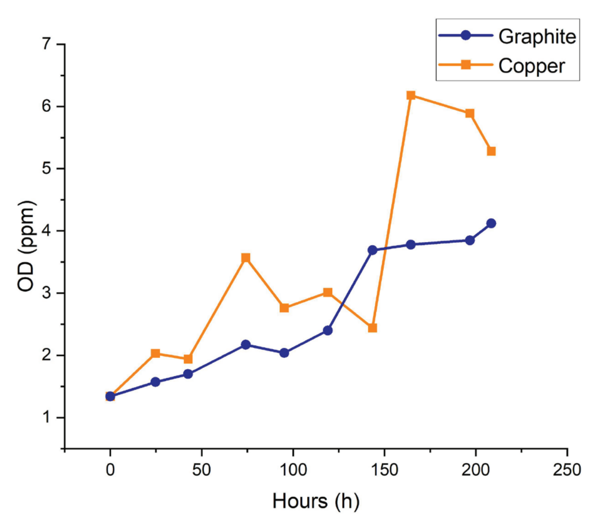
The results in *Fig. 5* show polarization curves with 10 Ω to 1 MΩ resistances. The polarization curve test in MFCs with graphite electrodes reaches its maximum point with a resistance of 1000 Ω, this being too high. This behavior is a problem for the dual-chamber type MFCs assembled in this investigation; although parameters such as substrate, Ph, proton membrane, and bacterial strains were controlled, current generation and power density were found to be at low levels. Meanwhile, the copper electrode test was more efficient. As we know, copper is a bactericide element, so bacteria could not develop on surfaces; however, the Av_C1 strain identified as *B. tropicus* adapted to the stressful environment as a copper electrode. This adaptive behavior to copper can be observed in the study of a new strain (Jimenez Pacheco et al., 2023).

Fig. 5. *Polarization curves for graphite and copper electrodes*



For the evaluations of dissolved oxygen, an increase was observed with the use of graphite and copper electrodes. For the cell with the copper electrode (orange), a maximum of 6.18 ppm was reached and for the cell with the graphite electrode (blue) a maximum of 4.12 ppm was reached (see *Fig. 6*).

Fig. 6. *Dissolved oxygen in graphite and copper electrodes MFCs*



Based on the electrochemical performance and the analysis of the microbial community observed, a better performance of the MFC operation was obtained (Sun et al., 2016). Emphasizing the type of electrode, it is the material that optimizes the MFC technology, since there is a positive correlation between the microbial community and electrochemical performance.

Conclusions

The use of different electrode materials, such as graphite and copper, shows a great contribution in the feasibility of producing bioelectricity from poultry residual biomass of chicken manure under anaerobic conditions. However, low power densities were obtained due to the MFC design, even though all parameters were under control. The fact that the strain Av_C1 had grown in stressful conditions was corroborated with DNA extraction, genetic sequencing, and evolutionary inference with tree phylogenies.

The assays showed a maximum production power density of 14 mW/m^2 for the cell with a graphite electrode, with a surface area of 28.08 cm^2, and a maximum production of 6.7 mW/m^2 for a cell with a graphite electrode, surface copper of 28.08 cm^2, less than the graphite, even though inoculum was the same in both experiments, but with different bacterial communities as we observed in molecular and bioinformatic section. These evaluations show that the copper electrode material must be considered of utmost importance when working with microbial fuel cells in relation to energy production, since they present acceptable conditions and do not generate any secondary effects or toxicity in this kind of bacteria presented on this research.

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