

Article

Use of Kiwi Waste as Fuel in MFC and Its Potential for Use as Renewable Energy

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Abstract: This research aimed to use kiwi waste as fuel to generate bioelectricity through microbial fuel cells. It was possible to generate an electrical current and voltage peaks of 3.807 ± 0.102 mA and 0.993 ± 0.061 V on day 11, showing an electrical conductivity of 189.82 ± 3.029 mS/cm and an optimum operating pH of 5.966 ± 0.121 . The internal resistance of the cells was calculated using Ohm's Law, resulting in a value of 14.957 ± 0.394 Ω , while the maximum power density was 212.68 ± 26.84 mW/m² at a current density of 4.506 A/cm². Through the analysis of the FTIR spectra carried out on the substrate, a decrease in the characteristic organic peaks was observed due to their decomposition during the electricity-generation process. In addition, it was possible to molecularly identify the bacteria *Comamonas testosteroni*, *Sphingobacterium* sp., and *Stenotrophomonas maltophilia* adhered to the anodized biofilm. Finally, the capacity of this residue to generate bioelectricity was demonstrated by lighting an LED bulb with a voltage of 2.85 V.

Keywords: agricultural waste; kiwi waste; microbial fuel cells; bioelectricity



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1. Introduction

The generation of organic waste has had an exponential growth in recent years due to the high demand for food products, influencing the production, industrialization, and modernization of agro-industries [1–3]; added to the increase in energetic demand, they have become two global problems without short-term solutions [4]. The United Nations' Food and Agriculture Organization (FAO) reported that approximately 1.3 million tons of food is wasted annually, and it is estimated that by 2025 it will be 2.6 billion tons, which would represent a third of global production of food [5,6]. Likewise, it is estimated that the waste from agro-industries reaches between 5 and 10% per year, and these wastes in developing countries are dumped into the environment without an adequate protocol, generating pollution in the environment and harmful effects on human health [7–9]. For this reason, large research centers have made proposals on the potential uses that can be given to these types of waste, with the production of biogas, compost, nutritional supplements, biomaterials, and bioelectricity being the most researched [10,11].

One of the most produced fruits in China, Italy, and New Zealand is the kiwi, which, with an annual production of between 1.5 and 1.6 million tons, represents approximately 90% of the world's production of this fruit, which is demanded throughout the world [12,13]. It has been reported that the annual production of China, Italy, and New Zealand is 1,765,847, 447,560, and 382,337 million tons, respectively, representing a great economic contribution for these countries from the agro-industrial point of view [14]. The high consumption of this fruit is mainly due to the high content of vitamins (C, B8, and K), dietary fiber, potassium, and polyphenols [15]. Just as its high demand generates great profits for the agro-industry, the collateral damage due to the residues generated in the harvest and sale generates losses [16]. This is why the search for new ways to use these types of waste has become a necessity for the industries dedicated to the production and sale of this fruit.

Microbial fuel cell (MFC) technology is presented as an innovative solution to this problem since it can use any type of waste as fuel to generate electrical energy, which could be used by companies to reduce expenses from their own waste [17,18]. Electrical energy is generated from the conversion of chemical energy into electrical energy thanks to the oxidation and reduction processes that occur in the anodic and cathodic chambers of MFCs [19]. The electrons are generated from oxidation that occurs in the substrate (anodic chamber), and these are conducted by the anode electrode to the outside and flow through the external circuit to the cathode electrode, thus generating a flow of electrons (electric current) [20,21]. Research on the use of different types of waste in MFCs has been reported, for example, by Idris et al. (2023), who used rotten sweet potato in their microbial fuel cells made with graphite electrodes, managing to generate a peak voltage and power density of 288 mV and 20.86 mW/m², showing an internal resistance of 232 Ω [22]. Likewise, Zafar et al. (2023) used apple in their single-chamber microbial fuel cells (MFC-SC), managing to generate voltage peaks and power density of 0.45 mV ± 0.50 mV and 221 mW/m² using carbon felts as electrodes [23]. Husain et al. (2022) used bakery waste in their MFC-SC made with graphite electrodes, managing to generate voltage peaks and power density of 700 mV and 1001 mW/m², for which the pH was adjusted to 7 [24]. Likewise, Yaqoob et al. (2022) used food waste from a cafeteria as a substrate in their MFC-SC, managing to generate voltage peaks and power density of 2900 mV and 41.58 mW/m², with an internal resistance of 813.78 Ω [25]. It has been found that the MFC-SC are being intensively investigated, and they are the most used due to their high values of electrical energy generated at a low manufacturing cost due to the fact that the cathode electrode is exposed to the environment (O₂) and does not need external aeration for the reduction process to occur [26,27]. Another way to increase the values of electrical energy is through the use of metallic nanoparticles incorporated in the electrodes fulfilling a catalyst function, which is similar to the way it is conducted in solar cells [28]; however, the production costs would increase, making scaling unsustainable today [29].

Because of this, the main goal of this research was to generate bioelectricity through kiwi waste in laboratory-scale single-chamber microbial fuel cells using zinc and copper as electrodes. The parameters of electrical current, voltage, pH, and electrical conductivity of the substrate were monitored for 35 days. Additionally, the values of internal resistance, power density, current density, and the FTIR spectrum (initial and final of the substrate) were measured. Finally, the micrographs obtained from the biofilm forming on the anode electrode at the molecular level were observed. This research was designed to try to contribute to the development of innovative ways to generate electricity using fruit waste, creating opportunities for governments and companies to reuse their own waste based on reducing their electricity costs in the near future.

2. Materials and Methods

2.1. Construction of Single-Chamber Microbial Fuel Cells

Single-chamber microbial fuel cells (MFC-SC) were fabricated from a 500 mL polymethylmethacrylate tube and used as a chamber for the substrate, where the Copper (Cu)

anode electrode was placed inside the tube. The zinc (Zn) cathode electrode was placed at one end (so that one of the faces was in contact with O_2), and both electrodes had an area of 78.54 cm^2 and were connected to an external circuit containing a 100Ω resistance, while inside, the electrodes were separated by the proton exchange membrane (Nafion 117; Wilmington, NC, USA), as shown in Figure 1. Three MFC-SC were performed in total.

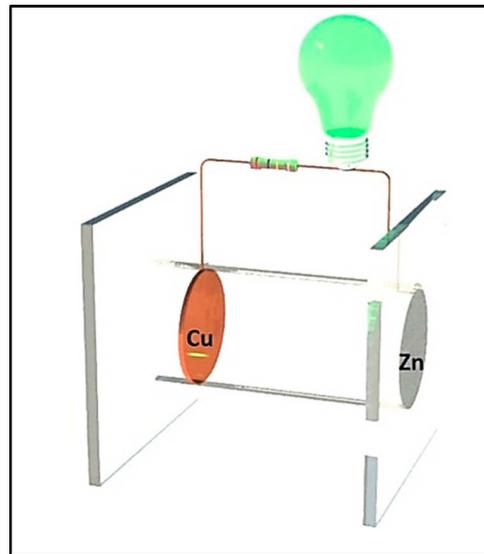


Figure 1. Prototype of the MFC-SC.

2.2. Collection and Preparation of Kiwi Waste

Kiwi waste (2 kg) was collected from the Mercado Zonal Palermo, Ex Mayorista, Trujillo, Peru. These wastes used as substrates were washed several times in order to eliminate any type of impurity acquired from the environment, and then allowed to dry at $26 \pm 1 \text{ }^\circ\text{C}$ in an oven (Labtron, LDO-B10, Michigan, USA) for 24 h. Finally, the waste passed through an extractor (Maqorito, 400 rpm, Michigan, USA) obtaining 1.8 L of kiwi waste extract, which was stored in hermetic vessels properly sterilized until use.

2.3. Characterization of Microbial Fuel Cells

The physical–chemical parameters monitored were carried out for 35 days, and a multimeter (Prasek Premium PR-85, USA) was used to measure the values of voltage and electric current (with external resistance (R_{ext}) of 100Ω). Asita also observed the values of electrical conductivity (conductivity meter CD-4301) and pH (pH meter 110 Series Oakton) of the substrate used. For the measurement of current density (CD) and power density (PD), the procedure carried out by Segundo et al. (2022) with R_{ext} was used, values of $0.3 (\pm 0.1)$, $0.6 (\pm 0.18)$, $1 (\pm 0.3)$, $1.5 (\pm 0.31)$, $3 (\pm 0.6)$, $10 (\pm 1.3)$, $20 (\pm 6.5)$, $50 (\pm 8.7)$, $60 (\pm 8.2)$, $100 (\pm 9.3)$, $120 (\pm 9.8)$, $220 (\pm 13)$, $240 (\pm 15.6)$, $330 (\pm 20.3)$, $390 (\pm 24.5)$, $460 (\pm 23.1)$, $531 (\pm 26.8)$, $700 (\pm 40.5)$, and $1000 (\pm 50.6) \Omega$ [30] were found. The FTIR transmittance spectra (Thermo Scientific IS50, Waltham, MA, USA) were used to observe the decrease in the peaks of the components present in the substrate and the internal resistance values of the MFC-SC, and an energy sensor was also used (Vernier- $\pm 30 \text{ V}$ and $\pm 1000 \text{ mA}$). The MFC-SCs operated at room temperature ($22 \pm 1.5 \text{ }^\circ\text{C}$), with a variable pH due to the fermentation process of the substrate.

2.4. Isolation of Anodic Microorganisms

The possible microorganisms responsible for generating electricity grow and develop on the anode (biofilms). Therefore, to isolate bacteria, a swab was performed under aseptic conditions of the anode plate (copper) and streaked in BHI agar medium. They were incubated at $36 \pm 1 \text{ }^\circ\text{C}$ for 24 to 48 h. The colonies that developed were streaked on

MacConkey agar to differentiate Gram-negative ones. On the other hand, the isolation of fungi was carried out on Sabouraud agar (plus chloramphenicol), with an incubation time of 30 ± 1 °C for 48 to 72 h. Finally, axenic cultures of the bacteria and yeasts isolated in inclined nutritive agar for bacteria and inclined Sabouraud agar for yeasts were performed. These were maintained at 4 °C and then molecularly identified.

2.5. Blast Characterization of Isolated Anodic Microorganisms

The molecular identification was carried out in the laboratory Ecobiotech Lab S.A.C., located in the city of Trujillo (La Libertad, Peru). Previous methods of rDNA extraction and amplification with universal primers for bacteria and fungi were performed. The sequencing was performed at the MacroGen laboratory in the United States. The analysis and correction of sequences were performed in the MEGA software (<https://www.megasoftware.net/>) (accessed on 21 March 2023), and then the sequence alignment computer program Blast was used to identify the percentage of identity of the isolated species. In the MEGA program, the dendrogram of the species identified with other related species (obtained from Blast) was constructed.

3. Results and Analysis

Figure 2a shows the monitored voltage values of the MFC-SC, where a value of 0.67 V on day one is shown to later increase to 0.993 ± 0.061 V on day eleven, after which continuous decreases were observed until the last day (0.23 ± 0.093 V). The diverse and abundant composition that this type of substrate contains according to the literature was beneficial for the bioconversion and biodegradability capacities of kiwi-waste extracts through MFC-SC. Compared with other investigations where vegetable waste [31], pig waste [32], mixtures of residual sludge and kitchen waste [33], and municipal solid waste [34] were used, this investigation showed much higher voltage values using less expensive materials. On the other hand, Figure 2b shows the values of electrical current monitored, managing to observe a value of 2.643 ± 0.03 mA on the first day up to 3.807 ± 0.102 mA on the eleventh day, which then decreased until the last day (1.451 ± 0.037 mA). The high values of electric current shown in the first days are due to the glucose content present in this fruit that acts as food for the metabolism of microorganisms. As these concentrations decrease, a decrease in the production of electrons is observed, thus generating a loss of electrical current in the last few days [35,36]. Rahman et al. (2021) mentions in his research that the main electron generators are microorganisms that oxidize the organic compound in the anodic electrode; this oxidation causes the electrons to move and travel through the external circuit to the cathodic electrode, generating electric current [37]. These values shown in this investigation are higher than those reported in other investigations; for example, Imwene et al. (2021) managed to generate electrical current peaks of 0.0210 ± 0.007 mA using mango debris as a substrate in their MFCs using graphite electrodes [38]. Additionally, Yaqoob et al. (2022) generated electrical current peaks of 0.026 mA using rambutan waste, langsung waste, and mango waste as fuel in their MFCs using graphite electrodes [39]. One of the important factors for the voltage and current values of this research is the electrodes used, which, being metallic in nature, have excellent properties that can easily conduct electrons, reducing electrical loss [40]. It has been reported that the energy gain by bacteria is directly related to the voltage coming from the anode and the redox potential coming from the substrate used [41]. In order to reduce manufacturing costs, metallic electrodes have been used in this research, but they should be coated with a compatible compound, without also ruling out the use of biocatalysts [42,43]. Likewise, the ubiquitous nature of microorganisms and their role in the decomposition of organic compounds make certain electrically active microorganisms proliferate, with the ability to generate electrons when they carry out the oxidation process of organic matter [44]. These types of microorganisms proliferate within a matrix with polymeric substances forming biofilms on the anode electrode, where bio-electrochemical reactions occur [45,46].

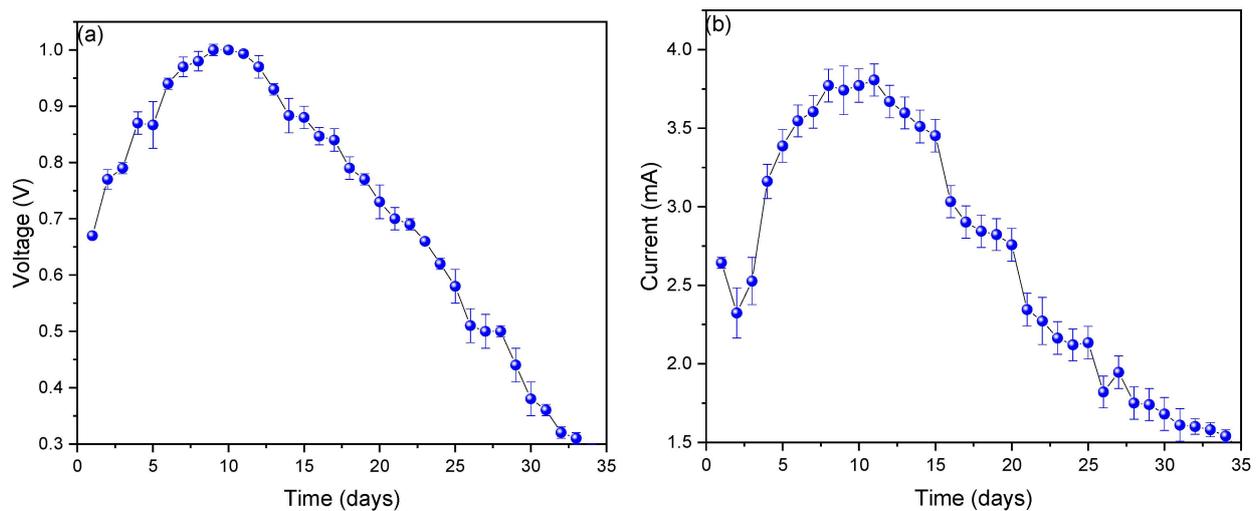


Figure 2. Values of (a) voltage and (b) electrical current obtained from the monitoring of the MFC-SC.

Figure 3a shows the values of the electrical conductivity of the substrate, and one can observe an increase from day 1 (120.14 ± 0.012 mS/cm) to day 10 (189.82 ± 3.029 mS/cm) and then a decrease until the last day (95.16 ± 3.055 mS/cm) of monitoring. The increase in electrical conductivity values is due to acidic conditions, according to Kalagbor et al. (2020), and these conditions were observed in the first days of monitoring, allowing a greater number of electrons in the first days [47]. The decrease in these values is due to the fermentation and decomposition of the compounds initially present in the substrate [48], which is sedimented at the end of the monitoring. Figure 3b contains the monitored pH values of the MFC-SC, and one can observe that the values vary from a slightly acidic level to a slightly alkaline level, with its optimum operating pH of 5.966 ± 0.121 on the eleventh day. In the literature, it has been reported that pH variations are due to different causes, one of the main ones being the fermentation of the different substrates used, and that for each substrate there is an optimum operating pH, and this can change if the design is varied. The O type of MFC is used for this; even the electrodes used have the capacity to have various pH values [49,50]. For example, Zafar et al. (2023) used fruit waste as a substrate in their single-chamber MFC, which operated at 3.7 ± 0.7 [51]. Additionally, Kebaili et al. (2021) used fruit-waste leachate as a substrate in their MFCs operating at a pH of 4 and graphite electrodes, managing to generate voltage peaks of 260 mV [52].

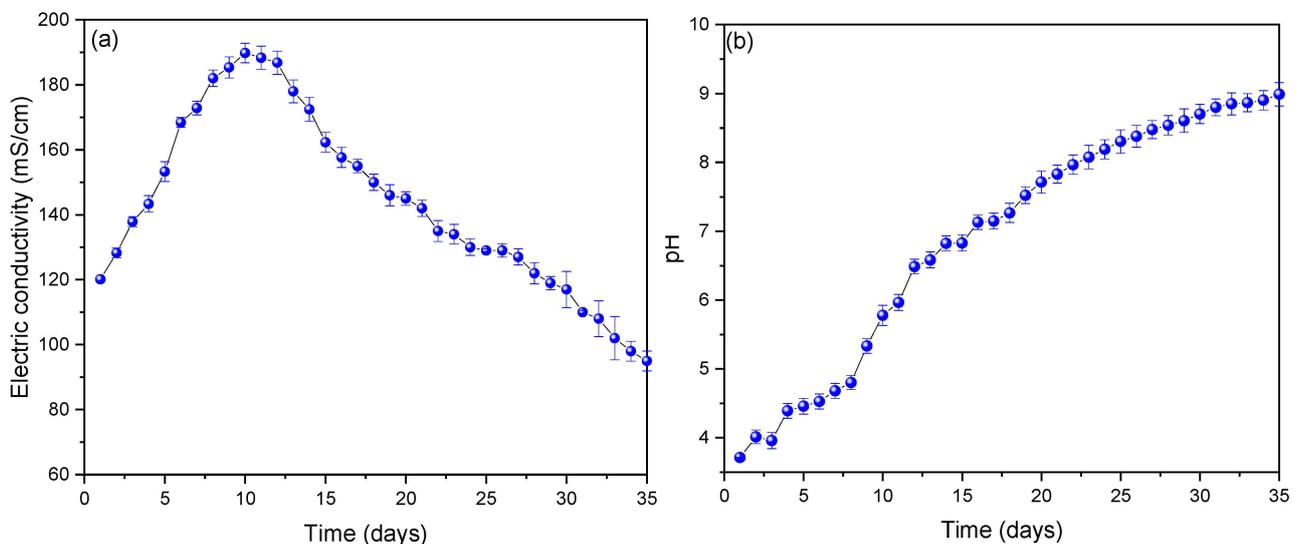


Figure 3. Values of (a) electrical conductivity and (b) obtained from the monitoring of the MFC-SC.

The value of the internal resistance of the MFC-SC is shown in Figure 4a, which was calculated using Ohm's Law ($V = RI$), where the voltage values (V) were placed on the "y" axis, and the values of the electrical current (I) were placed on the "x" axis, where the slope of the linear fit was the internal resistance ($R_{int.}$) of the MFC-SC, showing a value of $R_{int.}$ of $14.957 \pm 0.394 \Omega$. The low value of $R_{int.}$ obtained is due to the electrical conductivity shown by the substrate as well as by the electrodes with metallic characteristics used, which allowed less opposition to the flow of electrons [53,54]. The research carried out by Abbas et al. (2019) showed an internal resistance of 478.5Ω with voltage peaks of 300.5 mV using sediment from industrial plants as a substrate, mentioning that these values were obtained because the exo-electrogenic microorganisms in charge of generating the greatest number of electrons to the anode did not have the ideal conditions for their proliferation [55]. In this sense, Yaqoob et al. (2022) also managed to generate an internal resistance of 1557Ω , managing to generate voltage peaks of 112 mV using potato waste as a substrate in their MFC with graphite electrodes, mentioning that for good electron transport, the values of internal and external resistance have to be equal [56]. Figure 4b shows the power density (PD) values as a function of current density (CD), observing a $PD_{max.}$ of $212.68 \pm 26.84 \text{ mW/m}^2$ at a DC of 4.506 A/cm^2 with a peak voltage of $916.59 \pm 5.47 \text{ V}$. Luis et al. (2023) mentioned that the PD values depend on the internal resistance of the MFCs, as well as that variations of the substrate can increase or decrease these values as well, while the presence of a proton exchange membrane would increase the PD values [57]. On the other hand, the high values of this research are higher than those reported by other researchers with types of fruit waste; for example, Priya A. and Setty Y. (2019) managed to generate 31.57 mW/m^2 of PD at a DC of 350 mA/m^2 with a peak voltage of 0.4 V using apple debris as a substrate and a NAFION as a proton exchange membrane [58]. Likewise, Du H. and Shao Z. (2022) managed to generate PD peaks of 14.1 mW/m^2 at a DC of 320.1 mA/m^2 using white-potato residues as a substrate in their MFCs using carbon-felt electrodes with an internal resistance of 60.6Ω [59].

Figure 5 shows the initial and final FTIR spectra of the substrate used (kiwi waste), demonstrating peaks in the wave number of 3271, 2932, 2838, 1685, 1569, 1394, and 1028 cm^{-1} belonging to the C=C alkenes, C=C aromatic ring, C-H alkanes, C-O alcohols, -OH aromatic, C-O-H alcohols, -C-O alcohols, and -C-H alkenes, respectively [60,61]; all these peaks decreased at the end of the MFC-SC monitoring. In the process of generating electrical energy, the compounds initially present in the substrate degrade, breaking the compounds, which causes the decrease in the initial and final FTIR spectra [62].

From the anode plate (with microbial growth), three bacterial cultures were isolated in the plates with BHI agar. These were coded as M04, M05, and M09. The growth of these three bacteria on MacConkey agar means they are Gram-negative. Table 1 shows that the Blast characterization identified three species of possibly electrogenic bacteria: *Comamonas testosteroni*, *Sphingobacterium* sp., and *Stenotrophomonas maltophilia*. In the phylogeny of these bacteria, it is appreciated that both *S. maltophilia* and *C. testosteroni* belong to the group of Proteobacteria, while *Sphingobacterium* sp. belongs to the Bacteroidetes group (Table 1). In some studies, the dominium of the first group has been reported, followed by Firmicutes and Bacteroidetes in biofilms of MFC anodes [62,63].

The number of bacteria isolated could be influenced by the type of anode plate material, since copper has antimicrobial properties, which is a controversial issue in its use in MFCs. However, one study mentions that the conductivity of copper is 900-times greater than that of polycrystalline graphite, which represents an advantage over carbon-based electrodes. On the other hand, the bacterium *Geobacter* sp. is electrochemically active and can colonize copper; this could support the fact that the three types of bacteria isolated are electrogenic [64].

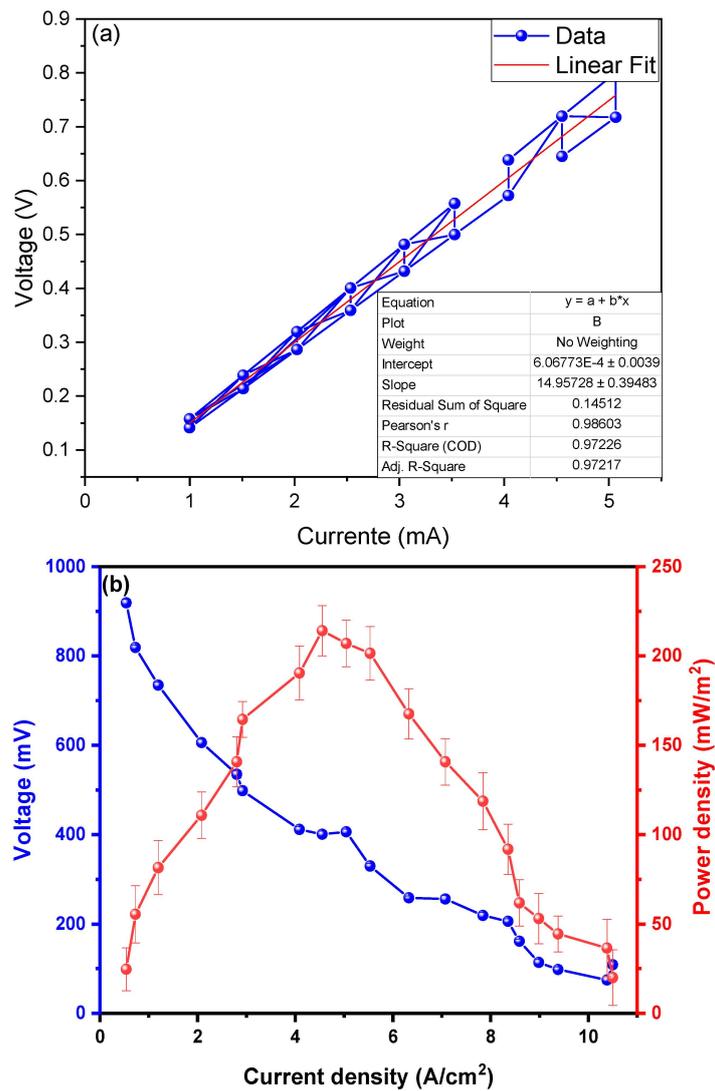


Figure 4. Characterization of (a) internal resistance and (b) power and voltage density regarding the current density of the MFC-SC.

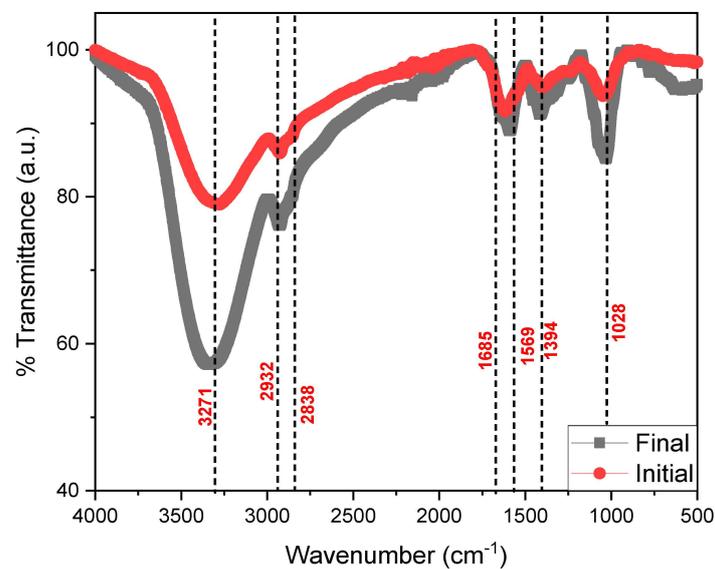


Figure 5. Initial and final FTIR spectrum of tomato debris used as substrate.

Table 1. Blast characterization of anodic microorganisms.

ID Sample	BLAST Characterization	Length of Consensus Sequence (nt)	% Identity	Accession Number	Phylogeny
M04	<i>Stenotrophomonas maltophilia</i>	1477	100.00	CP028899.1	Cellular organisms; Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; <i>Stenotrophomonas</i> ; <i>Stenotrophomonas maltophilia</i> group
M05	<i>Sphingobacterium</i> sp.	1451	99.44	KY492737.1	Cellular organisms; Bacteria; FCB group; Bacteroidetes/Chlorobi group; Bacteroidetes; <i>Sphingobacteriia</i> ; Sphingobacteriales; Sphingobacteriaceae; <i>Sphingobacterium</i> ; unclassified <i>Sphingobacterium</i>
M09	<i>Comamonas testosteroni</i>	1448	100.00	NR_113709.1	Cellular organisms; Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; <i>Comamonas</i>

Figure 6 shows the dendrogram of the groups of Gram-negative bacteria phylogenetically related to the three possible electrogenic bacteria identified. This dendrogram was made with the 16S rRNA gene sequences and analyzed with MEGA Software. The microorganisms identified in this study are Gram-negative bacteria (Figure 6), which are often isolated in microbial combustion cells with organic waste [65–69]. These bacteria appear to have a better ability to produce electrical energy in MFCs than Gram-positive bacteria [69]. On the other hand, these three types of bacteria are associated with the degradation of the substrate (kiwi waste) and the transfer of electrons to the anode plate, which generates an electric current. The evidence for this is the microbial growth formed on the anode plate, which is an important step for power generation within an MFC [70–72]. The *S. maltophilia* bacteria were isolated from biofilms developed on the anode plate of MFCs, which generated different voltage values from different organic waste substrates such as fruits, such as papaya (0.955 V) and golden gooseberry (1.03 ± 0.02 V), and vegetables, such as onion (0.991 ± 0.02 V). Consequently, the voltage values of the present work (0.993 ± 0.061 V) are similar to the voltage values generated by the aforementioned organic waste [26,73,74].

On the other hand, *C. testosteroni* and *S. maltophilia* are motile bacteria and could transfer electrons to the anode plate through their flagella [75–77]. *Sphingobacterium* sp. is a non-motile bacterium [78]; however, it is possible that some components of the biofilm can transport the electrons [79]. This is the first time that *Sphingobacterium* sp. was reported in biofilms of anodes of an MFC, so more research is needed to discover its electrogenic role in MFCs. Finally, another aspect that relates these three bacteria to the generation of electricity is that they are non-fermentative since within the structure of the biofilm there are two types of microorganisms, fermentative and non-fermentative [69,75,77]. These last microorganisms take advantage of the degradation products of fermentative microorganisms to generate electrons that are transferred to the anode plate [69].

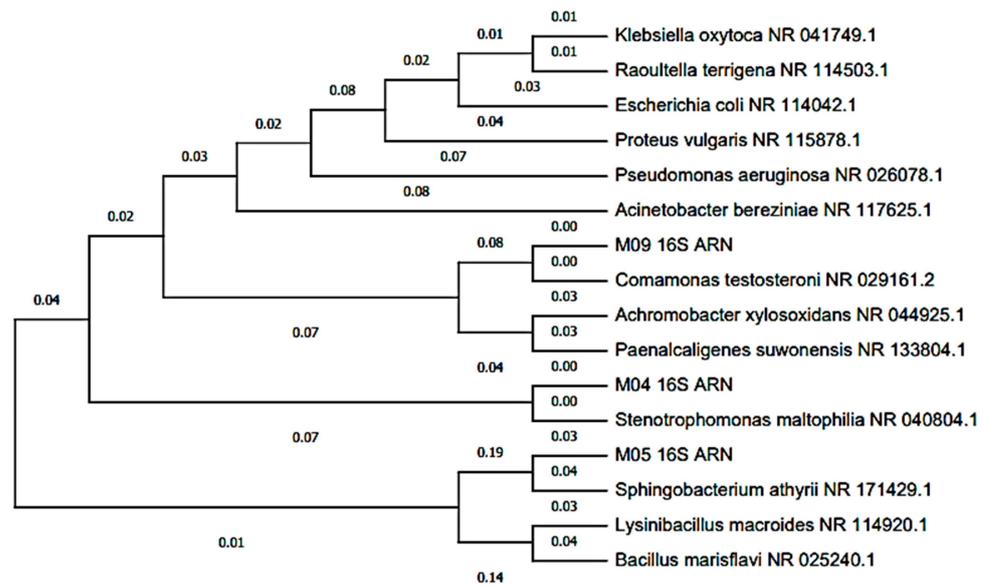


Figure 6. Dendrogram based on 16S rRNA gene sequences of possible electrogenic bacteria identified from an anode plate of MFCs fed kiwi waste.

On the other hand, in the last days of monitoring, the zinc cathode electrodes showed corrosion, which is why the editing of the microorganisms of this electrode could not be carried out. Finally, Figure 7 shows the schematization of the bioelectricity-generation process, where the single-chamber microbial fuel cells were connected in a series, managing to generate 2.85 V, which was enough to turn on an LED bulb.

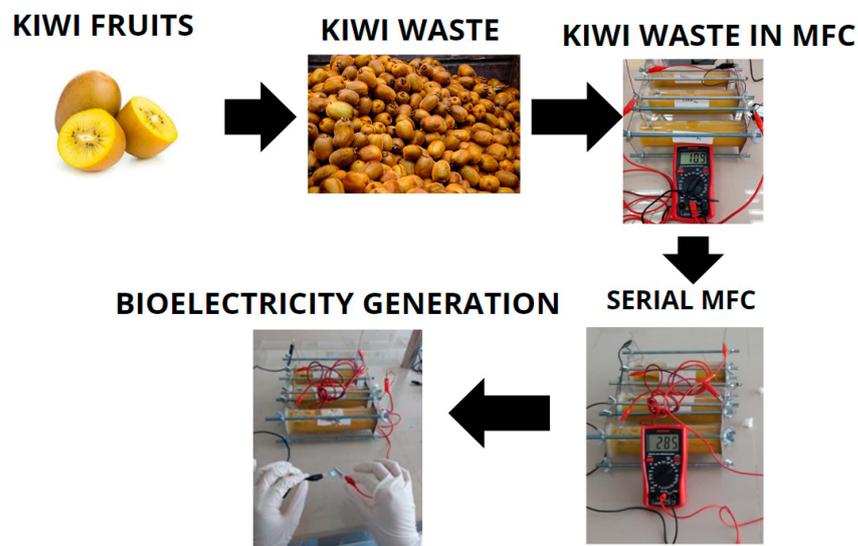


Figure 7. Schematization of the process of generating electrical energy from kiwi waste.

4. Conclusions

Bioelectricity was successfully generated using kiwi residue as fuel in low-cost microbial fuel cells on a laboratory scale. Maximum values of voltage and current of 0.993 ± 0.061 V and 3.807 ± 0.102 mA, respectively, were observed on the eleventh day, which operated with an optimum pH of 5.966 ± 0.121 , showing an electrical conductivity of the substrate used of 189.82 ± 3.029 mS/cm. The low internal resistance of 14.957 ± 0.394 Ω contributed to the high values of power density (212.68 ± 26.84 mW/m²) and current density (4.506 A/cm²) found. It was also observed that the peaks of the FTIR spectra shown decreased at the end of the monitoring, with the peak belonging to the wave number 3271 cm⁻¹ of the

C=C alkenes the one that decreased the most. Molecular identification found the bacteria *Comamonas testosteroni*, *Sphingobacterium sp.* and *Stenotrophomonas maltophilia* in the biofilm of the anode electrode. Finally, it was shown that the three microbial fuel cells connected in a series managed to generate 2.85 V, which is the amount necessary to turn on an LED bulb.

It is recommended that, for future investigations, the pH be adjusted to the value found in this investigation with some chemical compound, as well as that the use of some catalyst or biocatalyst be used to enhance the efficiency of microbial fuel cells. Likewise, the metallic electrodes should be coated to avoid harmful effects for the microorganisms present in the substrates, and this should be conducted with a non-toxic and environmentally friendly compound.

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