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Impact of Dragon Fruit Waste in Microbial Fuel Cells to Generate Friendly Electric Energy

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Abstract: Pollution generated by the misuse of large amounts of fruit and vegetable waste has become a major environmental and social problem for developing countries due to the absence of specialized collection centers for this type of waste. This research aims to generate electricity in an eco-friendly way using red dragon fruit (pitahaya) waste as the fuel in single-chamber microbial fuel cells on a laboratory scale using zinc and copper electrodes. It was possible to generate voltage and current peaks of 0.46 ± 0.03 V and 2.86 ± 0.07 mA, respectively, with an optimum operating pH of 4.22 ± 0.09 and an electrical conductivity of 175.86 ± 4.72 mS/cm at 8 °Brix until the tenth day of monitoring. An internal resistance of 75.58 ± 5.89 Ω was also calculated with a maximum power density of 304.33 ± 16.51 mW/cm² at a current density of 5.06 A/cm², while the FTIR spectra showed a decrease in the initial compounds and endings, especially at the 3331 cm⁻¹ peaks of the O–H bonds. Finally, the yeast-like fungus *Geotrichum candidum* was molecularly identified (99.59%). This research will provide great opportunities for the generation of renewable energy using biomass as fuel through electronic devices with great potential to generate electricity.

Keywords: dragon fruit; fruit waste; microbial fuel cells; generation; bioelectricity



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1. Introduction

Agricultural waste has generated large amounts of biomass due to the increase in the world population, which could cause serious environmental problems if not handled properly. It has been reported that approximately 140 Gt of waste is produced annually, most of which is not used [1,2]. One of the countries that has experienced this pollution the most is China, where waste expels approximately 11% of greenhouse gases into the environment. However, some countries, including the United States, Finland, Germany, the Netherlands, China, Korea, Japan, and South Africa, have begun to develop technologies to generate products with different residues from the cultivation processes of their respective countries [3–5]. Agro-industrial companies generate thousands of jobs around the world, from planting to harvesting and selling their products, becoming an economic engine for countries. In the production of fruits and/or vegetables, it has been shown that large amounts of waste are also generated because there are fruits that do not reach the final part of the sale process in good condition, generating large amounts of losses for companies.

Thus, by not having a specific process for the reuse of waste to generate a new product, companies cannot benefit [6]. According to the United Nations Organization, approximately 1.6 billion tons of organic waste is generated annually, of which 40 to 50% is food waste belonging to the human diet, whose collection varies depending on the country and its policies. However, in general, among the most commonly used methods are organic waste treatment plants, the suspension of biological pollutants, and the separation at origin for drop-off collection, but, at the same time, these systems are very expensive and necessary [7]. Several years ago, agro-industrial residues were seen as a problem in the food production chain due to disposal costs, and the residues were mostly used as composts or for the production of animal feed, but this has evolved significantly due to the emergence of new techniques and the help of technology, giving agro-industrial residues an added value and making them a sustainable and friendly resource for the environment [8,9]. When organic agro-industrial residues are used in bioconversion processes, not only is the amount of polluting organic matter in the environment reduced, but an added value is also given to these residues by using them as inputs for the production of electricity. On the other hand, the transition to a low-carbon economy is being facilitated by reducing the amount of carbon burned to produce energy, which contributes to the reduction of the carbon footprint [10,11].

In this sense, the technology of microbial fuel cells (MFCs), developed for the first time in the 1960s, has begun to take center stage because this technology allows for the use of different types of waste as fuel to generate bioelectricity [12]. Due to their great potential, microbial fuel cells have a large number of designs, but in general, they consist of two chambers (anodic and cathodic), which are almost always separated by a proton exchange membrane, and inside each chamber, electrodes are connected on the outside by an electrical circuit [13,14]. The generation of electric current originates because of oxidation/reduction reactions that occur within the anodic and cathodic chambers, where protons and electrons are released by microorganisms. The protons travel through the proton exchange membrane, while the electrons are captured by the anodic electrode, and through the external circuit, they travel to the cathode, generating a flow of electrons and electric current [15]. One of the most widely used microbial fuel cells is the one with a single chamber that, due to its versatility, offers several advantages [16]. According to the literature, in several investigations, single-chamber microbial fuel cells have been shown to generate higher power densities and reduce the internal resistance due to the direct contact of the cathode electrode with air (O₂), as well as reduce the costs for the manufacture of microbial fuel cells (MFCs) because they do not require a constant flow of air in the cathode chamber [17]. This type of design was carried out due to the need to aerate the cathode when oxygen is used as the final electron acceptor; in some works, they do not use the proton exchange membranes, but they do use a porous cathode because they take advantage of the atmospheric oxygen to allow for the protons to diffuse through them [18,19].

On the other hand, the pitahaya (*Selenicereus undatus*), or “Dragon Fruit”, has increased its production and export due to the fact that the importing countries of this fruit, such as the United States, Canada, Japan, and the European Union, have managed to consume approximately 17,000 tons in 2018. The growth in demand for the fruit is mainly due to its components, which include glucose, betalains, vitamins, organic acids, soluble dietary fiber, phytoalbumins, and constituent minerals [20]. It has been reported that in 100 g of this fruit, there is water (87 g), protein (1.1 g), fat (0.4 g), fiber (3 g), carbohydrates (11 g), iron (1.9 mg), vitamin B1 (0.04 mg), vitamin B2 (0.05 mg), vitamin B3 (0.16 mg), vitamin C (20.5 mg), calcium (8.5 mg), and phosphorus (22.5 mg), which have appropriate properties for medical and diuretic uses for the benefit of people [21,22]. The increase in the consumption of this fruit has generated an increase in waste products, creating a great problem for farmers and companies dedicated to the export and import of the fruit [23,24]. The use of organic waste, mainly fruit and vegetable waste, as fuel creates a great opportunity for governments and companies to reuse their own waste, even more so if this technology can be scaled for large

quantities. For this, it is necessary to parameterize the chemical, physical, and biological values for optimal operation. Some encouraging reports have been found in the literature on other types of fruits; for example, Din et al. (2020) used potato waste as fuel in their single-chamber MFCs, generating a maximum peak voltage and an electrical current of 1.12 V and 12.45 mA, respectively, at a pH of 7 [25]. In the same way, banana waste has been used as fuel in single-chamber MFCs with zinc and copper electrodes, managing to generate peak voltages and currents of 1.01 ± 0.02 V and 3.72 ± 0.05 mA, respectively, while operating at a pH of 4.02 ± 0.06 . Likewise, the maximum power density value of 5736.11 ± 12.62 mW/cm² [26] was achieved. Similarly, Tremouli et al. (2019) managed to generate voltage peaks of 0.4 V at a pH of around 8 using fermentable domestic waste extracts as fuel in single-chamber MFCs, concluding that metal electrodes generate better electrical conductivity, which would produce voltage values and currents greater than those obtained with carbon or graphite electrodes [27]. In the reviewed literature, it is observed that the various microorganisms present on the electrodes play an important role in the generation of electric current; however, they all depend on the pH of the medium used for their operation in the MFCs [28,29].

Microorganisms are ubiquitous in nature and play an important role in the recycling of organic waste from different industries due to their ability to exchange electrons, which is a property exploited by MFCs [30]. The presence of these microorganisms in the substrates of MFCs is an important factor in generating electricity because it transforms the chemical energy contained in organic matter into electric current [31]. In recent years, new species that can generate an electric current in MFCs have been reported; however, few strains can generate power densities as high as those generated by mixed communities [32]. Typically, in MFCs, electrons travel from the anode to the cathode through a closed circuit, combining with protons to finally be captured by an electron acceptor (usually oxygen) and produce reduced compounds. These electrons are obtained when electrically active microorganisms carry out oxidation processes on organic matter [33,34]. The microorganisms that transfer electrons to solid anodes are also called exoelectrogens, and the microorganisms that accept electrons are called electrophytes [35]. The microorganisms grow within a matrix of polymeric substances, forming biofilms and settling on the anodes, where bioelectrochemical reactions occur [36]. The reason for using organic waste is to maintain a sustainable cycle where the waste is used as a fuel source to generate bioelectricity and, after the process, the supernatant can be used for other functions, such as compost or fertilizer, through composting or another method [37].

The lack of knowledge on the use of red dragon fruit waste as a fuel source and the ignorance of its electrochemical potential motivate us to obtain the first results on its use in the generation of electricity. The main objective of this research is to generate electrical energy using pitahaya (red dragon) residues as fuel in laboratory-scale single-chamber microbial fuel cells using zinc and copper as the electrodes. The pH values, electrical conductivity, Brix degrees, voltage, and electrical current were monitored for a period of 30 days. Additionally, the internal resistance, current density, and power density of the microbial fuel cells were measured. The values of the initial and final FTIR transmission spectra were also reported, as well as the identification of the microorganisms attached to the anodic biofilm at the molecular level. This research presents a novel way of generating electrical energy and observing the optimal operating parameters of this type of microbial fuel cell so that, in the near future, it can be scaled successfully, becoming a sustainable and usable technology for society.

2. Materials and Methods

2.1. Fabrication of Single-Chamber Microbial Fuel Cells (scMFC)

The MFCs were acquired from the manufacturer, SAIDKOCC (SAIDKOCC-10091720; Fujian, China). The anodic (copper—Cu; area 50 cm²) and cathodic (zinc—Zn; area 50 cm²) electrodes were placed on the inside and outside (one side of the electrode is in contact with the environment) of the MFCs, respectively. The electrodes were joined by a 6.0 mm copper

wire on the outside, forming an external circuit. The anodic and cathodic chambers were separated by a proton exchange membrane (Nafion 117; Wilmington, DE, USA), which was attached to the cathode electrode (Figure 1). The microbial fuel cells were fabricated in triplicate.



Figure 1. Experimental setup of MFC.

2.2. Obtaining and Processing the Red Dragon Fruit Waste

A total of 3.2 Kg of pitahaya waste was collected from the La Hermelinda Market (Trujillo, Peru). To remove any impurities (dust, insects, etc.), the pitahaya waste was washed four times with distilled water. Then, it was dried at room temperature (21 ± 2 °C) for 24 h. The juice (800 mL) of the pitahaya waste was obtained by an extractor (LDO-B-10; Labtron, Camberley, UK). The obtained juice was separated and poured into sterilized tanks, where it was kept until use.

2.3. Characterization of Microbial Fuel Cells

The physical parameters (voltage and electrical current) were measured by a multi-meter (Prasek Premium PR-85). Moreover, the electrical conductivity was obtained using a CD-4301 conductivity meter. The power density (PD) and current density (CD) were calculated according to Segundo et al. (2022) [38], where $DP = IV/A$ and $DC = I/A$, where I is the electric current, V is the voltage, and A is the area of the electrode. Additionally, the following external resistances were used: 1.3 ± 0.15 , 5 ± 0.25 , 10 ± 0.27 , 20 ± 2 , 50 ± 4.2 , 100 ± 8.2 , 220 ± 19 , 500 ± 21.5 , 800 ± 24.5 , and 1000 ± 29 Ω . Finally, an energy sensor (Vernier ± 30 V and ± 1000 mA) was used for the internal resistance (R_{int}) measurements of the MFCs. On the other hand, the chemical parameters, such as the pH and degrees Brix ($^{\circ}$ Brix), were measured using a 110 series Oakton pH meter and an RHB-32 Brix refractometer, and all of the measurements were evaluated for thirty days.

2.4. Isolation of Microorganisms from the Anode

Nutritive, McConkey, and Sabouraud agar were employed to isolate the possible electrogenic microorganisms. A swabbing of the anode surface (with microbial growth) was performed. Then, streaking in the culture medium was performed. The culture medium for the isolation of the bacteria was incubated at 35 °C for 24 h. Meanwhile, for the isolation of the fungi, the culture medium was incubated at 30 °C for 24 h. Pure cultures were performed and stored for each microorganism colony until use, and Gram and lactophenol stains were used to observe the microscopic characteristics.

2.5. Molecular Identification

The molecular identification was performed at the Laboratory of Integral Solutions Limited Liability Company (Peru). The CTAB method was performed for the DNA extraction and amplification using the PCR technique. The amplified fragments were sent for sequencing using the Sanger method. Next, the MEGA X program and BLAST software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 21 March 2023) were used to analyze the sequences. Finally, the identification of the microorganisms was possible using the percentage of identification in BLAST.

3. Results and Analysis

In Figure 2a, the voltage values obtained in the 30-day monitoring period of the MFCs show that they managed to generate the maximum voltage peaks of 0.46 ± 0.03 V on the tenth day, but their values kept decreasing until the last day of monitoring (0.25 ± 0.02 V). Increases (in the first weeks) and decreases (in the last weeks) in the voltage values have been reported in other investigations, attributing the increase in the values to the process of conditioning and proliferation of the microorganisms due to the high carbon content present in the waste, while the decreasing voltage values are attributed to the decrease in organic matter because the same matter is consumed by the microorganisms in the process of their metabolism [39–41]. Likewise, Figure 2b shows the electric current values during the monitoring days. As it can be seen, the values increase from the first day (2.13 ± 0.003 mA) to the tenth (2.86 ± 0.07 mA) and then slowly decay until the last day (2.05 ± 0.08 mA). The values obtained are higher than those shown by other researchers; for example, in their single-chamber MFCs, Yoshimura et al. (2018) managed to generate peaks of approximately 0.05, 0.15, and 400 mV using mud, rice, and mixed waste (mud and rice) as the substrates, respectively, attributing the increases in electric current to the assimilation of low-molecular-weight compounds (sugars and amino acids) present in the used waste [42]. In his research, Parkash A. (2018) managed to generate peaks of approximately 350 mV and 1.2 mA in his MFCs using sludge enriched with blend cultures of microorganisms, which worked at a pH of 7.8 [41]. On the other hand, Malik et al. (2021) concluded that by using a carbon-rich source as fuel in MFCs, the electric current values will increase. In their research, they managed to generate peaks of 6.47 mA and 0.647 V using food waste from hotels, houses, and restaurants as the substrate [43]. Likewise, it has also been found in the literature that metallic electrodes, such as Cu and Zn, have a high electrical conductivity due to their inherent properties of the same material, thus allowing the passage of electrons through the external circuit and generating higher voltage values and electric currents. Thus, the presence of galvanic reactions during the process of generating electric power is not ruled out since the presence of this reaction has been observed in other fruit waste used as fuel in MFCs [28,29].

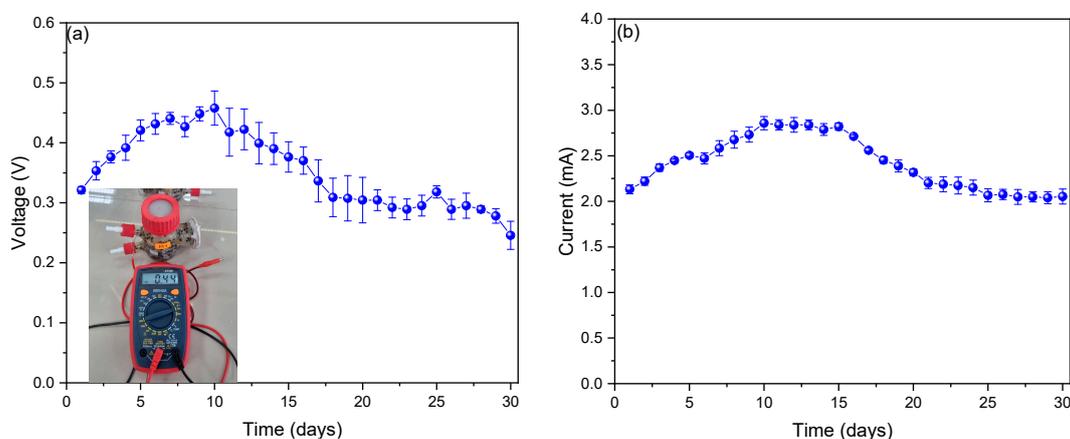


Figure 2. Values of (a) voltage and (b) electrical current of microbial fuel cells.

In Figure 3a, the pH values obtained from the monitoring period are shown, where it is observed that the values remain in the acidic and slightly neutral regimes, with an optimum operating pH of 4.22 ± 0.09 on the tenth day. Javed et al. (2021) mention the importance of pH in the generation of voltage in MFCs, considering a low or alkaline pH for the anodic chambers because using vegetable waste obtained better results at a low pH [44]. Likewise, it has been investigated that in papaya waste operating in MFCs, the optimal operating pH values are acidic, managing to generate voltage peaks of 1.1 V [45]. The importance lies in the fact that microorganisms need an adequate pH for their metabolism and growth, and for certain microorganisms reported in different investigations, the pH varies, which is why many investigations mention that the optimal pH will vary depending on the waste and type of MFC [46,47]. In Figure 3b, the electrical conductivity values of the MFCs are shown, where it can be seen that the values increased from the first day (117.29 ± 2.73 mS/cm) to the tenth day (175.86 ± 4.72 mS/cm) before decreasing until the last day (58.36 ± 4.51 mS/cm). Variations in electrical conductivity of the MFC have been reported by Stefanova et al. (2018) [48], who mention in their research that the variations are due to increases and decreases in the internal resistance of the MFCs, which were directly affected by the sedimentation process of the substrate used. Thus, it has also been shown that adding inorganic salts to the compound can increase the electrical parameters [48,49]. While Figure 3c shows the values of the °Brix observed, which remained constant for the first 3 days (14 °Brix) and then decreased, on day 7, where the voltage and electric current values were at their maximum, 11 °Brix were observed in the substrates of the MFCs, but on day 26, the values decreased to 0. It has been reported that fruits rich in galactose, glucose, sucrose, and organic acids (such as ascorbic and citric acid) are one of the main sources of energy that yeasts use for their growth [50,51]. These values serve to observe the amount of dry material in the waste, in this case, specifically the number of sugars, which were consumed by the microorganisms in the electricity generation process [52].

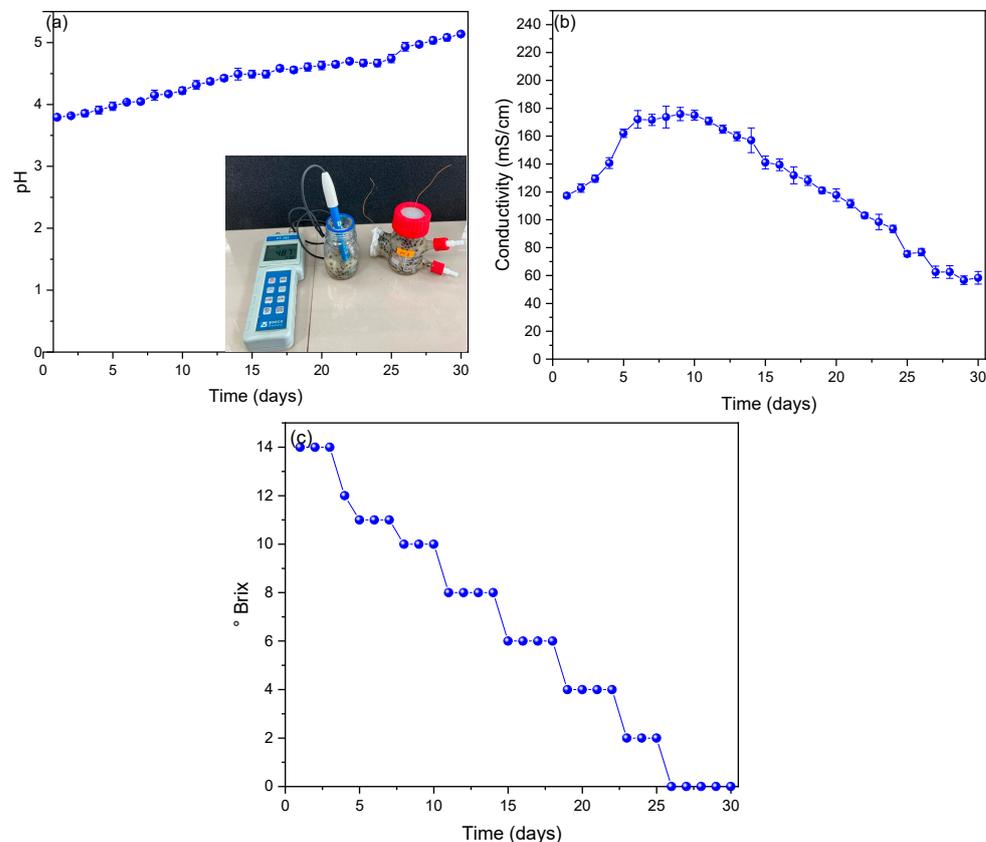


Figure 3. Monitoring of the values of (a) pH, (b) conductivity, and (c) Brix degrees of the microbial fuel cells.

In Figure 4a, the internal resistance (R_{int}) of the fuel cells is shown, for which Ohm's Law ($V = IR$) was used, where the voltage values were placed on the "Y" axis and those of the electric current on the "X" axis; in this way, the slope of the linear adjustment is the internal resistance of the MFCs. The value of the calculated internal resistance was $75.58 \pm 5.89 \Omega$; this value was calculated at the maximum peak of the voltage and electric current generation (on the seventh day). These resistance values were low compared to other investigations; for example, it has been reported that in MFCs with domestic water waste, the reported resistance was $256.81 \pm 3.4 \Omega$ (for a 3 mm electrode), but it can decrease by varying the thickness of the electrode [53]. Likewise, it has been reported that when manufacturing a carbon electrode with metallic nanoparticles, the internal resistance values decrease considerably. Ali et al. (2020) manufactured graphite electrodes with Fe nanoparticles, achieving an internal resistance of 3.2Ω [54]. On the other hand, Ullah et al. (2020) used synthetic waste with different glucose percentages in their MFCs as the substrates, obtaining an internal resistance of 301Ω for the highest glucose concentration, which reported the lowest R_{int} . [55]. According to the literature, metallic materials can help to generate high values of voltage and current by their very nature in the initial stage, but they play a role against them in the final stage due to the fact that the electrode used was copper and this material has a toxic effect on bacteria, which could explain the decrease in the values of the electrical parameters in the final stage [56,57].

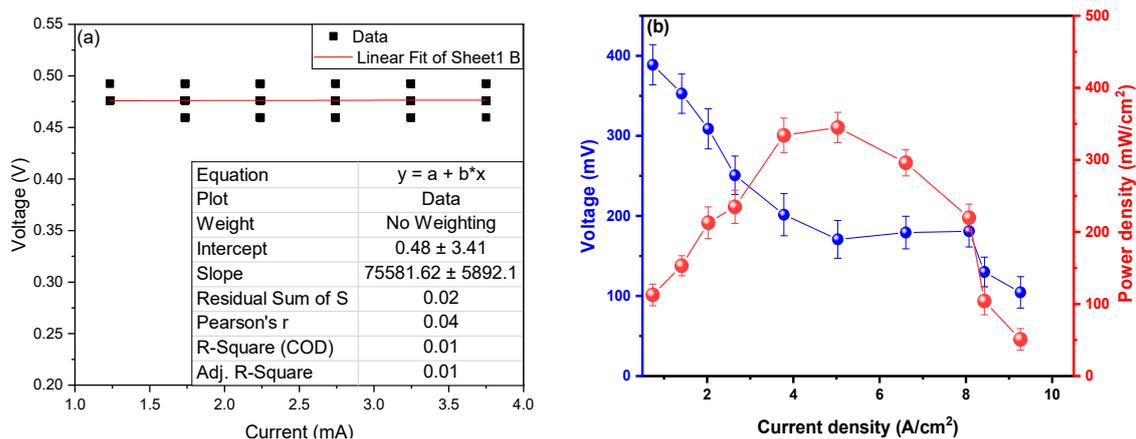


Figure 4. Values of (a) internal resistance and (b) power density as a function of current density.

In Figure 4b, the power density (PD) values are shown as a function of the current density (CD). The PD max. was $304.325 \pm 16.51 \text{ mW/cm}^2$ at 5.06 A/cm^2 DC, with a peak voltage of $387.27 \pm 11.87 \text{ mV}$. The PD values shown are relatively higher than those shown in other works; for example, Kebaili et al. (2021) managed to generate maximum PD peaks of 0.180 W/cm^2 in their single-chamber MFCs using leachate as the substrate [58]. Likewise, the research carried out by Gautam et al. (2021) managed to generate $2400 \pm 110 \text{ W/cm}^2$ in their MFCs using sewage sludge, compost leachate, and mess food waste and graphite rod electrodes as the substrate [59]. The high values obtained in the investigation may be due to the electrodes used, which, due to their metallic characteristics, have a high electrical conductivity, facilitating the passage of electrons through the entire electrode area [60,61].

The initial and final FTIR transmittance spectra of the used substrate are shown in Figure 5, where it can be seen that the most intense peak at 3331 cm^{-1} belongs to the O–H bonds, while the peaks at 2969 and 2805 cm^{-1} belong to the alkane (C–H) bonds; similarly, the 1686 cm^{-1} peak shows the presence of alkene compounds (C=C), and the 1496 and 968 cm^{-1} peaks show the presence of NO_2 and C–H bonds [62–64]. In addition, a decrease in the observed transmittance peaks is observed, which is due to the fact that the compounds are consumed in the metabolism of the microorganisms present in the waste during the processes of bioelectricity generation, fermentation, and degradation [65,66].

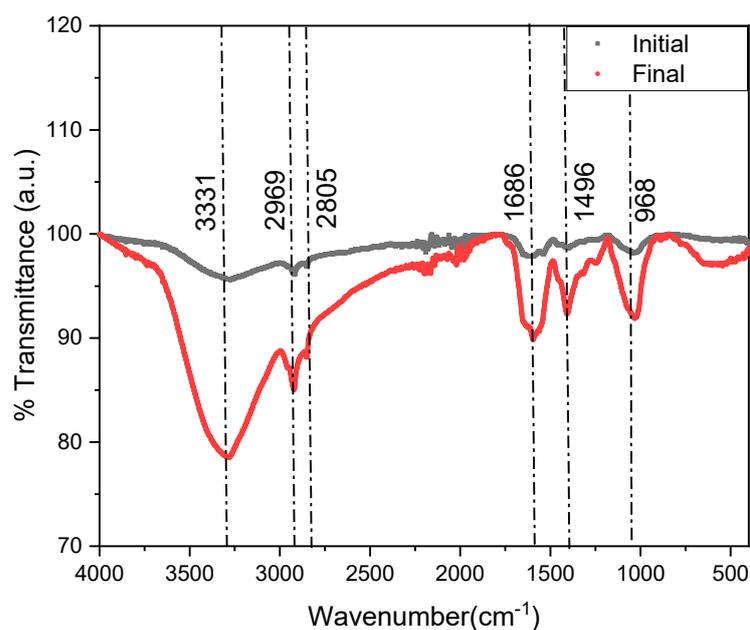


Figure 5. Initial and final transmittance spectra of pitahaya debris by FTIR.

Molecular identification was performed only for the anodic electrode because the resulting cathodic electrode would be damaged due to corrosion caused by the reactions that occurred within the MFCs for the generation of bioelectricity. Table 1 shows the yeast that was identified using molecular biology. This was possible through ITS (internal transcribed spacer) sequences [67]. In the BLAST analysis, *Geotrichum candidum* was identified with 99.59% of the identity percentage. This species is considered a yeast-like fungus or mold and can be isolated from plants, soil, fruits, etc. [68–70]. Only this species was isolated from the anode, probably due to some factors, such as the antibacterial activity of the anode (copper) and red dragon fruit (pitahaya) [71–73]. Another study showed *Saccharomyces cerevisiae* (yeast) growth in an MFC with an anode based on copper [74]. Concerning the electron transfers to the anode, some fungi are known to require electron mediators. However, there is evidence that some yeasts transmit electrons directly to the anode via redox enzymes located in the membranes [75–79]. On the other hand, a study reported the use of *Geotrichum candidum* and other species for the generation of electricity in MFCs, generating voltage peaks between 182.5 and 192.5 V and currents between 0.3 and 0.53 A, demonstrating that anaerobic microorganisms are capable of producing electricity under suitable conditions [80].

Table 1. Species identified from the anode of the MFCs with red dragon fruit waste.

Organic Waste	Identified Species	bp	Identity (%)	Access Number	Lineage
Pitahaya	<i>Geotrichum candidum</i>	243	99.59	MK381259.1	cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Saccharomycotina; Saccharomycetes; Saccharomycetales; Dipodascaceae; Geotrichum

In particular, there are some challenges. Only microorganisms that can grow on agar-based media were considered in this investigation. However, in future research, the involvement of other microbes that cannot grow in the culture media used must be considered. On the other hand, the use of another type of MFC, such as MFC type H, should also be considered.

4. Conclusions

It was possible to successfully generate bioelectricity through microbial fuel cells on a laboratory scale using dragon fruit waste as the fuel and zinc and copper as the electrodes, managing to generate electrical current and voltage peaks of 2.86 ± 0.07 mA and 0.46 ± 0.03 V, respectively, on the tenth day. The substrates used were operating in an acid regime with an optimum pH of 4.22 ± 0.09 , while their electrical conductivity was 175.86 ± 4.72 mS/cm and 8 °Brix. On the other hand, an internal resistance of 75.58 ± 5.89 Ω was calculated with a maximum power density of 304.33 ± 16.51 mW/cm² at a current density of 5.06 A/cm², while the FTIR spectrum showed a decrease in the final spectrum with respect to the initial one due to the consumption of the components by parts of the microorganisms present in the substrate. Likewise, it was possible to molecularly identify the yeast-like fungus *Geotrichum candidum* with a 99.59% identity (ID: MK381259.1).

For future work, the incorporation of metallic nanoparticles into porous carbon electrodes should be investigated to improve their electrical conductivity, including the type of electrode material, which must be economical, have good conductivity, and not be toxic to the electrogenic microorganisms. On the other hand, microbial biocatalysts should be used on other types of fruit and vegetable waste to increase the effectiveness of the MFCs, and the chemical-physical parameters (pH and temperature) should be standardized to increase the generation of electrical current in the MFCs.

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