

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

Rojas-Flores Segundo ^{1,*}, Santiago M. Benites ², De La Cruz-Noriega Magaly ², Juan Vives-Garnique ², Nélida Milly Otiniano ³, Walter Rojas-Villacorta ⁴, Moisés Gallozzo-Cardenas ⁵, Daniel Delfín-Narciso ⁶ and Félix Díaz ⁷

¹ Escuela de Ingeniería Mecánica Eléctrica, Universidad Señor de Sipán, Chiclayo 14000, Peru; segundo.rojas.89@gmail.com; jvives@crece.uss.edu.pe

² Vicerrectorado de Investigación, Universidad Autónoma del Perú, Lima 15842, Peru; santiago.benites@autonoma.pe; mdelacruz@autonoma.edu.pe

³ Instituto de Investigación en Ciencias y Tecnología de la Universidad Cesar Vallejo, Trujillo 13001, Peru; notiniano@ucv.edu.pe

⁴ Escuela de Medicina, Universidad Cesar Vallejo, Trujillo 13001, Peru; wrojasv@ucv.edu.pe

⁵ Departamento de Ciencias, Universidad Tecnológica del Perú, Trujillo 13011, Peru; c21228@utp.edu.pe

⁶ Grupo de Investigación en Ciencias Aplicadas y Nuevas Tecnologías, Universidad Privada del Norte, Trujillo 13007, Peru, daniel.delfin@upn.edu.pe

⁷ Escuela Académica Profesional de Medicina Humana, Universidad Norbert Wiener, Lima 15046, Peru; felix.diaz@uwiner.edu.pe

* Correspondence: segundo.rojas.89@gmail.com

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 waste. Reason why, this research aims to generate electricity in an eco-friendly way using red dragon fruit (pitahaya) waste as fuel in single-chamber microbial fuel 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 175.86 ± 4.72 mS/cm of electrical conductivity at 8 ° Brix to the tenth day of monitoring. Thus, an internal resistance of $75.58 \pm 5.89 \Omega$ 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 show 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.

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 have experienced this pollution the most is China, where waste expels approximately 11% of greenhouse gases into the environment; while countries such as the United States, Finland, Germany, the Netherlands, China, Korea, Japan, and South Africa have begun to develop technologies to generate products with the different residues of 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 in good condition, generating large amounts of losses for companies, which, by not having a due process for its reuse 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 or 50% is food waste belonging to the human diet, whose collection of these types of waste varies depending on the country and the policies of each one, but in general among the most common methods are organic waste treatment plants, suspension of biological pollutants and separation at Origin for Drop-Off Collection; but these systems are very expensive and a necessary good at the same time [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 composting or for the production of animal feed, but this has evolved significantly, due to the emergence of new techniques with the help of technology giving agro-industrial residues an added value; making them a sustainable and friendly resource with 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 being 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 60s, has begun to take center stage because their technology allows the use of different types of waste as fuel to generate bioelectricity [12]. Due to its great potential, it has a large number of designs, but in general, it consists of two chambers (anodic and cathodic) almost always separated by a proton exchange membrane and inside each chamber with electrodes 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 protons 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 generating 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, it was observed that in several investigations it has been shown to generate higher power densities than microbial fuel cells single chamber, as well as the reduction of internal resistance due to the direct contact of the cathode electrode with air (O₂) and the reduction of costs for the manufacture of the microbial fuel cell (MFC) because it does 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 membrane, but they do use a porous cathode because they take advantage of atmospheric oxygen to allow for 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 importing countries of this fruit such as the United States, Canada, Japan, and the European Union have managed to consume approximately 17 thousand tons in 2018; the growth in demand of the fruit is mainly due to the components it contains, for example, glucose, betalains, vitamins, organic acids, soluble dietary fiber, phytoalbumins and constituent minerals [20,21]. It has been reported that in 100 gr of this fruit there is approximately water (87 gr), protein (1.1g), fat (0.4g), fiber (3 g), carbohydrate (11g), 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); appropriate properties for medical and diuretic use for the benefit of people [22]. The increase in the consumption of this fruit has generated an increase in waste products, generating a great problem for farmers and companies dedicated to the export and import of 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 scalable 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 maximum peak voltage and electrical current of 1.12 V and 12.45 mA 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 operating at a pH of 4.02 ± 0.06 . Likewise, maximum power density values of 5736.11 ± 12.62 mW/cm² [26] were achieved. So too, Tremouli et al. (2019) managed to generate voltage peaks of 0.4 V at a pH of around 8, using fermentable domestic waste extract as fuel in single-chamber MFCs, concluding that metal electrodes generate better electrical conductivity, which would produce voltage values and current 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 the MFCs is an important factor to generate electricity because it transforms 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 are the strains that can generate a power density 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 of organic matter [33,34]. These microorganisms that transfer electrons to a solid anode are also called exoelectrogens, and microorganisms that accept electrons are called electrophytes [35]. Microorganisms grow within a matrix with polymeric substances forming biofilms and settling on the anodes where bioelectrochemical reactions occur [36]. The sense of 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 use the supernatant for some other function, such as compost or fertilizer through composting or another method [37].

The non-existent knowledge about the use of Red Dragon waste as fuel sources and its ignorance of its electrochemical potential motivates us to obtain the first results on its use in electricity generation. 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 electrodes as electrodes. The pH values, electrical conductivity, Brix degrees, voltage and electrical current were monitored for a period of 30 days. Also, 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 microorganisms attached to the anodic biofilm at the molecular level. This research presents a novel way of generating electrical energy, observing the optimal operating parameters of this type of microbial fuel cell so that in the near future it can scale 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 was 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 inside and outside (one side of the electrode in contact with the environment) of the MFC, 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, USA), which was attached to the cathode electrode (Figure 1), where 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 total was collected from La Hermelinda market (Trujillo, Peru). To remove impurities (dust, Insects, etc.), the pitahaya waste was washed four times with distilled water. Then they were dried at room temperature (21 ± 2 °C) for 24 hours. Juice (800 mL) of pitahaya waste was obtained by an extractor (Labtron, LDO-B-10-Camberley, Uk), this obtained juice was separated and poured into sterilized tanks where it was kept until its use.

2.3. Characterization of Microbial Fuel Cells

Physical parameters (voltage and electrical current) were measured by a multimeter (Prasek Premium PR-85). Moreover, the electrical conductivity was obtained by 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 the voltage and A is the area of the electrode; thus, 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}) measure of the MFCs. On the other hand, chemical parameters like pH and degrees Brix (°Brix) were measured with pH meter 110 series Oakton and RHB-Refractometer/32 brix, respectively, and all 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 a streaking in the culture media was made. The culture media for bacteria isolating was incubated at 35°C for 24 hours. Meanwhile, for the isolation of fungi, the culture media was incubated at 30°C for 24 hours. A pure culture was performed and stored for each colony of microorganism until use Gram and Lactophenol stains were necessary to observe microscopic characteristics.

2.5. Molecular Identification

The molecular identification was performed in the Laboratory of Integral Solutions Limited Liability Company (Peru). The CTAB method was made for DNA extraction and amplification was employed the PCR technique. The amplified fragments were sent to sequence by the Sanger method. After the MEGA X program and Blast software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) were necessary to analyze the sequences. Finally, the identification of the microorganisms was possible by the percentage of identification in Blast.

3. Results and Analysis

In Figure 2 (a) the voltage values obtained in the 30-day monitoring of the MFCs can be observed, managing to generate maximum voltage peaks of 0.46 ± 0.03 V on the tenth day, whose values decreased until the last day of monitoring (0.25 ± 0.02 V). The increase (in the first weeks) and decrease (in the last weeks) of the voltage values have been reported in other investigations, attributing the increase in values to the process of conditioning and proliferation of microorganisms due to the high carbon content present in the waste, while decreasing voltage values decrease organic matter because the same matter is consumed by microorganisms in the process of their metabolism [39–41]. Likewise, Figure 2 (b) shows the values of electric current during the monitoring days, being able to observe that the values increase from the first day (2.13 ± 0.003 mA) to the tenth (2.86 ± 0.07 mA) for then slowly decay until the last day (2.05 ± 0.08 mA). The values obtained are higher than those shown by researchers, for example, Yoshimura et al. (2018) in their single-chamber MFCs managed to generate peaks of approximately 0.05, 0.15 and 400 mV, using mud, rice, and both mixed waste (mud and rice) as substrate, respectively; attributing the increases in electric current to the assimilation of low molecular weight compounds (sugars and amino acids) present in 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 the 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 a substrate [43].

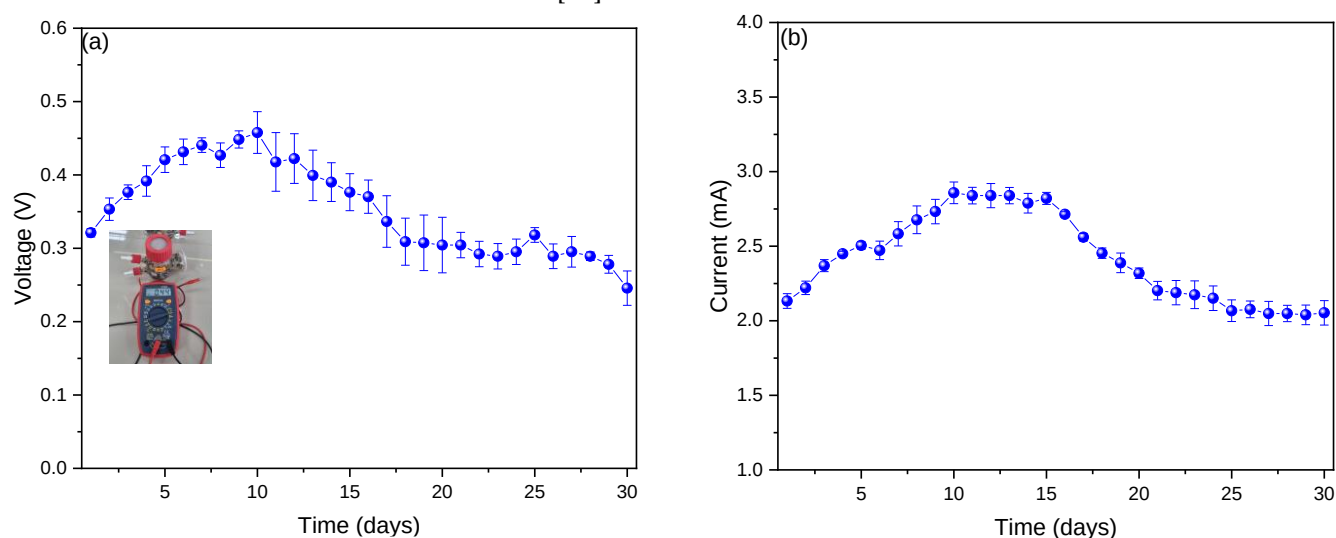
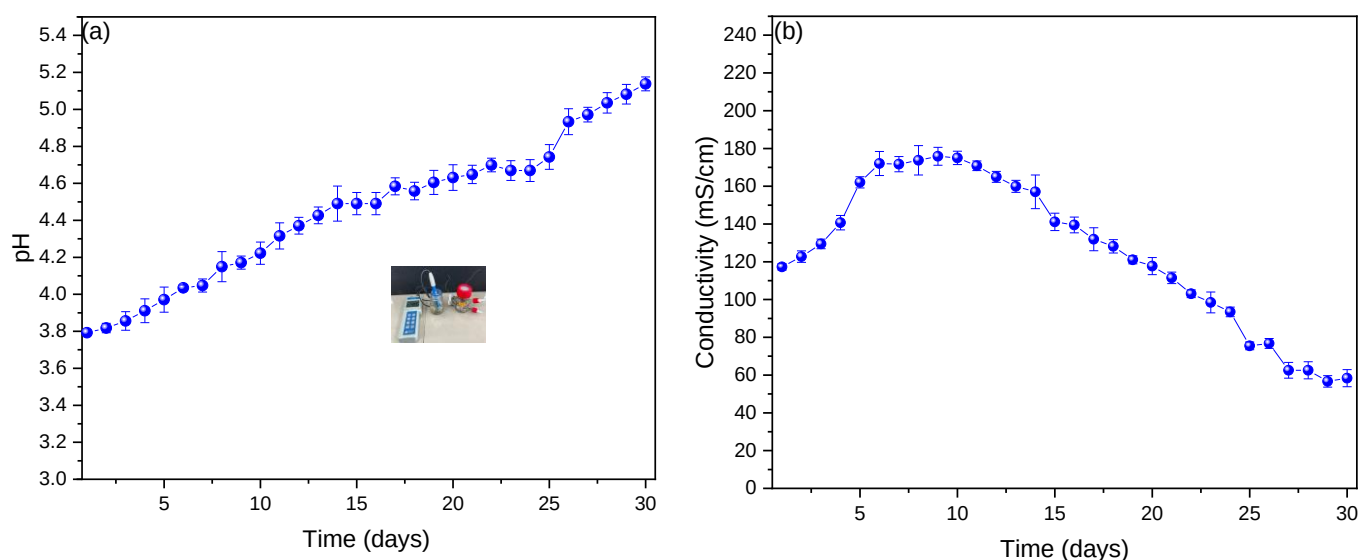


Figure 2. Values of (a) voltage and (b) electrical current of microbial fuel cells.

In Figure 3 (a) the pH values obtained from the monitoring carried out are observed, where it is observed that the values remain in the acidic and slightly neutral regime, with its 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 low or alkaline pH for the anodic chambers because using vegetable waste obtained better results at low pH

[44]. Likewise, it has been investigated that in papaya waste operating in MFC, the optimal operating values are acidic pH, managing to generate voltage peaks of 1.1 V [45]. The importance lies in the fact that microorganisms need 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 for waste and type of MFC, the optimal pH will vary [46,47]. In Figure 3 (b) the values of the electrical conductivity of the MFCs are shown, managing to observe that the values increased from day 1 (117.29 ± 2.73 mS/cm) to the tenth day (175.86 ± 4.72 mS/cm). for after falling until the last day (58.36 ± 4.51 mS/cm). Variations in electrical conductivity in the MFC have been reported by Stefanova et al. (2018), who mention in their research that the variations are due to increases and decrease 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 3 (c) shows the values of the °Brix observed, which remained constant the first 3 days (14 °Brix) and then decreased, on the seventh day, where the values of voltage and electric current were maximum, 11 °Brix were observed in the substrates of the MFCs, but on day 26 the values decreased to zero. 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 microorganisms in the electricity generation process [52].



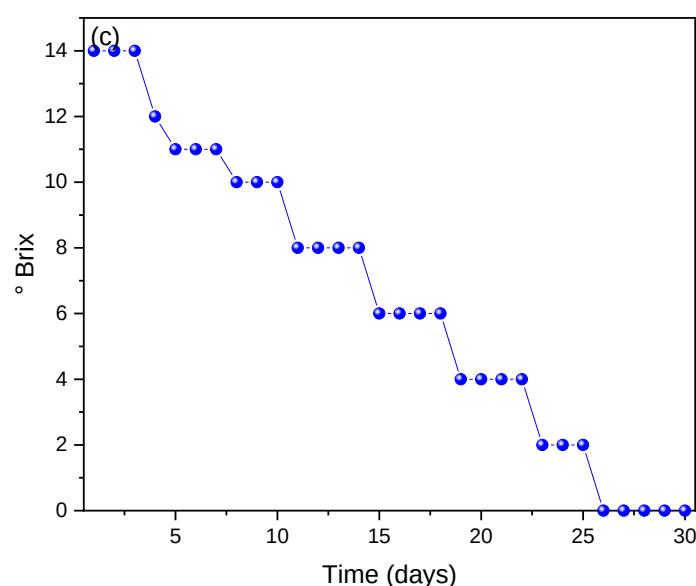


Figure 3. Monitoring of the values of (a) pH, (b) conductivity and (c) Brix degrees of the microbial fuel cells.

In Figure 4 (a) 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 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 voltage and electric current generation (seventh day). These resistance values found 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 3mm 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 wastes with different glucose percentages in their MFCs as a substrate, 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, they play a role against it in the final stage, due to the fact that the electrode used was copper, 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].

In Figure 4(b) the power density (PD) values are shown as a function of current density (CD), the PD max. found was $304.325 \pm 16.51 \text{ mW/cm}^2$ at $5,06 \text{ A/cm}^2$ 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 a 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 rods electrodes as substrate [59]. The high values obtained in the investigation may be due to the electrodes used, due to their metallic characteristic, it has a high electrical conductivity; facilitating the passage of the electrodes through the entire electrode area [60,61].

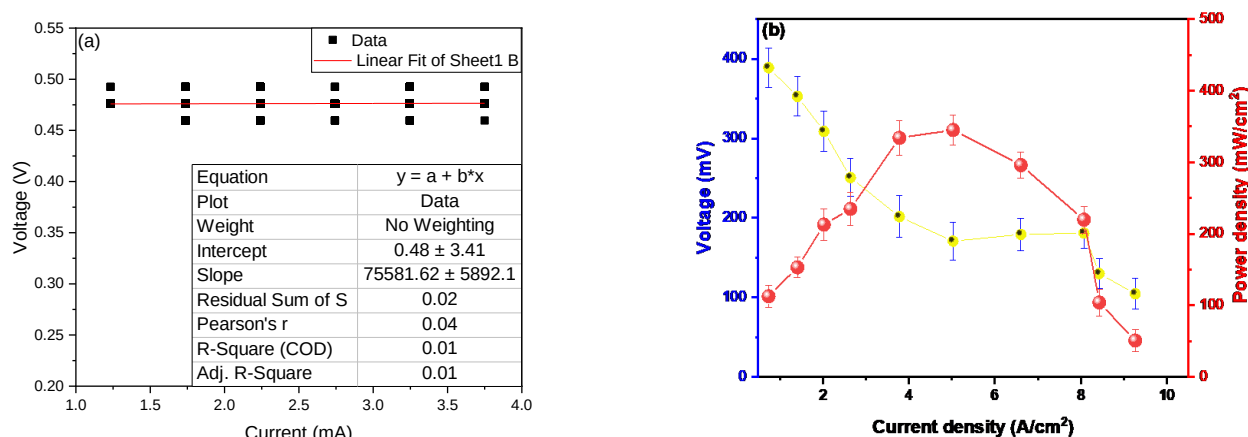


Figure 4. Values of (a) internal resistance and (b) power density as a function of current density.

The initial and final FTIR transmittance spectra of the used substrate are shown in Figure 5; being able to observe the most intense peak at 3331 cm^{-1} belonging to the O-H bonds, while the peak at 2969 and 2805 cm^{-1} belongs to the alkanes (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 in the process of bioelectricity generation, fermentation, and degradation [65,66].

Table 1 shows the yeast identified by molecular biology. This was possible through ITS sequences (Internal Transcribed Spacer) [67]. In 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 specie was isolated from the anode due to some factors, probably, for example, 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 electron transferences to the anode, some fungi, are known to require electron mediators. However, there is evidence of some yeasts transmitting 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 an MFC, generating a voltage peak between 182.5 and 192.5 V and a current between 0.3 and 0.53 A, demonstrating that anaerobic microorganisms are capable of producing electricity under suitable conditions [80].

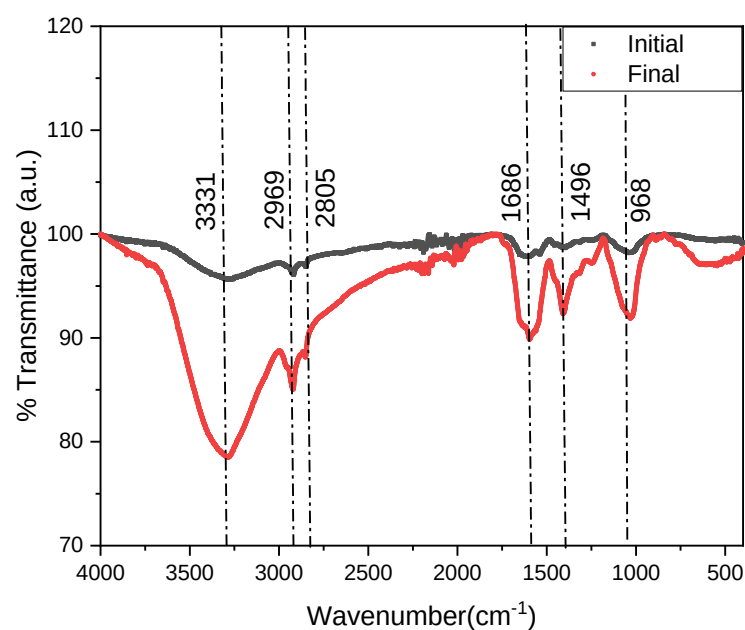


Figure 5. Initial and final transmittance spectrum of Pitahaya debris by FTIR.

Table 1. Specie 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 ; Saccharomycetes ; Saccharomycotina ; 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 must be considered. On the other hand, 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 fuel and zinc and copper as electrodes. Managing to generate electrical current and voltage peaks of 2.86 ± 0.07 mA and 0.46 ± 0.03 V 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 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 works, the incorporation of metallic nanoparticles into porous carbon electrodes should be investigated to improve their electrical conductivity, as well as the type of electrode material, which must be economical, have good conductivity and not to be

toxic for electrogenic microorganisms. On the other hand, the use of microbial biocatalysts is being intensely investigated in order to increase the electrical parameters and standardize chemical-physical parameters (pH and temperature) to increase the generation of electrical current in MFCs should be investigated.

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