


Article

Golden Berry Waste for Electricity Generation

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Abstract: The environmental problems caused by the excessive use of fossil fuels for electricity generation have led to the development of new technologies. Microbial fuel cells constitute a technology that uses organic sources for electricity generation. This research gives a novel means of using Golden Berry waste as fuel for electricity generation through microbial fuel cells made at low cost, achieving current and voltage peaks of 4.945 ± 0.150 mA and 1.03 ± 0.02 V, respectively. Conductivity values increased up to 148 ± 1 mS/cm and pH increased up to 8.04 ± 0.12 on the last day. The internal resistance of cells was 194.04 ± 0.0471 Ω , while power density was 62.5 ± 2 mW/cm² at a current density of 0.049 A/cm². Transmittance peaks of the Fourier-transform infrared (FTIR) spectrum showed a decrease when comparing the initial and final spectra, while the bacterium *Stenotrophomonas maltophilia* was molecularly identified with an identity percentage of 99.93%. The three cells connected in series managed to generate 2.90 V, enough to turn on a TV remote control. This research has great potential to be scalable if it is possible to increase the electrical parameters, generating great benefits for companies, farmers, and the population involved in the production and marketing of this fruit.

Keywords: waste organic; golden berry; bioelectricity; generation; electricity



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1. Introduction

The generation of bioelectricity from organic waste solves several critical problems in today's society, such as organic waste pollution, global warming, acid rain, haze, and the lack of electricity in remote cities [1]. Due to this, a great variety of emerging technologies have been developed in the last few decades to provide a solution, among which are gasification, co-combustion, solar energy, biomass energy, etc. [2]. A potential solution to this is the use of microbial fuel cells (MFCs), taking organic waste as a substrate [3]. MFC is a technology that uses the chemical energy of the waste to convert it into electrical energy. It is generally made up of two chambers (anode and cathode), almost always separated by a proton exchange membrane, where the anode electrode collects the electrons generated due to the oxidation of the substrate and, through an external circuit, they travel to the anode electrode, generating an electron flow (electric current) [4,5]. There are different types of MFCs, with the single-chamber MFC being the most promising one, because the operation is simplified since it does not require chemical regeneration of the catholyte. Moreover, a higher volumetric power density is obtained due to the smaller cell volume, and it can operate, in some cases, without proton exchange membranes. All this minimizes the manufacturing cost of this type of cell [6,7].

Physalis peruviana L. is known as Golden Berry (GB) in English-speaking countries [8]. This fruit has excellent nutritional and bioactive properties, and its popularity in medicine has increased exponentially in recent years due to its anti-asthmatic, diuretic, antiseptic, anti-inflammatory, anti-proliferative, sedative, analgesic, and anti-diabetic uses [9]. Although it was initially marketed as a fresh product, it has begun to be used in syrups and jams or dehydrated (similar to raisins) for use in bakeries, cocktails, etc. [10–12]. According to Vega et al. (2020), the export of this fruit between 2015 and 2016 increased by 160%, and these figures continue to increase due to the high demand from Asian and European countries [13]. Because of this, many agro-industrial companies have begun to produce this fruit, generating waste in the process, which can be used as fuel in MFCs. Rahman et al. (2021) used mango, banana, and orange waste in single-chamber MFCs, generating voltage peaks of approximately 357 mV for the cell with the orange substrate; the cells with mango generated the lowest voltage (100 mV) [14]. Similarly, Kebaili et al. (2021) used fruit waste leachate in MFCs, achieving approximately 240 mV in the cell with anaerobiosis and a maximum power density of 80 mW/cm² on day 21 [15]. Dhulipala et al. (2020) used kitchen food waste in MFCs whose external resistance was 100 Ω, achieving power generation of 0.47 V and a power density of 0.040 W/m² on the eighth day, maintaining a constant pH of 7 [16]. Likewise, Ramadan et al. (2020) created MFCs in the absence of a proton-exchange membrane with aeration in the cathodic chamber and used agro-industrial waste as a substrate, managing to generate maximum peaks of 890 mV on day 24 with an external resistance of 500 Ω and a power density of 140 mW/m² [17].

In this sense, the main objective of this research was to generate bioelectricity by using GB waste (substrate) in a single-chamber microbial fuel cell created at low cost, which was monitored for 35 days. The parameters monitored were the generated values of voltage, current, pH, conductivity, degrees Brix, and conductivity. In the same way, the values of power density, current density, internal resistance, and energy of microbial fuel cells were determined. The initial and final substrates were also characterized by Fourier-transform infrared spectroscopy (FTIR), and the electrogenic bacteria adhered to the anode were identified molecularly. In this way, added value can be given to the waste of this type of fruit, generating a good, such as electricity generation, for the benefit of companies or farmers dedicated to the harvest and sale of GB, in a manner that is beneficial to the environment.

2. Materials and Methods

2.1. Construction of Microbial Fuel Cells

Single-chamber microbial fuel cells (three in total) were created by using zinc (Zn— anode) and copper (Cu—cathode) electrodes with 78.54 cm² of area and 0.05 cm of thickness, while a polymethylmethacrylate tube of 30 cm × 5 cm in length and radius, respectively, was used as a container for the GB waste (substrate). The anode was placed inside and the cathode was placed at one end of the chamber; one side of the cathode was in contact with the environment, in the absence of the proton-exchange membrane. The electrodes were joined with copper wire (12 mm diameter) by means of an external circuit with an external resistor of 1000 Ω; see Figure 1.

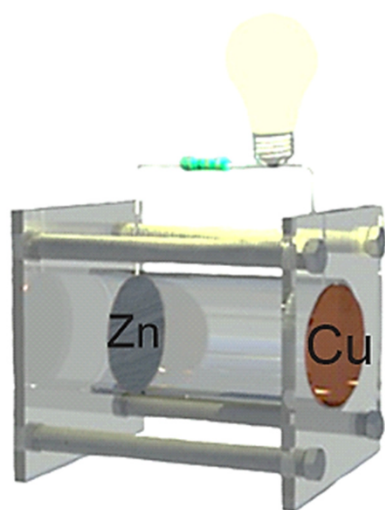


Figure 1. Scheme of single-chamber microbial fuel cell.

2.2. Collection and Preparation of Golden Berry Waste

The substrates (fuel) were collected from Golden Berry waste from La Hermelinda market, Trujillo, Peru. This waste was washed with distilled water three times to remove any impurities from the medium (dust, insects, among others), and then dried at 23 ± 2 °C in a LabTron oven (LDO-B10, Basingstoke, UK) for 24 h. In total, 4 kg of all GB waste was crushed in an extractor (Maqorito, CS600, Lima, Peru) to obtain approximately 1.5 L of waste extract, which was placed in bottles (1.5 L, pyrex) that were properly sterilized until their use in the MFCs. Each MFC contained 400 mL of GB waste, to which no more substrate was added during the whole monitoring process, and the chamber orifice was sealed with a cap to create anaerobic conditions.

2.3. Isolation of Electrogenic Microorganisms from the Anode Chamber

To isolate the electrogenic microorganisms, the anode plate was swabbed and diluted in tubes with BHI broth and then samples were placed in Petri dishes by using the stretching technique in Sabouraud Agar, Mac Conkey Agar, and Nutrient Agar culture media. They were incubated at temperatures of 30° for yeasts and 35 and 44.5 °C (for the isolation of total coliforms). The procedure for the isolation of microorganisms was carried out in duplicate [18].

2.4. Molecular Identification of Bacteria

Molecular identification was performed by the Analysis and Research Center of Biodes laboratory, for which pure cultures of the isolated bacterial strains were sent. The protocol for molecular identification was proposed by Rojas-Flores et al. (2021) [18], based on 16S rRNA analysis, which is specifically used for bacteria [19].

2.5. Physicochemical Characterization of MFCs

Voltage and current values in MFCs were monitored for 35 days by using a multi-meter (Prasek Premium PR-85, Lima, Peru), with an external resistor of 1000 Ω , whose values were taken at 10 am every day. Power density (PD) and current density (CD) values were calculated by using the formula of Rojas-Flores et al. (2021) with external resistors of 0.2 (± 0.1), 0.6 (± 0.18), 1 (± 0.3), 1.5 (± 0.31), 3 (± 0.6), 10 (± 1.3), 20 (± 6.5), 50 (± 8.7), 60 (± 8.2), 100 (± 9.3), 120 (± 9.8), 220 (± 13), 280 (± 21.4), 350 (± 31.5), 390 (± 24.5), 460 (± 23.1), 531 (± 26.8), 700 (± 40.5), 1200 (± 90.2) Ω [18]. Conductivity (conductivity meter, Lutron, CD-4301, Taiwan, China), pH (pH-meter, Oakton, 110 Series, Vernon Hills, IL, USA), and brix values (brix refractometer, Yhequipment Co., RHB-32, Shenzhen, China) were monitored during the 35 days. Transmittance spectra were measured by Fourier-transform infrared spectroscopy (FTIR) (Thermo Scientific, IS50, Waltham, MA, USA) and an energy

sensor (Vernier- ± 30 V & ± 1000 mA, Beaverton, OR, USA) was used to obtain internal resistance values.

3. Results and Analysis

Voltage values generated are shown in Figure 2a, where it is possible to observe the high values generated in the first few days, reaching a maximum peak of 1.03 ± 0.02 V on the fifth day; then, they decreased slowly until the last day (0.554 ± 0.055 V). The rapid generation of voltage in the initial several days was due to the bacteria and chemical compositions of the solutions present in the system, which needed some time to form the electroactive biofilm on the anode electrode [20,21]. According to Ma et al. (2020), voltage values gradually decrease in this type of substrate, because biodegradable components are easily exhausted, and the components that are more difficult to decompose are used to produce electricity at the end of the process [22]. These results are better than those obtained by Prasadha W. (2020), in which the author used food waste (fruit and vegetable waste) as leached fuel, obtaining peak voltages of 404 mV on the eighth day, and then they slowly decayed until the last day of monitoring; he attributed this phenomenon to the fact that the bacteria began to die due to nutrient depletion [23].

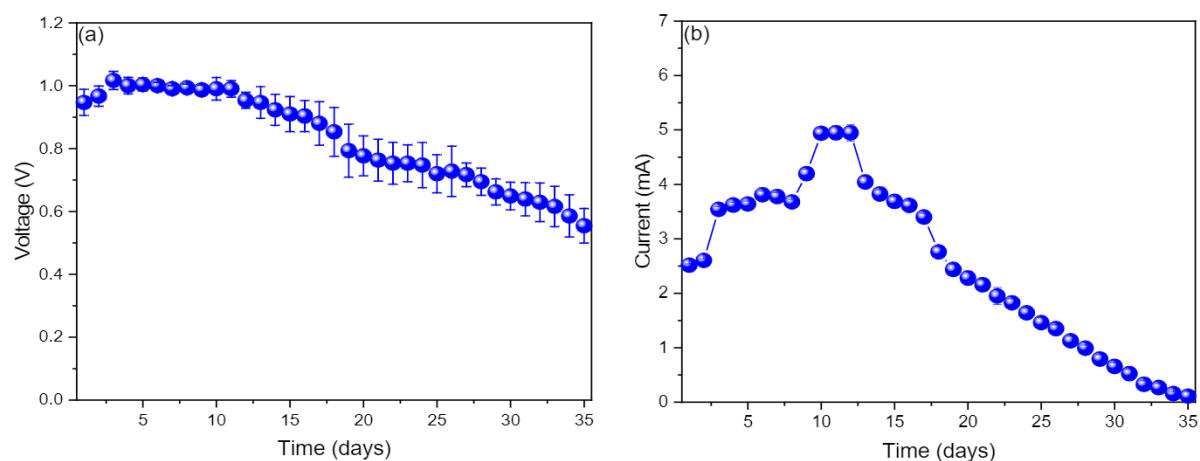


Figure 2. Values of (a) voltage and (b) current generated during the 35 days of monitoring.

Figure 2b shows the current values generated during the monitoring period. As can be observed, on the first day, the current was 2.516 ± 0.02517 mA and then it increased rapidly to 4.945 ± 0.150 mA on the twelfth day; then, it gradually decreased until the last day (0.11 ± 0.0854 mA). The high electric current values may be due to the fact that single-chamber MFCs without a membrane help the biofilm to homogenize on the electrodes because the active live microbes can move freely through the liquid of both electrodes [24]. This is important because the anodic biofilm is responsible for producing electrons, protons, and CO_2 [25]. In the same way, the use of metallic electrodes helps to improve the electrical conductivity of the electrode and to improve electron transfer [26].

Conductivity values are shown in Figure 3a, which increased from the first day (148 ± 1 mS/cm) to the eighth day (179.33 ± 2.08 mS/cm), and then they decreased continuously until the last day of (22.34 ± 3.06 mS/cm) monitoring. The high values of electrical conductivity of the substrate in the initial several days demonstrate its low internal resistance and explain the high values of current shown [27]. Meanwhile, the decrease in this parameter may be due to the sedimentation of organic compounds from the substrate at the bottom of the anode chamber in the last few days of monitoring [28]. Figure 3b shows the values of degrees Brix ($^\circ\text{Brix}$) observed during the 35 days of monitoring, which, from the first to the fourth day, showed constant values (10 $^\circ\text{Brix}$) for all cells, and then they declined to zero on the twenty-sixth day. After this, there were no variations until the last day of monitoring. It is worth mentioning that Golden Berry contains 78.9% moisture and 15% soluble solids (mainly sugars), such as fructose and glucose [29], using it as a carbon

source for the growth of bacterium *Stenotrophomonas maltophilia* [30]. pH values increased from 4.21 on the first day to 8.04 ± 0.12 on the last day, as shown in Figure 3c; the optimal pH of operation for voltage generation was 4.34 ± 0.072 , corresponding to the fifth day of monitoring. According to Margaria et al. (2017), the microorganisms responsible for generating electrons must have certain specific pH conditions for their acclimatization and growth to be conducive to such activity [31]. Levels between 5.27 ± 0.065 and 8 ± 0.12 are the least favorable for bacterial growth, since they affect the metabolism of microorganisms for this type of substrate [32].

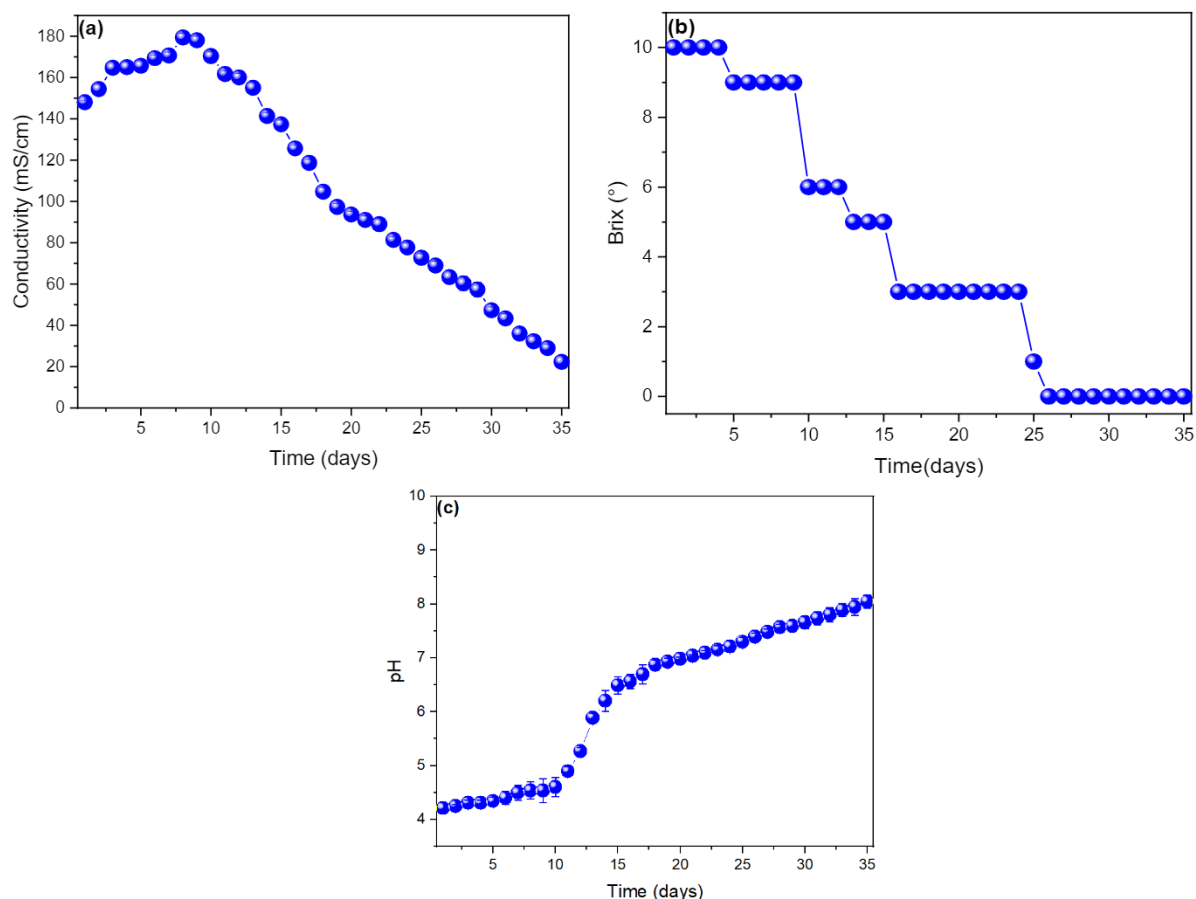


Figure 3. Values of (a) conductivity, (b) degrees Brix, and (c) pH of MFCs during the 35 days of monitoring.

Figure 4a shows the output power (P_s) of MFCs with different external resistances ($R_{ext.}$). With a $R_{ext.}$ of $1 \text{ k}\Omega$, P_s increased from 8.96 to 9.629 mW in the first 42 h; when $R_{ext.}$ increased to $5 \text{ k}\Omega$, P_s increased from 7.147 to 8.35 mW from 42 to 100 h, respectively. Finally, when $R_{ext.}$ was $10 \text{ k}\Omega$, P_s was from 3.09 mW at 100 h to 3.18 mW at 180 h. Previous studies conducted under the influence of $R_{ext.}$ by Kamau et al. (2017) indicate that the variation of $R_{ext.}$ from 2.7 to $2.2 \text{ k}\Omega$ results in a decrease in power from 1.69×10^{-3} to 1.27×10^{-3} mW. These variations of P_s and $R_{ext.}$ are similar to those of our work, because the increase in $R_{ext.}$ decreases P_s and vice versa [33]. Figure 4b shows the modeling of Ohm's law, where the potential (V) results from the multiplication of current (I) and resistance (R) or $V = I.R$, where the current was assigned to the X axis and potential to the Y axis [34]. The experimental data fit the equation $y = 194.04 \pm 0.0471 x + 0.50129 \pm 0.00863$ with $R^2 = 0.9375$, where the slope represents the average slope of MFC systems ($194.04 \pm 0.0471 \Omega$), whose value is lower than the value of the actual external resistor (1000Ω) applied during the experiments. The formation of the electroconductive biofilm on electrode surfaces is one of the main reasons for the low value of the internal resistance [35]. Figure 4c shows the PD

and CD values, indicating a PD_{max} of $669.60 \pm 10.84 \text{ mW/cm}^2$ at a CD of 5.65 A/cm^2 , with a maximum voltage of $1032.4 \pm 39.45 \text{ mV}$. These results exceed those found by Flores et al. (2020), who used citrus waste and managed to generate a PD_{max} of $62.5 \pm 2 \text{ mW/cm}^2$ at a CD of 0.049 A/cm^2 with a peak voltage of $940 \pm 11 \text{ mV}$ [36]. Results are also similar to those presented by Kamau et al. (2018), who managed to generate a PD_{max} of 12 mW/m^2 on day 18. Possibly, its value was lower because the graphite rod electrode was tied without a certain shape [37].

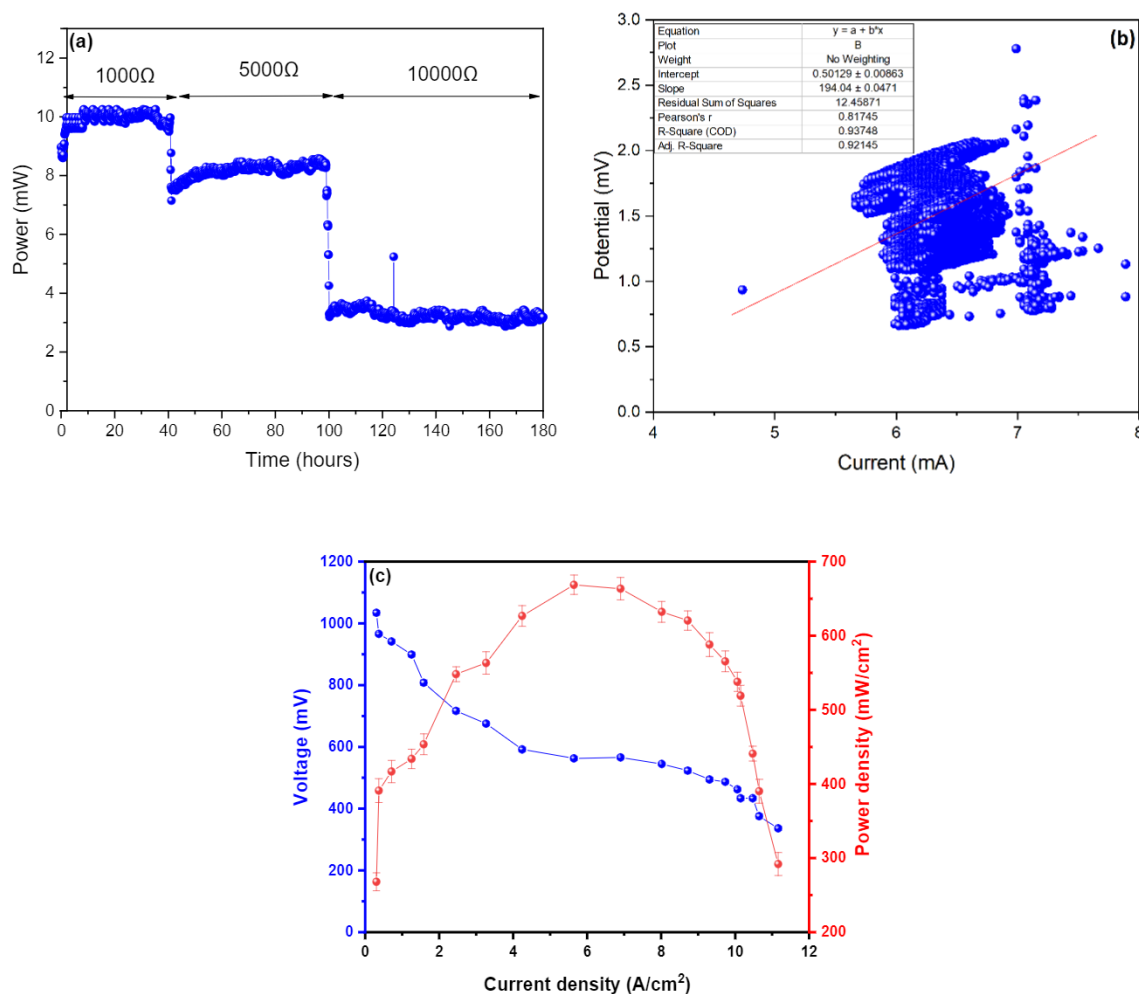


Figure 4. (a) Power, (b) internal resistance and (c) power density versus current density of MFC.

In Figure 5, the variation between the initial and final FTIR spectra of the substrate waste is shown. The most intense band around 3275 cm^{-1} corresponds to those of OH stretching, corresponding to polysaccharides or lignins [38]. Symmetric and asymmetric stretching vibrations of CH_2 groups are found at 2920 and 2853 cm^{-1} , which are mainly associated with lipid hydrocarbon chains [39]. Similarly, the bands around 1635 cm^{-1} are associated with the stretching of $\text{C}=\text{OO}^-$ groups and $\text{C}=\text{C}$ aromatic groups (phenolic compounds) [3]. The region between the bands from 1030 to 1540 cm^{-1} corresponds to organic compounds, such as sugars, alcohols, and organic acids, present in the sample [40]. The decrease in the band intensity of the spectrum of substrate waste is mainly due to the fact that many organic compounds were consumed or degraded by breaking the chemical bonds in bioelectricity generation during the monitoring of the MFCs [41].

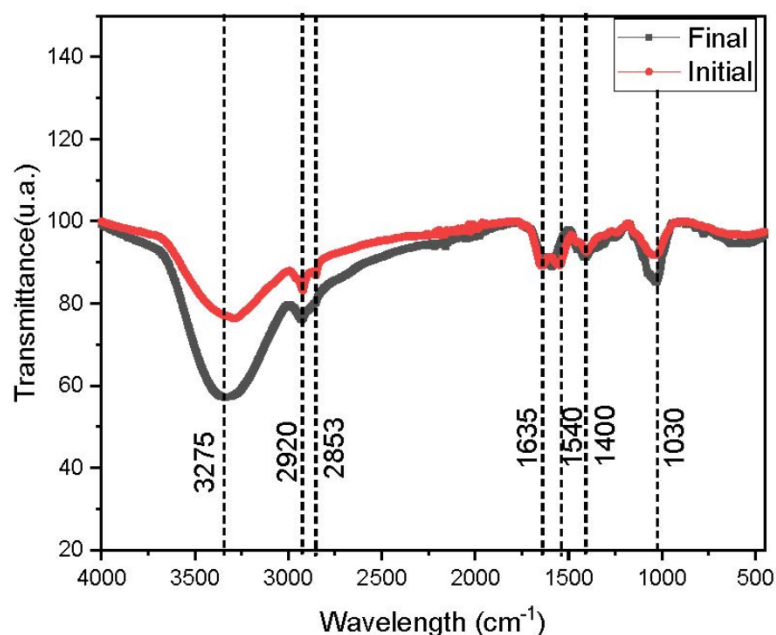


Figure 5. Initial and final transmittance spectrum of GB waste.

The region sequenced and analyzed in the BLAST program of the isolated bacterium obtained an identity percentage of 99.93% for *Stenotrophomonas maltophilia* (Table 1). Zafar et al. (2019) reported electrochemically active bacteria for the degradation of unwanted pollutants, such as petroleum-contaminated soils and sludge activated for electricity generation by using microbial fuel cells, where *Stenotrophomonas maltophilia* (89%) and *Shewanella* sp. (15%) were the predominant bacterial species on the anodic surface in the respective reactors [42].

Table 1. BLAST characterization of the rDNA sequence of the bacterium isolated from the MFC anode plate.

BLAST Characterization	Consensus Sequence Length (nt)	% Maximum Identity	Accession Number	Phylogeny
<i>Stenotrophomonas maltophilia</i>	1474	99.93%	CP040434.1	Cellular organisms; Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas; Stenotrophomonas maltophilia group

The dendrogram was created in MEGA X software by using the Bootstrap method with 1000 replicates, which was based on the 16S rDNA of a *Stenotrophomonas maltophilia* culture isolated from the anode plate of microbial fuel cells with Golden Berry juice, showing levels of similarity (or distance) between phylogenetically related species (see Figure 6). It is worth mentioning that a BLAST characterization of the rDNA sequence of such a bacterium isolated from the MFC anode plate was performed. *Stenotrophomonas maltophilia* is a Gram-negative, non-glucose-fermenting, aerobic bacterium; it is motile since it has polar flagella [43], and has the ability to adhere and form biofilms on different surfaces, including the abiotic ones. As for its main habitats, this bacterium is found in the soil, where it fulfills important ecosystemic functions in plant growth [44], as well as in water, and as a part of the microflora of food and in various other microbiota [45]. Finally, Figure 7 shows the schematization of bioelectricity generation through the three microbial fuel cells connected in series, using GB waste as fuel. As can be observed, 2.90 V was generated. This novel means of generating electricity provides an option for producers and exporters and

importers of this product to give a second use to the waste of this type of fruit for their own benefit.

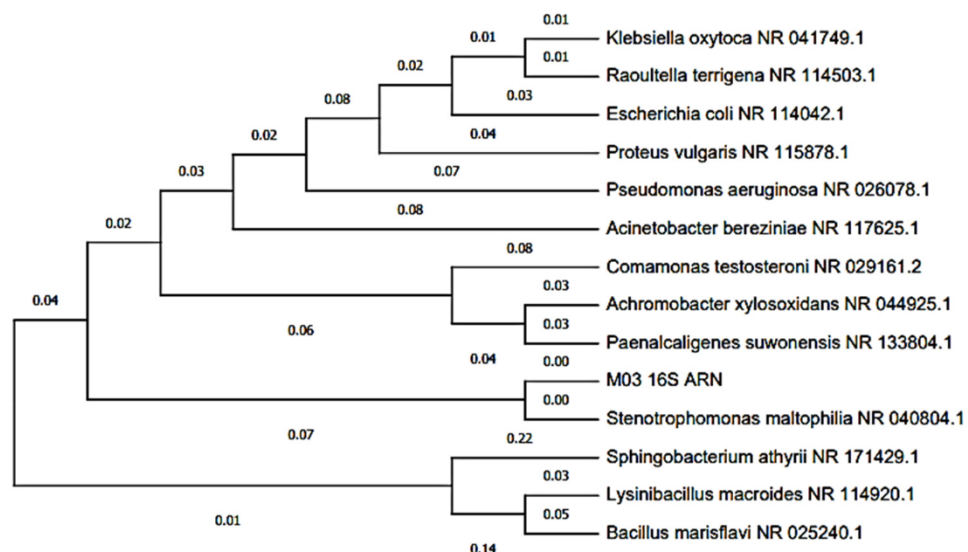


Figure 6. Dendrogram based on the 16S rDNA of a *Stenotrophomonas maltophilia* culture isolated from an MFC anode plate.

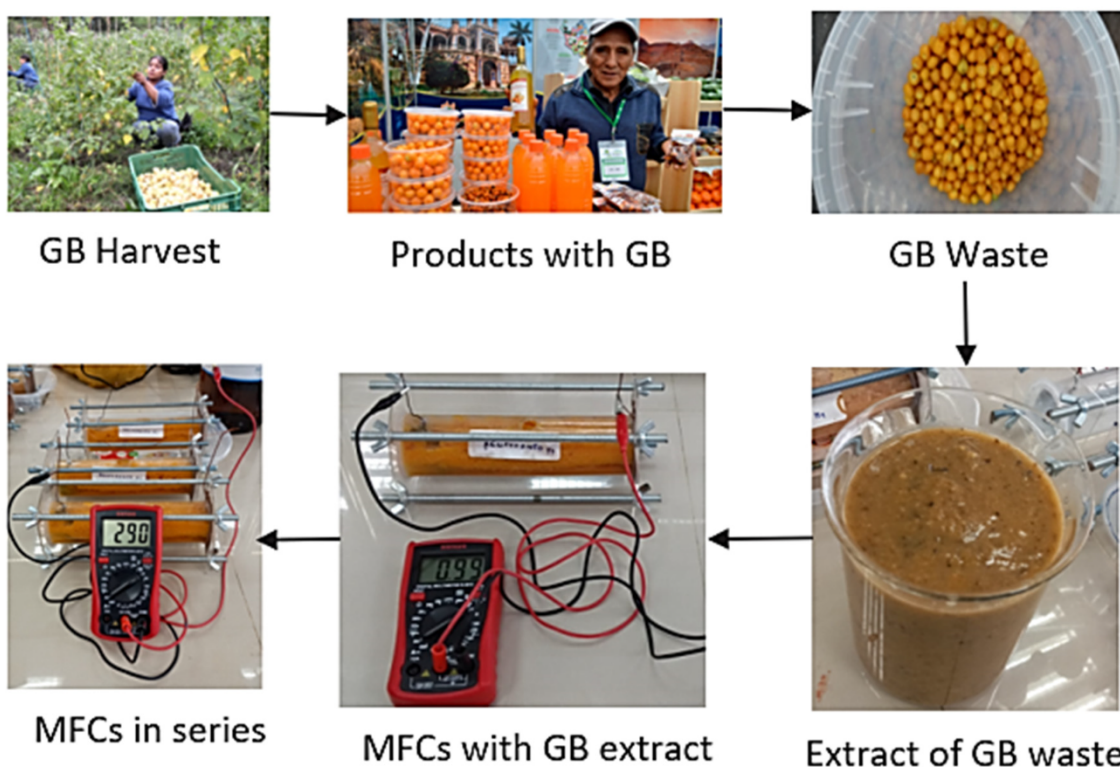


Figure 7. Scheme of bioelectricity production by using the three MFCs connected in series.

4. Conclusions

Bioelectricity was successfully generated by using Golden Berry waste as fuel in low-cost microbial fuel cells. Maximum voltage and current peaks of 1.03 ± 0.02 V and 4.945 ± 0.150 mA were generated on the fifth and twelfth day, respectively. Conductivity values increased to 148 ± 1 mS/cm on the eighth day and the optimum operating pH of the cells was found to be 4.34 ± 0.072 , while °Brix gradually decreased from the

first day of operation. The power of cells decreased as external resistances increased in value and the internal resistance of cells was found to be $194.04 \pm 0.0471 \Omega$. The maximum power density was $62.5 \pm 2 \text{ mW/cm}^2$ at a current density of 0.049 A/cm^2 with a peak voltage of $1032.4 \pm 39.45 \text{ mV}$. Transmittance peaks of the FTIR spectrum decreased compared to the initial and final ones, with the band around 3275 cm^{-1} being the most noticeable. *Stenotrophomonas maltophilia* was identified by molecular techniques with an identity percentage of 99.93%. This research provides a potential use of this type of waste for bioelectricity generation, benefiting farmers, exporters, and importers, because they could generate their own energy in the future and achieve a reduction in expenses in an eco-friendly manner. For future works, it is recommended to use the optimal pH of the research operation, which has to be stabilized with some chemical compound, at the same time as using zinc–copper electrodes coated with some compound in order to increase the durability of the device.

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