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Using Lime (*Citrus × aurantiifolia*), Orange (*Citrus × sinensis*), and Tangerine (*Citrus reticulata*) Waste as a Substrate for Generating Bioelectricity

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This paper focuses on using lime, orange, and tangerine waste as fuel for the generation of bioelectricity. The tangerine microbial fuel cell evidenced a maximum voltage peak of 1.1 V on day 13, while displaying abrupt voltage losses on the following days and throughout the remaining period of substrate monitoring. On the other hand, orange-based fuel denotes lower current generation during these 28 days with values close to 1.6 mA and 0.25 mA on the first and last day, respectively. Still, orange generated 3.5 mA on the first day with values sharply declining in the following days until reaching 0.91 mA on the last day. In general, substrate volumes in the microbial fuel cells diminished over time, which is consistent with the voltage and current values reported.

In addition, pH values increased from day 1 for all substrates, reaching acidic pH values on the last day of measurement due to the consumption of carbon sources. Tangerine reported the highest current density (CD) at 72 mW/cm^2 and a maximum voltage of 1.06 volts, while orange generated a CD of 62.5 mW/cm^2 and 0.94 volts.

Keywords: microbial fuel cells, voltage, current, acids, organic waste and bioelectricity.

Introduction

Environmental issues faced by the society due to climate change and excessive consumption of fossil fuels are vigorously degrading our ecosystem (Victor, 2017). As a side consequence, one third of the food production (433.33 million tons) worldwide is wasted annually, which roughly accounts to $750 billion (Dahiya et al., 2018). In Peru, wholesale fruit and vegetable markets inefficiently manage their organic waste. In fact, fruit and vegetable waste is usually discarded in surrounding areas, thus causing contamination due to the foul odor and rodents attracted by the fruit and vegetable waste (Centeno et al., 2019). Since their discovery in 1984 (Lefebvre et al., 2010), microbial fuel cells (MFC) have been intensively studied due to the increased interest in using wastewater for the generation of electricity (Shah et al., 2019). MFCs use organic substances as substrates (fuel) by converting chemical energy into electrical energy supported by catalytic reactions involving microorganisms (Rahimnejad et al., 2015). These values of electricity generated by the MFCs can be used in integrated circuits for future simulations with algorithms (Gokul et al., 2020; Gokul et al., 2019).

MFCs are composed of a cathode and an anode, which are commonly separated by a proton exchange membrane (Slate et al., 2019). The electrons (e^-) and protons (p^+) resulting from microbe oxidation in the anode are transferred to the cathode through an external circuit and the membrane itself. These two charges are then consumed, thus reducing the oxidant, which is typically O_2 (Gajda et al., 2018; Yu et al., 2018). This type of technology offers a viable solution for leveraging waste, which is usually carelessly discarded by both households and companies. In their work, Li et al. (2016) proved that 120 mL of food waste can generate 0.5 volts from day 18 to day 30, for a period of 18 hours until the next fuel supply (Li et al., 2016). Recently, it has been identified that anthocyanin-rich extracts (such as Vaccinium Spp., C. Terntea Linn, and L. Ruthenicm Murr) used as redox mediators exhibit electrochemical activities, which significantly increase the generation of electricity in MFCs. When comparing C. Terntea Linn and Vaccinium Spp. with L. Ruthenicm Murr, the latter shows the most significant redox mediation capacity and a favorable antioxidant activity due to its higher polyphenol content (anthocyanin) (Xu et al., 2019).

Dual chamber MFCs have also been used as *palm tree* oil fuel, generating voltage peaks of 0.647 ± 0.03 V within the first 80 hours of starting the experiment with a 3.5 g/mL sample (Kondaveeti et al., 2019). In the studies conducted by Toding et al. (2018), *banana* and *orange* peels were individually used as fuel for a single chamber MFC without chemical pre-treatments or adding mediators, but using copper plates coated with activated carbon powder in the cathode and zinc plates coated with graphite powder in the anode. The experiment results yielded 0.492 V and 0.101 mA for the *banana* peels and 0.563 V and 0.017 mA for the *orange* peels. Grape waste has also been used as fuel in single chamber MFCs using zinc, copper, and magnesium electrodes, as well as thionin and toluidine (red and blue) as mediators. In this case, thionin generated higher voltages peaking at 2.5 V when using magnesium as electrode, while using toluidine red and blue as mediators generated 2.35 and 2.2 volts, respectively (Sivaa et al., 2014). MFCs have also been designed without requiring proton exchange membranes, using *papaya* waste as fuel, as well as carbon felt and magnesium oxide as anode and cathode, generating 0.75 to 0.81 mW/cm^2 and a peak voltage of 0.7 volts. The authors concluded that pulp causes variations in cell performance, without causing damage to the electrodes (Khan and Obaid, 2015).

This research seeks to reuse food waste extracts, such as *lime (Citrus × aurantiifolia), orange (Citrus × sinensis)*, and *tangerine (Citrus reticulata) juices* in affordable MFCs (without proton exchange membranes). This study focuses on reporting voltage, current, pH, conductivity, current density (CD), and power density (PD) throughout a period of 28 days, thus generating eco-friendly electricity, reducing environmental pollution, and lowering production costs for implantation in the most remote places.

Materials and Methods

Sample collection and preparation

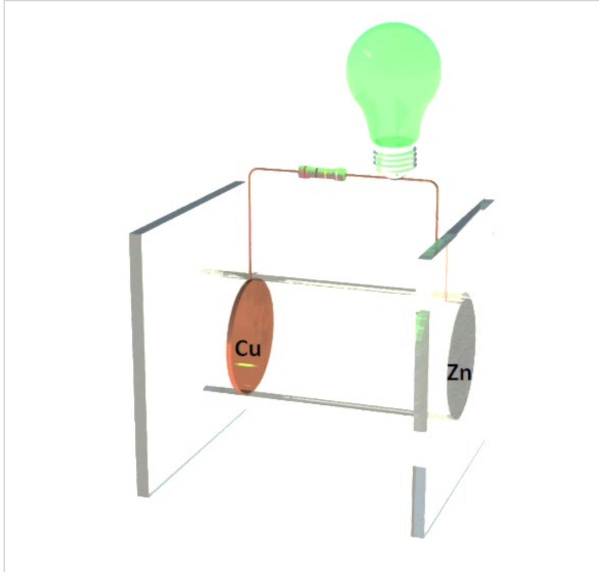
Organic *lime*, *orange*, and *tangerine* waste was collected from La Hermelinda Market, Trujillo, Peru. This organic waste was subsequently washed several times with distilled water to remove any impurities (dust, insects, or other pollutants). After washing, the fruits were superficially dried at 22 ± 2°C in a Labtron kiln (LDO-B10) for 24 h. Then, the dried fruit waste was filtered through an extractor (Maqorito-400 rpm), obtaining 250 mL of waste, before being ultimately transferred to properly sterilized beakers.

Microbial fuel cell production

Three cells were built for each substrate using 10 cm acrylic squares serving as fasteners for 16 × 5 cm^2 PVC pipes. In addition, zinc and copper electrodes, each 5 cm in diameter, for an area of 19.63 cm^2, were used, as shown in Fig. 1. A total of 5 holes were made at the top of the electrodes through which they are attached to the upper part of the PVC pipe using copper wire (0.3 mm thick). In addition, a 9-cm^2 hole was made in the center of the tube to facilitate substrate influx, as well as conductivity and pH measurements.

Voltages were measured using a multimeter (Prasek Premium PR-85) for 30 minutes per day over a period of 28 days. On the other hand, current was measured through resistors at 10.4 (±0.8), 23.5 (±0.5), 46.4 (±0.7), 99.1 (±3.1), 197.1 (±5.6), 264, 387 (±6.5), 687 (±8.7), 808 (±8.2), and 988 (±9.3) Ω. Furthermore, to measure CD and PD, we used the procedure suggested by Zhuang et al. (2012). Finally, conductivity

Fig. 1. *Microbial fuel cell (MFC) prototype*



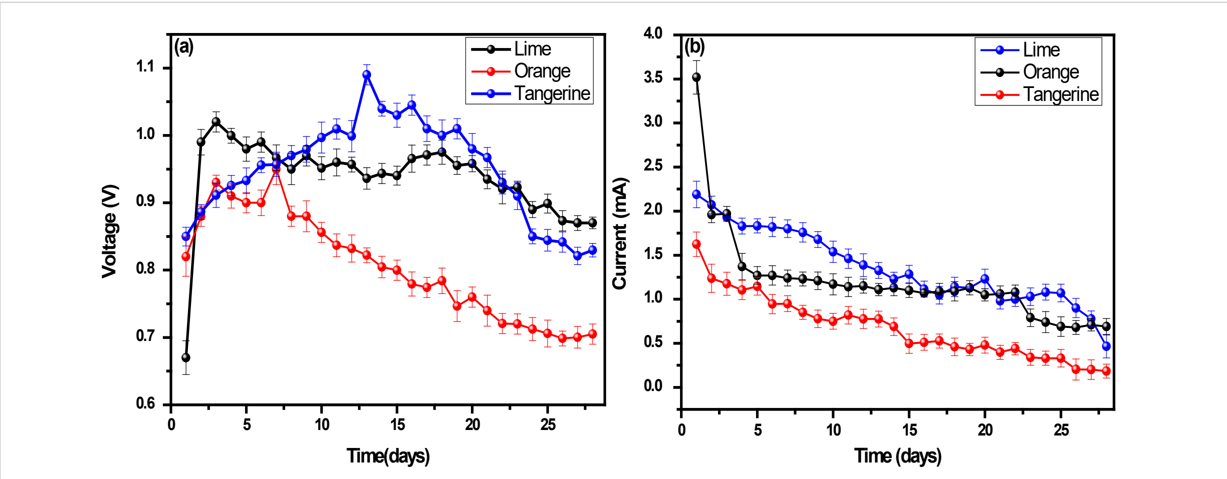
(CD-4301 conductivity meter) and pH (110 Oakton Series pH meter) changes were also monitored and measured. The microorganisms found in MFCs were identified using a microbiological analyzer, VITEK2 COMPACT (Biomerieux, VK2C-18806, France), and a scanning electron microscope (SEM - TECSAN VEGA 3 LM) equipped with a SPI 11430-AB gold coating system (TESCAN USA) was used for the micrographs. The data points of the voltage, current, and PD and CD figures represent the average values from three replicates and the error bars represent the corresponding standard deviations.

Results and Discussion

Fig. 2 (a) denotes average voltage (V_p) generation reported by the open MFC circuit for the three types of organic waste. On the first day, *tangerine* reports the highest V_p generation with values of approximately 0.85 ± 0.02 volts. However, *lime* generates a higher V_p as of the second day with values exceeding 1 volt until decreasing on the seventh day. Thereafter, these values are surpassed by *tangerine* until day 23 (0.92 ± 0.01 V), where its V_p decreases until reaching 0.83 ± 0.015 V on day 28. The substrate that consistently reported lower V_p was *orange,* only reaching values under 0.95 ± 0.019 volts as of the seventh day. Substrate voltage variations may be influenced by the development of microorganisms in the MFC since the substrates used may or may not degrade easily, which affects microorganism growth (Logroño et al., 2015; Khan and Obaid, 2015; Zhuang et al., 2012)

Fig. 2 (b) denotes average current (C_p) generation. *Orange* generates 3.5 ± 0.15 mA on the first day but drops rapidly on the second day to under 2 ± 0.10 mA. On the other hand, *tangerine* reports lower generation of electric current with values under 0.20 ± 0.09 mA on day 28. Still, the *lime* MFC maintains C_p values ranging from 2.2 ± 0.14 to 0.5 ± 0.08 mA as of day 1 and until the last day of monitoring. The constant decrease in C_p can be explained by the fact that waste microbes feed from the substrates available; however, as soon as the fresh substrate is depleted, the microbes start to die, thus causing a downward trend in current generation (Kamau, 2018). In addition, the higher generation of C_p in the first few days can also indicate the presence of soluble compounds, which were initially oxidized and consumed progressively, forming an electrochemically active biofilm (Cercado et al., 2010). Also, the fruits used are known for containing vitamin C, which has antioxidant and reducing properties; which would generate a flow of electrons in the cell and produce the electricity (Vilaplana, 2007).

Fig. 2. *Monitoring the generation of (a) voltage and (b) current in microbial fuel cells (MCFs) over 28 days*



As depicted in Fig. 3, some volume variations may be observed for the MFC during the 28 days, wherein the *orange* MFC loses the most volume, in agreement with the voltages reported in Fig. 2 (a), because lower volumes generate lower voltages. On the other hand, the tangerine and lime MFCs generated the highest voltages because they lost the least volume during the monitoring period, which indicates a direct proportional relationship between degraded substrate volumes and voltage generation (Rabaey et al., 2004). However, Lyon et al. (2012) report that the influence of this parameter is small compared with the changes in the microbial community

Fig. 3. *Microbial fuel cell (MFC) volume changes over 28 days*

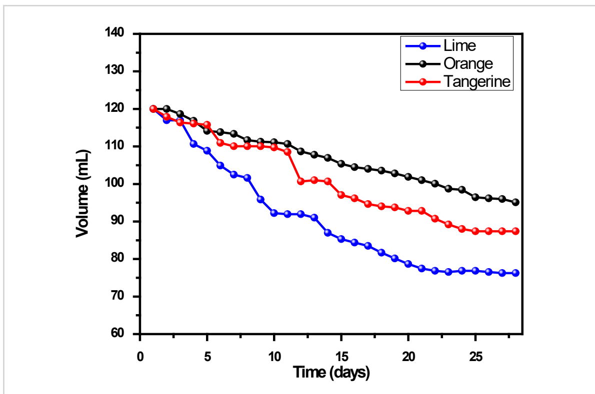
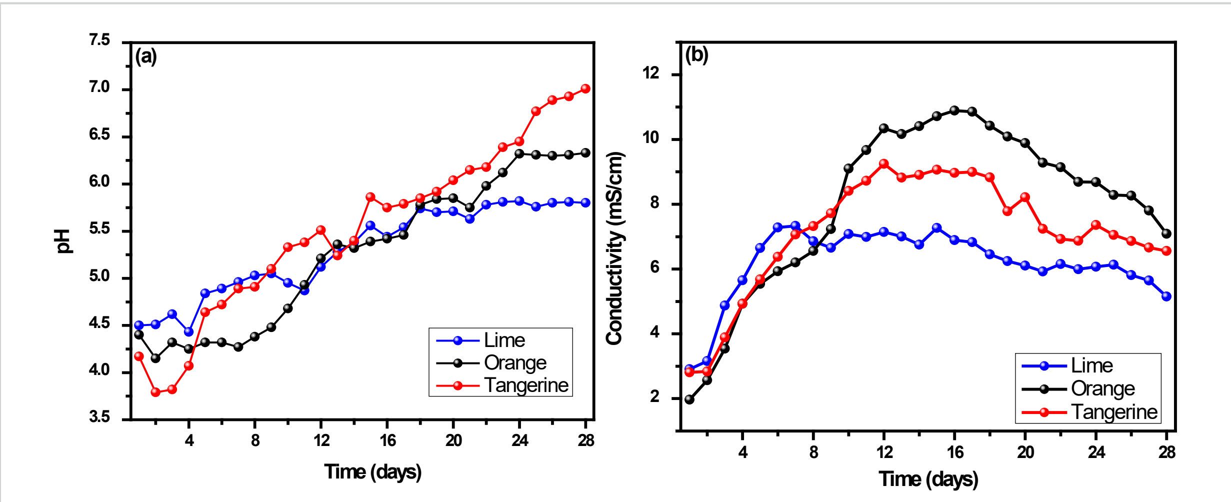


Fig. 4 (a) denotes pH values, wherein values increased on the first day of monitoring for three substrates. In fact, *tangerine*, *lime*, and *orange* increased from 4.4, 4.24, and 4.51 on the first day to 6.25, 6.98, and 5.65 on the last day, respectively. Acidic pH values such as those reported by the fruit waste in the final days of the experiment are consistent with current generation since acidic conditions mean that carbon sources were consumed faster. Raghavulu et al. (2009) observed that the transfer of extracellular electrons (e^-) is more efficient in an acidophilic microenvironment than in a neutral environment. Hence, the increase in electron transfers triggers the oxidation process and, therefore, the cycles of electricity generation will become shorter.

Fig. 4 (b) depicts conductivity for the three substrates. Herein, considerable generation of electricity is reported from day 11 to 17, with values exceeding 10 mS/cm for *tangerine*, while peak values did not exceed 7 mS/cm for *orange*. In these cases, conductivity variations are caused by dissolved ions that contribute to electrons and degrade substrate resistance (Karthikeyan et al., 2016). As the conductivity values started to increase for these substrates, current values did not increase. Consequently, the limiting electricity generation can be attributed to other factors. For example, reduced mass transfers from electron donor may be significant, as well as slow proton flow rates hindering production (Malvankar et al., 2012).

Fig. 4. *Microbial fuel cell (MFC) (a) pH and (b) conductivity over a period of 28 days*



MFC polarization values for the three different substrates are shown in Fig. 5. These values were calculated using the polarization curve to characterize current as a function of voltage (Cheng and Logan, 2011; Mohan et al., 2008). Current values were measured directly using the resistors described in the Materials and Methods section (Deepika et al., 2015). When using *orange* as a substrate, the system was able to generate a PD of 0.0625 ± 0.002W/cm^2 at 0.049 A/cm^2 of CD and a maximum of 0.94 ± 0.011 V. Meanwhile, the *lime* substrate generated a maximum PD of 0.066 ± 0.0012 W/cm^2 at 0.047A/cm^2 of CD and a maximum voltage of 0.96 ± 0.11 V. Finally, the *tangerine* substrate reported 0.072 ± 0.0014 W/cm^2 at 9x10^{-4} A/cm^2 of CD and a maximum voltage of 1.06 ± 0.18V. The power generated by this system slightly exceeds previous works not using a mediator, as shown in *Table 1*. In some cases, the system exceeds the voltage, CD, and power values reported in the literature, which mostly use wastewater and fruit and vegetable waste as substrates.

The corrosive nature of the metal electrodes used (Zn and Cu) did not affect their excellent production rates during the monitoring period. Dunas et al. reported

Table 1. *Performance of the microbial fuel cells (MFCs) against the literature*

Substrate type	MCF	Maximum voltage (V)	Power density (PD) (mW/m^2)	Current density (CD) (mA/cm^2)	Reference
Sewage sludge	Double chamber	0.66	59	175	(Madhavan et al., 2016)
Wastewater	Double cylindrical chamber	0.03	1.2	0.043	(Wang et al., 2016)
Orange peel waste	Double chamber	0.59	358.8	847	(Miran et al., 2016)
Kitchen waste	Single chamber	0.620	60	--	(Moqsud et al., 2014)
Plant waste extract	U-shaped chamber	0.5413	88,990	314.4	(Javed et al., 2017)
Food waste	Double chamber	0.260	19,151	195	(Hou et al., 2016)
Wastewater from vegetable oil industries	Double chamber	0.563	1,260	--	(Firdous et al., 2018)
Food waste	Double chamber	0.046	29.6	88	(Chatzikonstantinou et al., 2018)
Potato, tomato, and lettuce waste	Double chamber	0.200	3.5	100.2	(Du et al., 2015)
Fermentable household waste extract	Single chamber	0.482	3.14	14	(Tremouli et al., 2019)

the corrosion of the copper electrodes of their MFCs. Still, this may be due to the high saline content to which their electrodes were exposed, which accounts for reporting lower PD values than the ones reported in this research (Dumas et al., 2007). On the other hand, Nurettin reports that zinc electrodes in an MFC using organic matter as substrate generate a biofilm, rust, and corrosion, thus evidencing the electrochemical reaction of Zn (Nurettin, 2017)

The micrographs for the Cu and Zn electrodes after 28 days are shown in Fig. 6. As it can be observed, the Cu anode (Fig. 6 (a)) evidences a cracked layer, which may be due to corrosion or because of a biolayer formed by the oxidation of microorganism activity in the production and transfer of electrons (Rojas et al., 2020). This transfer takes place after forming the biolayer (Wang et al., 2012) although not all microorganisms attach to the anode since the upper layers of these microorganisms are connected to each other by endogenous electron transporters known as nanowires (Zhang et al., 2012; Rabaey et al. 2004).

Fig. 6 (b) denotes the zinc electrode (cathode), in which lower adhesion of the biolayer is observed. This may be due to the fact that the cathode receives and does not generate electrons (Tamakloe et al., 2015), and the thickness variations may be due to the reduction of the material but not due to oxidation (Meignanalakshmi and Kumar, 2016) or failures in the manufacture of the electrical circuit (Gokul et al., 2019). The pure microorganism cultures obtained on day 28 yielded gram-positive bacilli identified as *Brevibaciullus laterosporus* by the VITEK-2 system, as well as *Candida boidini*, as identified by the API 20C AUX system. Finally, the three MFCs with the highest voltage generation were selected and connected in series, generating 2.87 volts on the last day of monitoring and managing to switch on a green LED, as shown in Fig. 7. This fuel production provides a new way of leveraging waste from these three fruits as substrates (fuel). Hence, the companies that export and import these agricultural products may now reuse their damaged products (not suitable for consumption) and economically generate their own electricity.

Fig. 5. *Power and current density (PD and CD) from microbial fuel cells (MFCs) using (a) orange, (b) lime and (c) tangerine*

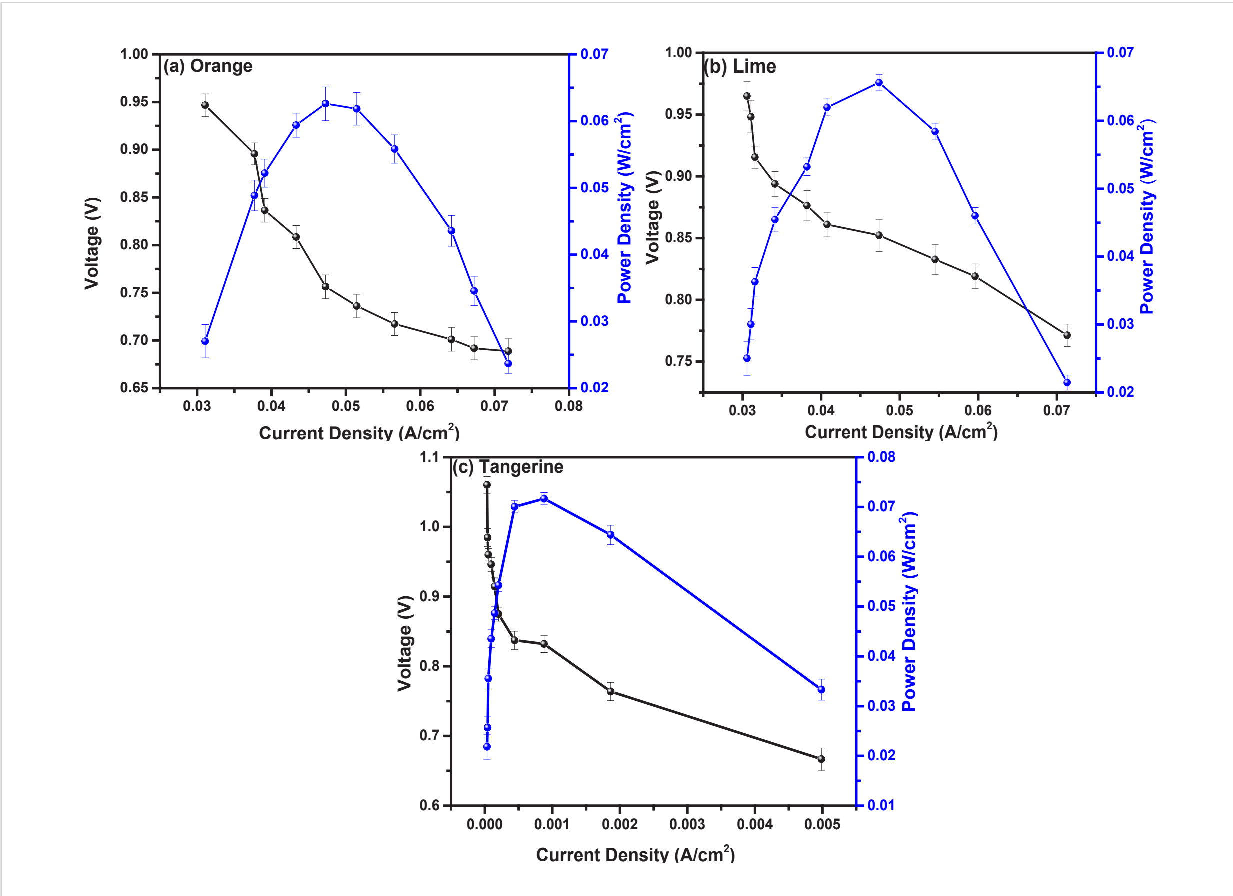


Fig. 6. *SEM micrographs of the (a) anode and (b) cathode electrodes of the MFC*

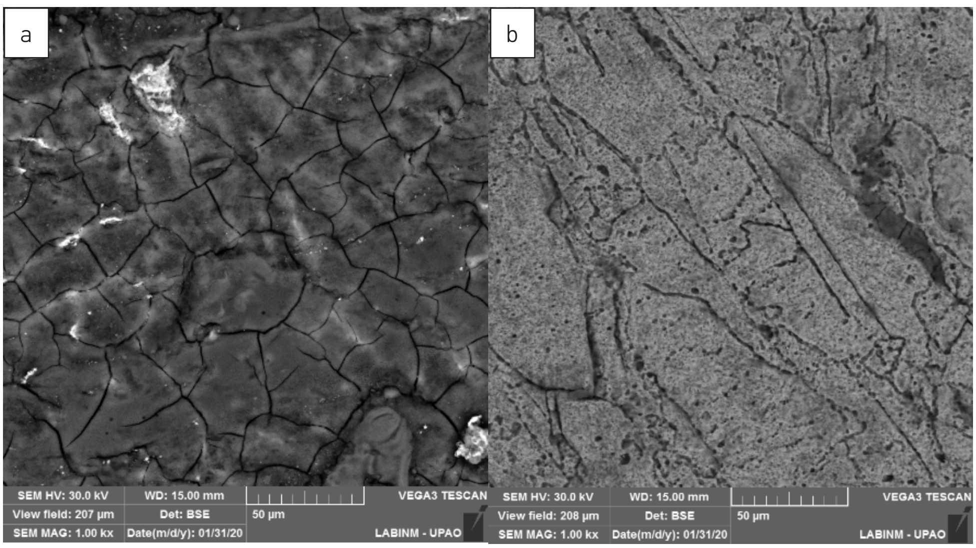
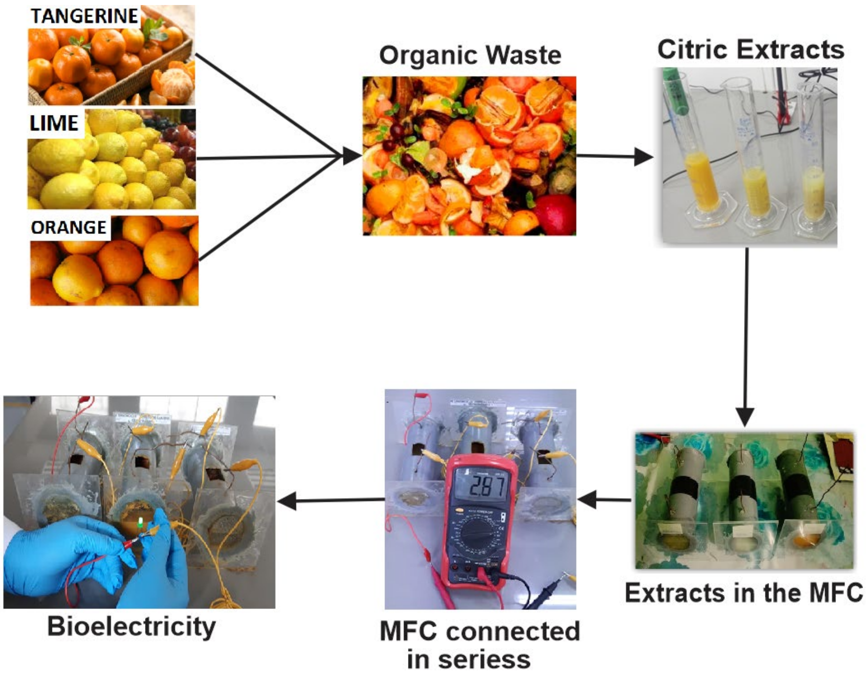


Fig. 7. *Bioelectricity generation from agricultural waste*



Conclusions

This research demonstrates that *lime, orange*, and *tangerine* waste can be repurposed to generate electricity through affordable MFCs using alternative zinc and copper electrodes instead of the conventional coal, graphite, and graphene electrodes. The research proved that *tangerine* and *orange* waste may generate maximum voltages of 1.06 and 0.95 volts, respectively, and the substrate volume and pH values are directly linked to current and voltage generation by the MFCs. The maximum PD generated was 0.072 ± 0.0014 W/cm^2 at a CD of 9x10^{-4} A/cm^2 in the *tangerine* MFC and the lowest PD generation was reported by the *orange* MFC with 0.0625 ± 0.002W/cm^2 at CD of 0.049 A/cm^2. In addition, the *Brevibaciullus laterosporus* and *Candida boidini* microorganisms were identified. The research not only provides a new electricity generation alternative for fruit distribution centers, but also for companies that export and import this fruit. This way, these companies may reduce their electricity consumption costs by leveraging the fruit products damaged during the storage process or when being shipped to other countries.

{Gurauskiene, 2006, Eco-design methodology for electrical and electronic equipment industry}

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