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Generation of Bioelectricity Using Molasses as Fuel in Microbial Fuel Cells

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The large amount of molasses that are generated in sugar-processing companies are not always redistributed for commercialization in by-products. Because of this, the present research uses these wastes as fuel in low-cost, lab-scale, single-chamber microbial fuel cells. Zinc and copper electrodes were used as electrodes and 100 mL of molasse in the chamber as fuel, managing to generate current and voltage peaks of 1.73 ± 0.13 mA and 0.953 ± 0.142 V. In monitoring the conductivity of the substrate, a maximum peak of 111.156 ± 8.45 mS/cm was observed, and a slightly acidic pH was observed throughout the monitoring. It was possible to obtain a power density of 5.45 ± 0.31 W/cm^2 for a current density of 308.06 mA/cm^2, while the yeast count showed a logarithmic curve throughout the monitoring. Finally, the molecular technique identified 100% of the special C. boidinii present in the anodic electrode. This research will give great benefits to sugar companies because they will be able to generate electricity using the molasses that cannot generate by-products.

Keywords: molasse, microbial fuel cell, generation, electricity, waste.

Introduction

The demand for electrical energy has exponentially increased over the last decades (Bedi and Toshniwal, 2019), fossil fuels being one of the main sources used for power generation, accounting for about 80% of the world's energy supply. Due to their constant production and consumption, fossil fuel reserves have decreased and will be depleted in the coming decades (Guo et al., 2018; Rahman, 2020). Likewise, the use of fossil fuels generates a series of environmental problems. Among the most significant ones is global warming due to the emission of greenhouse gases and climate change, which are current problems faced by humanity (Ye et al., 2018; Balcilar et al., 2019). Many research groups have been working hard to find new ways to generate electricity while decreasing or eliminating greenhouse gases that cause climate change (Akay et al., 2020; Iodice et al., 2018).

For these reasons, there is a great interest in developing new sustainable energy sources to replace fossil fuels (Boyle, 2004). Thus, microbial fuel cells (MFCs) have emerged as a promising technology to meet the growing energy needs (Nawaz et al., 2020). These bioelectrochemical systems employ microorganisms as biocatalysts that oxidize organic matter at the anode and reduce oxygen or a suitable electron-accepting compound at the cathode, resulting in the generation of electricity (Kakarla and Min B, 2019). There are various designs of an MFC, for example, single-chamber MFCs which have a simple and low-cost design compared with other designs. These cells have only an anode chamber without the requirement of aeration in a cathode chamber (Goel, 2018). It is worth mentioning that power generation in MFCs depends on several factors such as their configuration, electrode types, membrane types, microbial function, and substrate type (Chakraborty et al., 2020; Zhou et al., 2017). It is important to highlight that MFCs present several advantages in relation to other power generation systems. Since these systems work at room or lower temperatures, they generate less CO_2, and do not require energy input as long as the cathode is aerated (Yang et al., 2018). In addition, a wide range of substrates (among which wastewater stands out), soluble or dissolved complex organic wastes such as molasses, a by-product of sugar industry that is generated as a result of the refining of sugars from sugarcane, can be used. It is a dense, viscous, dark-colored liquid (Xu et al., 2019; Chen et al., 2020; Corbella et al., 2019; Mardiana et al., 2020).

In this regard, sugarcane is of utmost importance to the economy of many world regions due to its versatility and capacity to adapt to different climate conditions (Lagos and Castro, 2019). Thus, worldwide in 2019, a total of 194 million tons of sugarcane were generated in a harvested area of 26 million hectares. Most of this production was concentrated in America with 52.1%, followed by Asia with 40.2% and Africa with 5.5%. In terms of countries, Brazil, India and China stand out with the most significant productions (Gordillo de Anda and Suarez, 2021). In Peru, sugarcane is grown in three regions (coast, highlands and jungle). Due to agroclimatic conditions, this plant is sowed and harvested throughout the year (Espinoza Cortez, 2021). In the first quarter of 2019, the production in Peru reached 2.5 million tons distributed; among all the departments, La Libertad (41%) and Ancash (6%) stand out (Castrillón Liñan, 2021). In this context, it is worth mentioning that sugarcane cultivation produces agricultural residues, such as cane tops and green leaves (8%), sheaths and dried leaves (20%), and industrial by-products such as molasses, a residue that can be used as substrate in MFCs to generate bioelectricity (Hao et al., 2017).

There are several research studies on this topic. For example, Hassan et al. (2019) have demonstrated the possibility of producing bioelectricity from sugarcane molasses by using MFCs. For this purpose, they isolated and identified a bacterial strain (*Brevibacillus borstelensis* STRI1) from the substrate, which was employed as a biocatalyst, obtaining as a result 990 ± 5 mV in open-circuit voltage and 453 ± 6 mV in closed-circuit voltage after 10 days of operation. As for power density, 188.5 mW/m^2 was obtained and the calobiotic efficiencies ranged from 59.8% to 28.03%, related to initial concentrations from 0.3 ± 0.05 to 2.0 ± 0.15 g/mL. Finally, the DOC (Dissolved Organic Carbon) removal was determined with a value of 11.7% at 5 days and 81.7% at 30 days. Likewise, Manjerkar et al. (2018) have investigated electricity generation by using sugarcane molasses and kitchen waste as fuel. In that research, they used electrodes made with aluminum mesh and bacteria intrinsically contained in the substrates to be used, resulting in a maximum potential of 365 mV in the case of molasses and 260 mV in the case of kitchen waste. At the same time, they observed that, as the days went by, the BOD

(Biochemical Oxygen Demand) and organic matter content decreased. Thus, the generation of electricity using molasses as substrate is a promising aspect, since molasses is a waste that is readily available for use (Sivasankar et al., 2018).

The main objective of this research is the generation of bioelectricity in a single-chamber MFC by using copper and zinc electrodes and sugarcane molasses as substrate, in which the values of voltage, current, conductivity, pH, degrees Brix, current density (CD) and power density (PD) were monitored. The biofilms of the cathodic and final anodic electrode were also observed, and the yeast count was performed in the molasses sample and the microorganisms present in the anodic electrode were molecularly identified. This research work will add value to molasses as an environmentally friendly way of generating bioelectricity.

Materials and Methods

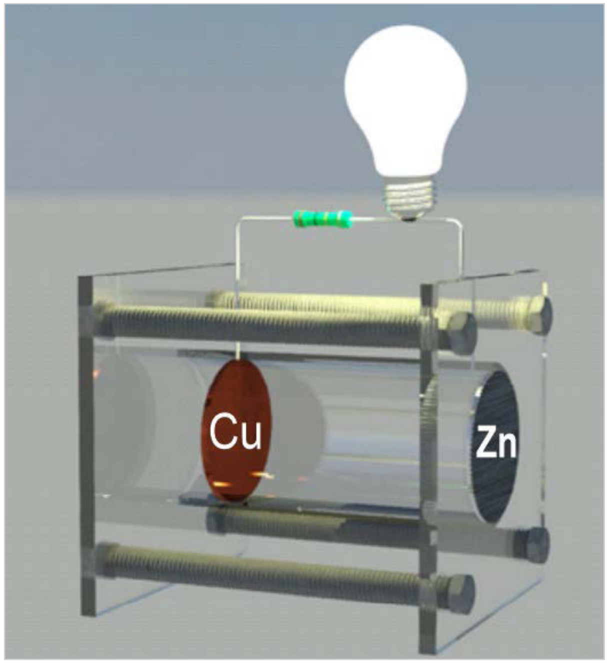
Construction of single-chamber microbial fuel cells

The microbial fuel cells (three) were constructed by using a polymethylmethacrylate tube of 5 cm and 20 cm diameter and length, respectively, as chamber. The 80 cm^2-area electrodes were made of zinc (Zn) and copper (Cu), where the anode (Cu) was placed inside the tube at approximately 5 cm from one end and the cathode was placed as a cap on the other end of the tube, being one side of the electrode in contact with the substrate and the other one with the environment (O2). The electrodes were connected by an external circuit with an external resistor of 100 Ω and in the absence of a proton exchange membrane. See *Fig. 1*.

Sampling and bioelectrochemical analysis

A sample of 1 L of molasses waste from Empresa Agroindustrial Laredo was collected in airtight flasks for being used as substrate, of which 100 mL was placed in each MFC. Then, the initial parameters of pH, degrees Brix, voltage, electric current, current density, power density and yeast count were measured.

Fig. 1. *Scheme of the MFC (microbial fuel cells) prototype.*



Isolation and identification of microorganisms

Isolation in solid media of bacteria coming from the anode chamber

The isolation of microorganisms was performed with a sterile swab, rubbing the surface of the copper electrode, and then placing the sample on the swab in Petri dishes containing Nutrient Agar, MacConkey Agar incubated at 35°C and 45°C (for isolation of gram-negative bacteria) and Sabouraud Agar incubated at 30°C for yeasts. This procedure was performed in duplicate. Colonies were observed to grow on Sabouraud Agar plates, and then they were sub-cultured in Sabouraud Agar with 4% dextrose, until obtaining axenic cultures for subsequent identification.

Identification and axenic cultures

Molecular identification was performed by the Analysis and Research Center of “Biodes Laboratorios”. For this purpose, an axenic culture of the isolated yeast was sent for the subsequent extraction of genomic DNA by means of the CTAB extraction method (Gustincich et al., 1991), which was subsequently subjected to the amplification of fungal-specific sequences, the ITS (*Internal Transcribed Spacer*) region by the PCR technique (Wahyuningsih et al., 2000). The PCR products were sequenced in the Macrogen laboratory (USA); then these sequences were analyzed with MEGA X (*Molecular Evolutionary Genetics Analysis*) bioinformatics software; and then, they were aligned and compared with other sequences in BLAST (*Basic Local Alignment Search Tool*) bioinformatics program, through which the identity percentage for the identification of the isolated yeast was obtained.

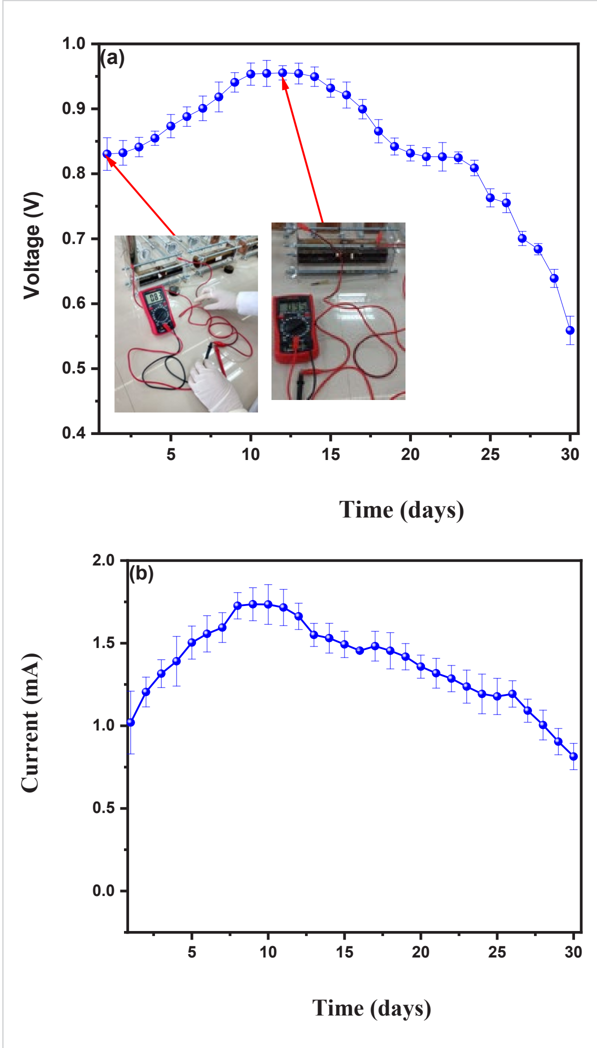
Physico-chemical characterization of MFCs

MFCs were monitored for 30 days at room temperature (~ 22 ± 2°C), in which voltage and current variations were observed by means of a multimeter (Prasek Premium PR-85), while for current and power density measurements, the formulae used by Rojas et al. (2021) were applied, where power density (PD) and current density (DC) were calculated by using external resistors ($R_{ext.}$) of 2.4 ± 0.23, 9.9 ± 2.41, 19.8 ± 3.06, 29.6 ± 5.84, 49.2 ± 8.48, 192.2 ± 19.17, 384 ± 25.34, 564 ± 36.81, 812 ± 53.19 and 996 ± 60.23 Ω, by means of the formulae $PD = V^2_{cell}/(R_{ext.}A)$ and $CD = V_{cell}/(R_{ext.}A)$; where V_{cell} is MFC voltage and the area (A) is 78.54 cm^2. Conductivity (Conductivity meter CD-430), pH (pH meter 110 Oakton series) and degrees Brix (RHB-32 brix refractometer) were also monitored.

Results and Discussion

Fig. 2 (a) shows the average values of the voltages generated over a period of 30 days. The voltage values increased progressively from the first day (0.832 ± 0.098 mV) until day 12 (0.953 ± 0.142 mV). After this day, they slowly decreased until the last day (0.551 ± 0.275 mV). According to An et al. (2017), it was shown that a voltage decrease was mainly due to the decrease of electricity-generating microorganisms because of the parameters of temperature, pH, electrodes used, etc. Likewise, Sreelekshmy et al. (2020) conclude that the accumulation of electrons on the anode surface and the growth of bacteria are due to the degradation of organic compounds through the

Fig. 2. *Monitoring of the generation of (a) voltage and (b) current of MFCs*



interaction of a wide variety of microorganisms and intermediates, such as CH_4 and CO_2, as well as the interrelation of organic substances by exoelectrogens.

Fig. 2 (b) shows the generated current values of MFCs over a period of 30 days. As it can be seen, the values increase from the first day (1.012 ± 0.21 mA) until day 15 (1.73 ± 0.13 mA), and then slowly decay until the last day (0.81 ± 0.172 mA) of monitoring. The capacity to maintain continuous electricity generation may be due to the abundance of fuel (substrate) in the first 15 days. In the initial stage of MFC operation, it can be correlated with the delay period of microorganism formation on the anode surface (Yuvraj and Aranganathan, 2017). Thus, dead biomass is accumulated at the bottom of the MFC and inside the anode fibers producing scale by clogging the anode electrode pores (Kang et al., 2017). *Fig. 3 (a)* shows the average pH values of the MFCs, observing slight increases in pH values from 5.03 to 5.81 ± 0.34 on the first and last day, respectively, staying within the limits of slightly acidic. In the same graph, it was observed that the optimum pH for the highest voltage generation (0.953 ± 0.142 mV) was 5.25 ± 0.12 on day 12. Margaria et al. (2017) showed that for different design and substrate there was an optimum pH at which a voltage and current peak was generated, which basically depends on the optimum pH for the growth of the microorganisms generating this property. Sreelekshmy et al. (2020) showed that, for dual-chamber fuel cells, using wastewater effluents as substrate and titanium-doped steel electrodes obtained higher voltage generation (~ 1100 mV) at an alkaline pH and the change of that variable causes the performance to decline, which is due to the change of the ionization state of the functional groups (carboxyl and amino) in the bacterial biofilm formed on the anode electrode. *Fig. 3 (b)* shows the values obtained for MFC conductivity for 30 days. As it can be observed, the values increased from the first day (88.67 ± 1.11 mS/cm) until day 22 (111.156 ± 8.45 mS/cm). After that period, the value decayed to 101.67 ± 13.45 mS/cm on the last day. Variations in ionic conductivity were mainly due to redox processes at the electrode-electrolyte interface, which could result in the reduction of ohmic resistance (Cruz et al., 2021). Likewise, *Fig. 3 (c)* shows the values of degrees Brix. As it can be observed, the values decayed from day 4 (20.07 ± 0.57°Bx) to day 30 (11.33 ± 2.18°Bx), depending on the monitoring time. This coincides with Rojas et al. (2021), who showed that degrees Brix decreased from the first (22.01 ± 0.57°Bx) to the last day (8.26 ± 0.36°Bx) with grape waste substrate in the MFCs, sugars being the main constituents because they are consumed by yeasts for the fermentation and growth process (Dumont et al., 2021).

Fig. 3. *Monitoring of (a) pH (b) conductivity and (c) degrees Brix values of the MFCs for 30 days*

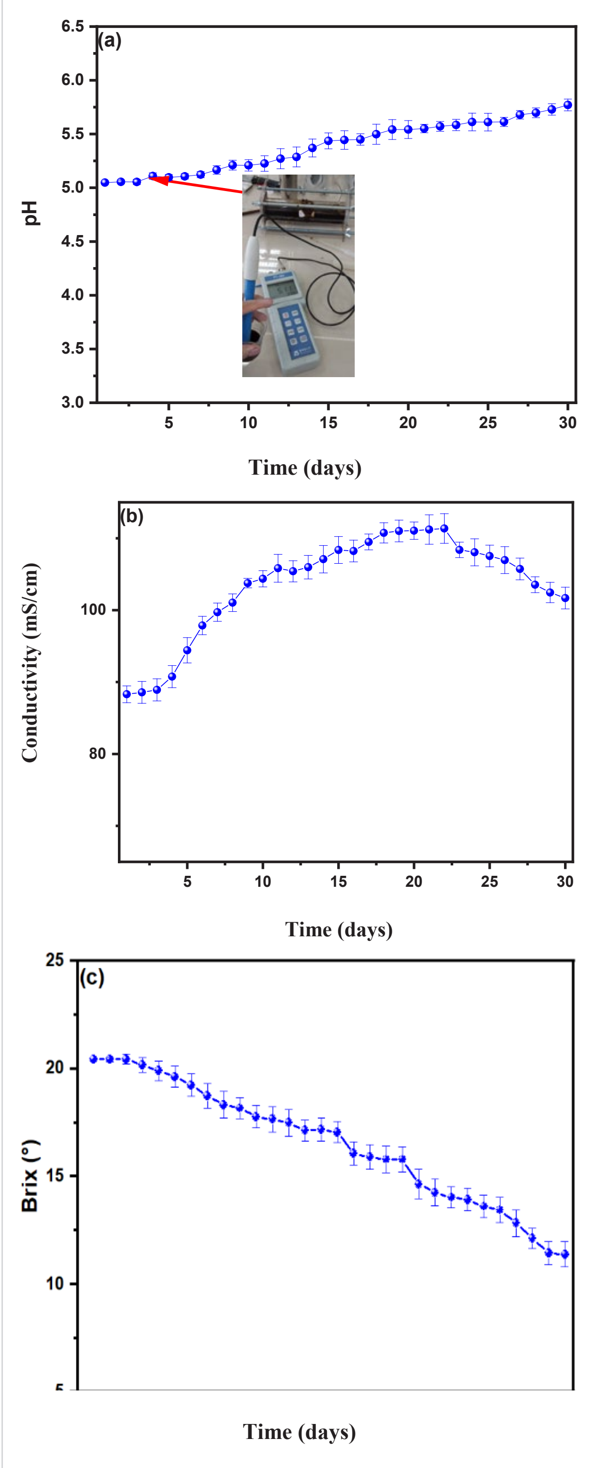


Fig. 4 shows power density (PD) and current density (CD) values of microbial fuel cells. The $PD_{MAX.}$ shown was 5.45 ± 0.31 W/cm^2 at a PD of at a CD of 308.06 mA/cm^2, with a voltage peak of 0.934 ± 0.081 V. The values obtained in this work exceed those investigated by Gazali et al. (2020), in which they used persimmon fruit wastes and organic wastes mixed as substrate (fuel), obtaining a PD_{max} of 0.162W/m^2 at a CD of approximately 1.4 A/m^2 with a voltage peak of 0.22 V (Gazali et al.). Similarly, Moharir et al. (2018) generated lower PD (974.89 mW/m^3) and CD (~85 mA/cm^2) values by using leached food waste as substrate in cells of two types of circulation (with and without air). However, the authors showed that higher PD and CD values can be generated in cells with air circulation.

Fig. 4. *Current density and power density values of MFCs*

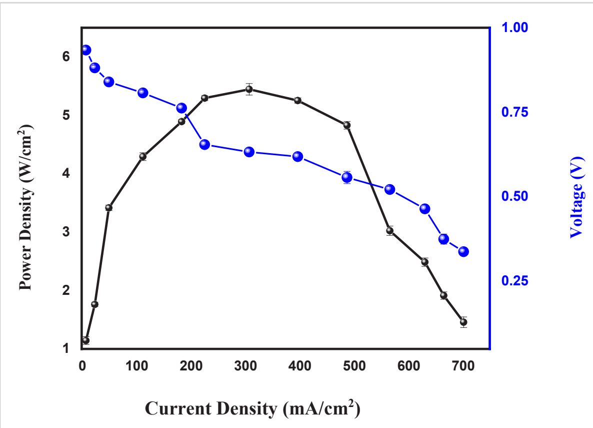
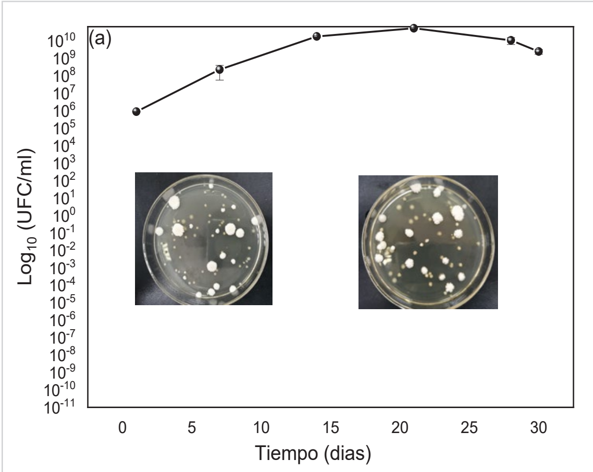


Fig. 5 shows the graph regarding the results of biomass during a 30-day period, where a logarithmic phase was observed that lasted for 20 days. This is due to the fact that yeasts consume the nutrients of the environment (substrate), the composition of molasses being 75%–85% total solids, 30%–36% sucrose, 10%–17% (fructose + glucose), 10%–16% ash, and some smaller amounts of polysaccharides, oligosaccharides, organic acids, proteins and nitrogenous compounds (Shuler, 2002).

For molecular identification, it was necessary to identify ITS regions, which are specific to identify fungi (Schlenzig, 2009). The regions sequenced and

Fig. 5. *Growth curve of the yeast Candida boidinii*

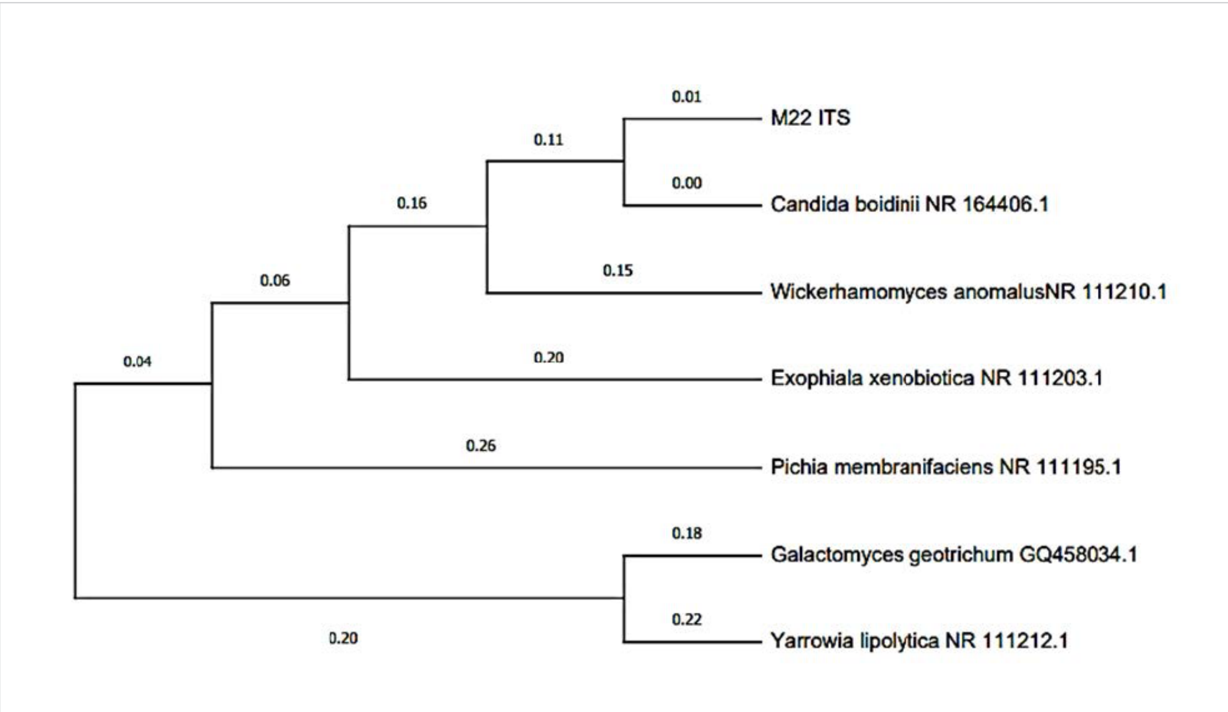


analyzed in the BLAST program obtained an identity percentage of 100.0%, which corresponds to the *C. boidinii* species (*see Table 1*). The genus Candida is of great interest in the generation of bioelectricity in MFC due to the stability and improved catalytic activity of the enzyme Formate dehydrogenase (FDH; Lekseeva et al., 2011). NAD+-dependent FDH enzymes are generally homodimeric proteins and each monomer has a specific active site for both NAD+ and formate. In addition, it plays an important role in the energy supply of methylotrophic microorganisms. On the other hand, Candida species cause the formation of biofilms (Guo et al., 2016). These two features are important in the mechanisms of electron transfer by means of redox mediators or soluble electron transporters and biofilms in order to generate electrical energy (Kumar et al., 2015). The dendogram was based on the ITS regions of the rDNA regions of the yeast isolated from the anode plate where the molasses was located (*see Fig. 6*). This yeast species can be found in other fruits; for example, Mambusca et al. (2013) isolated *C. boidinii* from blackberry and pineapple fruit juices being the natural microbiota of fruits. Rojas et al. (2021) isolated *C. boidinii* from cranberry juice with 100% of identity by molecular techniques generating electrical energy with maximum voltage and current peaks of 1.127 ± 0.096 V and 1.130 ± 0.018 mA on day 8, respectively.

Table 1. *BLAST characterization of the rDNA sequence of yeast isolated from the anode plate of MFC with molasses substrate*

BLAST characterization	Length of consensus sequence (nt)	% Maximum identity	Accession number	Phylogeny
Candida boidinii	658	100.00%	KY101986.1	Cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Saccharomycotina; Saccharomycetes; Saccharomycetales; Pichiaceae; Ogataea; Ogataea/Candida clade

Fig. 6. *Dendrogram based on the ITS regions of the rDNA regions of a C. boidinii culture isolated from the anode plate of MFC with molasses substrate*



Conclusions

Electrical current was successfully generated by low-cost, laboratory-scale microbial fuel cells, using copper-zinc electrodes and sugarcane molasses waste. A voltage and current peak of 0.953 ± 0.142 mV and 1.73 ± 0.13 mA were generated on day 12 and 15, respectively. Ph values remained slightly acidic throughout the monitoring, while conductivity values increased to a peak of 111.156 ± 8.45 mS/cm on day 22 and the degrees Brix decreased from day 4 to the last one. On the other hand, the maximum power density shown was 5.45 ± 0.31 W/cm2 at a current density of 308.06 mA/cm2, while yeast count values showed a logarithmic curve that remained for 20 days.

This research is a solution for large companies in the sugar area, due to the large current values generated and the low cost, which would lead to a sustainable and beneficial system not only for society but also for companies.

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