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Sugar Industry Waste for Bioelectricity Generation

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Microbial fuel cells are presented as the promise of technology to generate electricity by using organic waste. In this research, molasses waste from Laredo Agroindustrial Company was used as fuel, as well as graphite and zinc electrodes, managing to build low-cost cells. It was possible to generate voltage and current peaks of 0.389 ± 0.021 V and 1.179 ± 0.079 mA, respectively. The cells showed that acid pH levels and conductivity values were around 100 mS/cm during the period of the highest bioelectricity generation. The maximum power density was 3.76 ± 0.62 W/cm² for a current density of 247.55 mA/cm², showing a peak voltage of 0.459 ± 0.52 V. The yeasts showed a logarithmic phase up to day 25 reflecting an increase in cell growth. Microbial fuel cells are projected to be the most viable solution for organic waste and clean energy generation problems.

Keywords: waste, molasses, generation, agro-industry, electricity.

Introduction

The main source of energy for economic and social development in the past century was fossil fuels. Their discovery has led society to develop its various activities based on these fuels, which has generated great problems, on the one hand, due to their finite nature and the negative impacts they generate on the environment because of the emission of greenhouse gases (Matsuoka et al., 2015), such as carbon dioxide (CO₂), nitrogen oxides NO_x (N₂O, NO and NO₂), sulfur oxides SO_x (SO₂ and SO₃), volatile organic compounds, among other gases (Sayed et al., 2021). It is therefore vital to develop new sources of energy generation that meet the world's energy needs without compromising the sustainability of the planet. Within the development of energy alternatives, we find the energy obtained from biomass that represents a source of sustainable and renewable production in the future (Do et al., 2020). This is how microbial fuel cells (MFC) emerge, which have gained great attention due to their capacity to generate energy from organic or inorganic compounds through microorganisms (Das, 2017). In these electrochemical devices, organic waste degrades to smaller molecules (Hemalatha et al., 2020) because of microorganisms that play an essential role as they form biofilms in the anode chamber where substrates are oxidized generating electrons, protons and other metabolites. There are single-chamber microbial fuel cells, which have a cathode electrode that is, on one side, in contact with liquid and, on the other, is directly exposed to air. Various studies, such as the one conducted by Guo et al. (2021) showed that single-chamber MFCs, in the absence of a membrane, generate higher output power; therefore, in this study, this design was chosen because they are simpler, as well as economical to build. MFCs can also operate at room temperature and atmospheric pressure (Hemalatha et al., 2020); it is possible to use a wide range of substrates, such as soil sediments, organic waste, among others. Molasses is among these substrates that can be used in MFCs. This is an important by-product resulting from the refinement of sugar cane (*Saccharum officinarum*). This substrate has great potential for power generation, for its high content of fructose and glucose, and is readily available (Liang et al., 2020).

In this sense, worldwide, in 2019, 194 million tons of sugar cane were generated, standing out America, Africa and Asia with the most significant productions; as for countries, Brazil stands out with a production of 604 million tons of sugar cane between 2000 and 2019, followed by India and China with lower production (Risco, 2019). In the case of Peru, sugar cane is produced in three regions of the country: on the coast, with high production, concentrated in the north, such as La Libertad and Lambayeque; in the jungle, in the department of San Martín; and, in the highlands, all year round (Carpio Carrillo, 2019). There are a variety of research works on the generation of energy from molasses in microbial fuel cells. For example, Pandit et al. (2014) studied improved energy recovery from cane molasses by using microbial fuel cells, where they generated biohydrogen from the fermentation of cane molasses and electrical energy, obtaining an output power of 3.02 W/m³. They concluded that the integration of an MFC is a promising way to use the energy of that substrate.

This research aims to give added value to one of the residues of sugar cane, molasses, by using it as substrate in a single-chamber microbial combustion cell. Therefore, the values of voltage, current, conductivity, pH, degrees Brix, current density and power density were monitored. A yeast count was also performed and the biofilms of the cathode and anode electrode were analyzed. This molasses treatment alternative represents an eco-friendly method.

Materials and Methods

A. Construction of single-chamber microbial fuel cells

MFCs were constructed by using polymethylmethacrylate tubes of 5 and 20 cm in diameter and length, respectively, and at their ends, electrodes of zinc (Zn) and graphite (GR) of 5 and 0.05 cm in diameter and thickness were placed. Cu wires (12 mm diameter) were welded to the electrodes. Finally, the MFCs were encapsulated by using an acrylic box to avoid any external contamination, as shown in Fig. 1.

Fig. 1. Scheme of the MFC prototype

B. Sampling and bio-electrochemical analysis

One liter of molasses waste was collected in hermetic bottles from Laredo Agroindustrial Company. Initial parameters of pH, brix, voltage, electric current, power, current density, power density, volumetric power, and yeast count were measured.

B.1 Isolation and identification of microorganisms

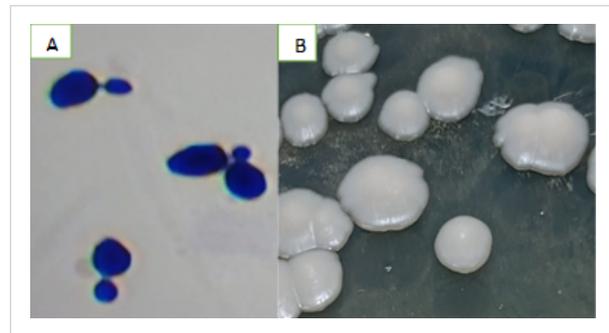
B.1.1. Isolation of microorganisms from the cathode in solid media

Swabs of the cathode plate were performed and sown by stria in culture media of Agar MacConkey, Nutrient Agar and Sabouraud Agar. They were incubated at temperatures of 35°C and 44.5°C (for the isolation of total coliforms) and 30°C for yeasts. The cultures were performed in duplicate.

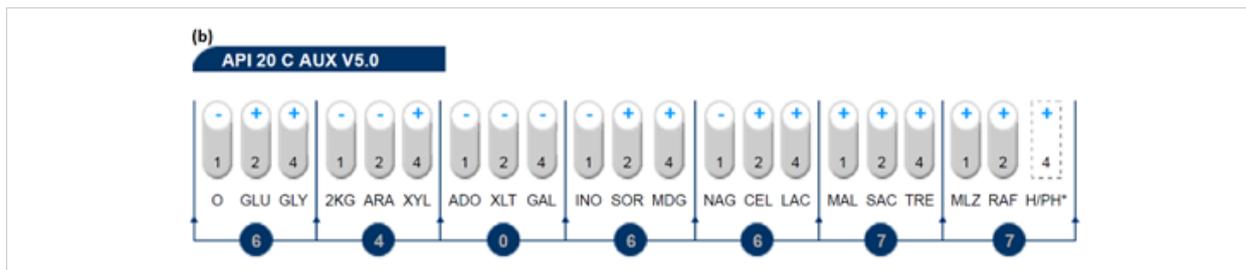
B.1.2. Biochemical identification of yeasts

Axenic (or pure) cultures were made from characteristic yeast colonies. Sabouraud Agar slant culture media contained in screw cap tubes were used. The

identification of genus and species was carried out, recording the microscopic characteristics of oval cells in the process of gemmation (see Fig. 2 (a)), macroscopic white-colored smooth glossy colonies, with edges that vary from entire to lobulated, which are characteristic of the genus *Candida* (see Fig. 2 (b)).

Fig. 2. a) Microscopy of *Candida pelliculose* with Gram staining (100X) and b) A macroscopic view of *C. pelliculose* colonies on Sabouraud Agar with black background

On the other hand, Fig. 3 shows the reading of the microbial identification made by using the API 20 C AUX system of BIOMERIEUX (González Castroagudín, 2017), in which a score is obtained based on each test either positive or negative. The numerical identification of an observed profile is supported by the calculation of its proximity to the different taxa in the database and to the most typical profile in each taxon. Finally, the classification of taxa allowed obtaining an identification result (Gobernado et al., 2021). Subsequently, the results were entered in the API WEB software to determine the genus and bacterial species (API® Reference) confirming that the yeast under study is *C. pelliculose*, with an ID of 98.9 % in the microbial identification system API 20 C AUX. According to Jimoh et al. (2012), it is indicated

Fig. 3. API 20 C AUX identification for *C. pelliculose* (98.9 % ID)

that, as spontaneous fermentation advances, there are several species of yeast involved. In the production of fermented beverages in Nigeria, the following yeasts were isolated: *S. cerevisiae*, *C. colliculosa*, *Candida utilis*, *Candida magnolia*, *R. mucilaginoso*, *Trichosporon asahii*, *Candida pelliculose* and *Cryptococcus albidus*.

C.1 Physico-chemical characterization of MFCs

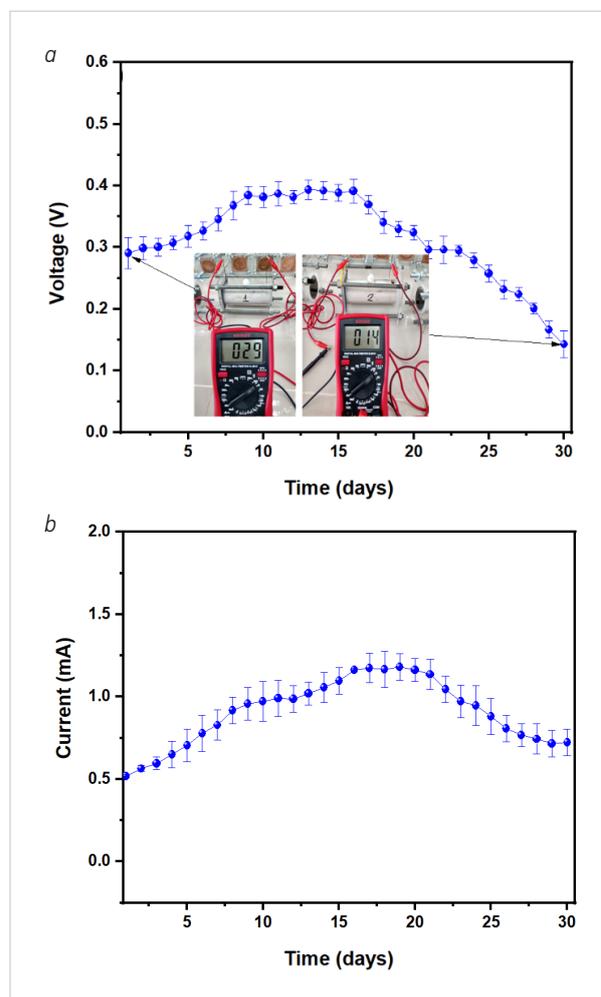
MFCs were monitored for 30 days at room temperature ($\sim 21.5^\circ\text{C}$), in which voltage and current variations were observed by using a multimeter (Prasek Premium PR-85). Meanwhile, for current and power density measurements, the formulae used by Rojas et al. (2020) were used, where power density (PD) and current density (CD) were calculated by using external resistors ($R_{\text{ext.}}$) of 2.4, 9.9, 19.8, 29.6, 49.2, 192.2, 384, 564, 812 and 996 Ω in the formulae $\text{PD} = V_{\text{cell}}^2 / (R_{\text{ext.}} \cdot A)$ and $\text{CD} = V_{\text{cell}} / (R_{\text{ext.}} \cdot A)$; where V_{cell} is the voltage of MFC and the area (A) is 78.5 cm^2 . Conductivity (Conductivity meter CD-430), pH (pH meter 110 series Oakton) and degrees Brix (RHB-32 brix refractometer) were also monitored.

Results and Discussion

In Fig. 4 (a), the average values of the generated voltages of MFCs during a period of 30 days are observed. MFCs increased their values from the first day of monitoring until day 16, where a maximum peak of approximately $0.389 \pm 0.021 \text{ V}$ is observed and, then, it falls until the last day ($0.144 \pm 0.032 \text{ V}$). This research generated higher voltage compared with others. For example, in the research work carried out by Guo et al. (2016), its double chamber cells with catalyst substrates for oxygen reduction generated a peak voltage of 305 mV. Likewise, the research work carried out by Morris et al. (2007), in which groundwater containing petroleum hydrocarbons was used as a substrate, managed to generate 250 mV after the formation of the biofilm. In Fig. 4 (b), the electrical current values generated during the monitoring period are shown; the values increase slightly from the first day ($0.52 \pm 0.039 \text{ mA}$) to day 19 ($1.179 \pm 0.079 \text{ mA}$), after which the loss of electrical current until the last day ($0.707 \pm 0.104 \text{ mA}$) of monitoring is noticed. The generation of electricity is mainly due to the oxidation of organic matter (molasses waste) from where they produce

electrons and are captured by the anode electrode, and pass to the cathode electrode generating a flow of electrons (Saha et al., 2019). The type of substrate is important for any type of biological process, because it serves as a source of carbon (nutrient) and energy (Oyiwona et al., 2018), likewise, the efficiency and economic feasibility of changing organic waste into bioenergy depending on its characteristics and component of the waste material (Igboamalu et al., 2019; Ong and Yamagiwa, 2018).

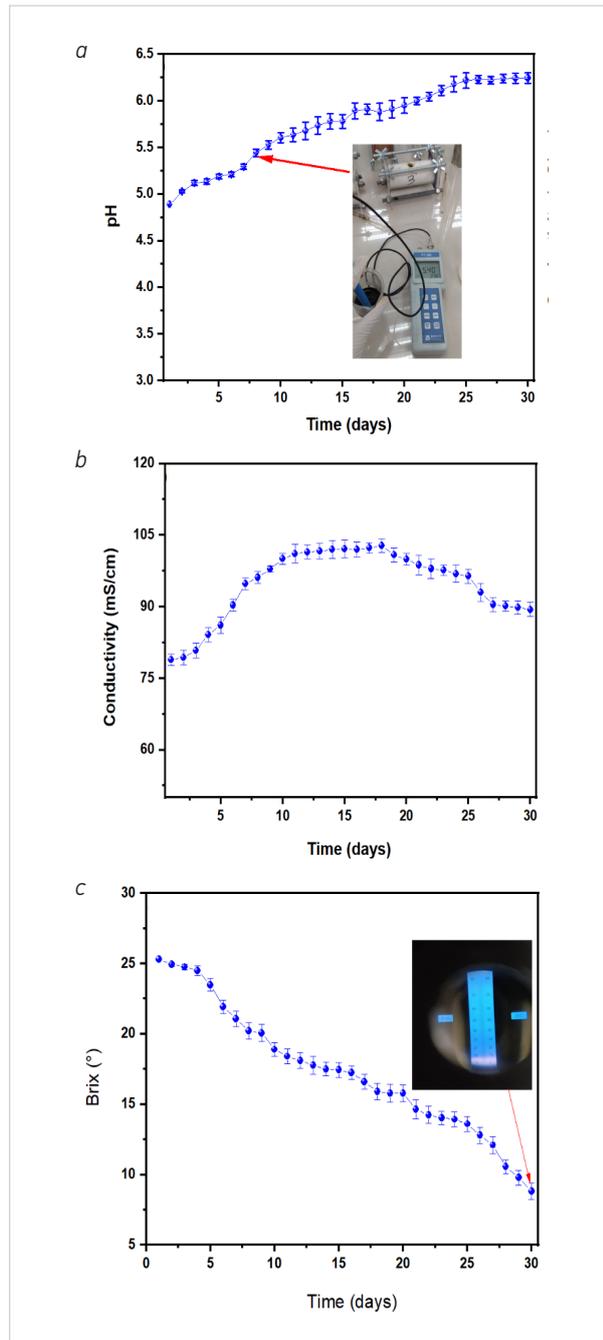
Fig. 4. Monitoring the generation of (a) voltage and (b) current of MFCs



In Fig. 5 (a), the pH values of MFCs are observed. The values increase slightly from the first (4.98) to the last (6.25 ± 0.079) day, resulting in a pH of 5.90 ± 0.081 (from day 19) where a peak voltage value was generated. The increase in pH values is mainly due to microbial activity,

exactly when bacteria approached the stationary or death phase. During this period, large numbers of dead microorganisms were observed settling at the bottom of the reactor (Li et al., 2021; Tamilarasan et al., 2017). In Fig.

Fig. 5. Monitoring of values of (a) pH, (b) conductivity and (c) degrees Brix of MFCs for 30 days

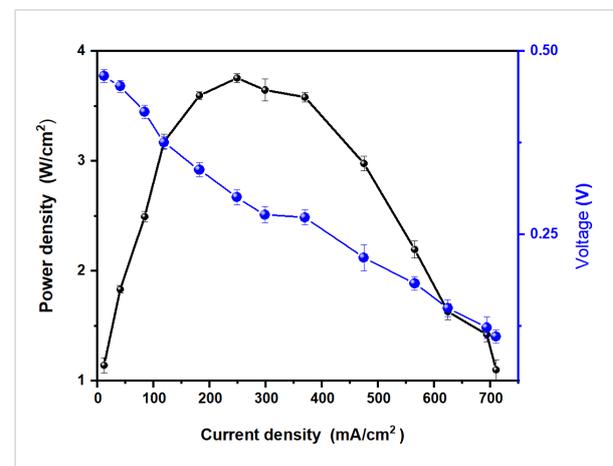


5 (b), conductivity values are observed, which increase from day 1 (79.1 ± 0.03 mS/cm) to day 10 (100 ± 0.23 mS/cm), time in which it remains slightly constant until day 18 (102 ± 0.31 mS/cm), and, finally, it descends slightly to the last day (89.12 ± 0.65 mS/cm). In Fig. 5 (c), the decrease of the values of degrees Brix, from 25.4 to 8.6 on the first and the last day, respectively, is observed.

The values of power density (PD) and current density (CD) of microbial fuel cells are shown in Fig. 6. The PD_{MAX} shown was 3.76 ± 0.62 W/cm² in a CD of 247.55 mA/cm², with a peak voltage of 0.459 ± 0.52 V. The values obtained in this work exceed those reported in Tamilarasan et al.'s (2017) study, in which wastewater from the treatment of surgical cotton was used as substrate (fuel), achieving a PD_{max} of 0.119 W/m² in a CD of approximately 130.5 mA/cm². In the same way, Michalopoulos et al. (2017) generated lower values of PD (~54 mW/m³) and CD (~150 mA/cm²) by using the liquid fraction of a mixture of organic residues and cheese whey as fuel in a double-chamber cell.

In Fig.7 (a), the graph is observed in relation to the biomass concentration over a period of 30 days, where a logarithmic phase that lasted for 25 days was observed, reflecting increased cell growth due to the fact that, at this phase, yeasts consume nutrients by releasing electrons from organic substrates (Ayodele et al., 2020; Sedky et al., 2019), such as sugar cane molasses with approximately 50 % fermentable sugar content, 30–36 % sucrose, 10–17 % (fructose + glucose), polysaccharides, organic acids, proteins, among others (Rodicio

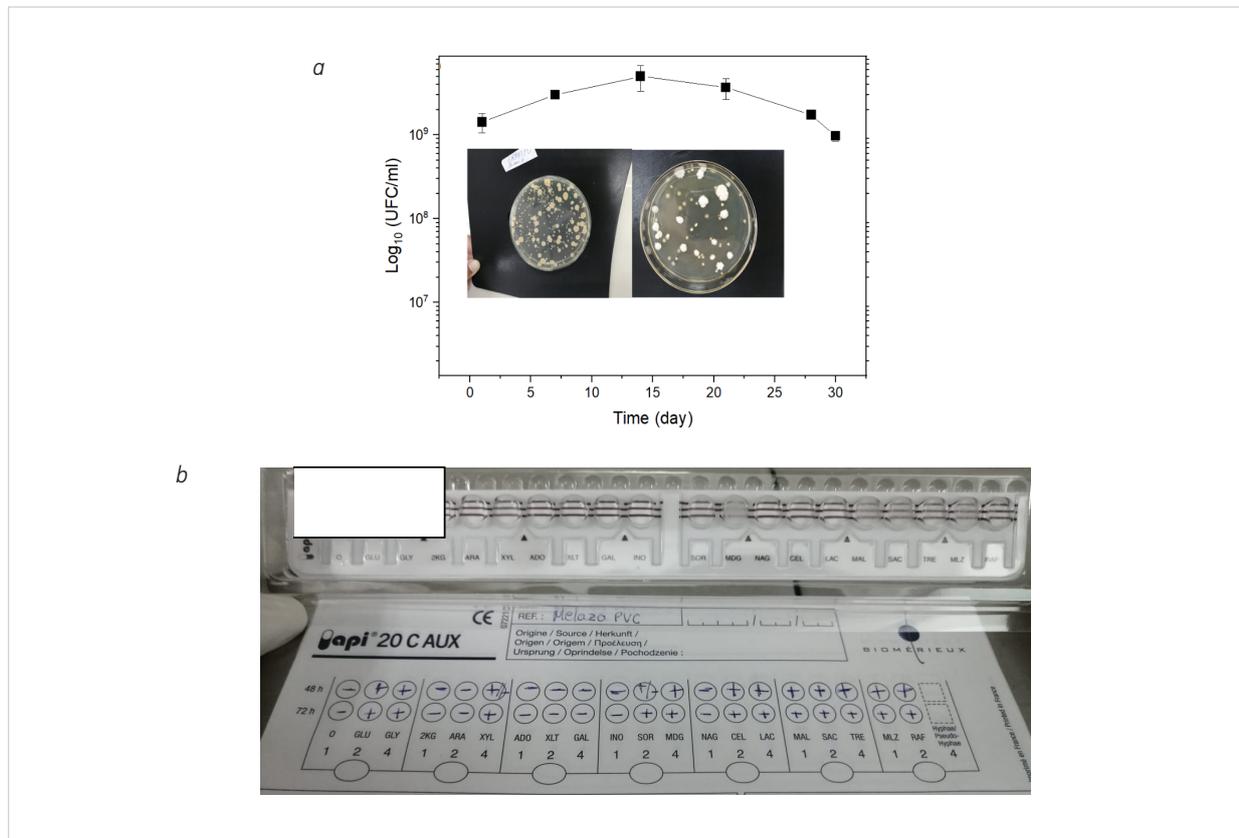
Fig. 6. Values of current density and power density in MFCs



and Heinisch, 2017). These sugars are an excellent source of carbon for yeasts that present different enzymatic complexes responsible for electron transport reactions (Suarez-Diez et al., 2020). These enzymes are able to decompose various substrates, mainly, sugars, through two metabolic pathways, respiration and fermentation, which begin with glycolysis, resulting in the

production of two molecules of pyruvate and ATP per glucose (Perez-Samper et al., 2018). Fig. 7 (b) shows the result that was entered in the API WEB software to determine the genus and bacterial species (API® Reference) confirming that the yeast under study is *C. pelliculose*, with an ID of 98.9 % in the microbial identification system API 20 C AUX.

Fig. 7. Values for (a) yeast count and (b) API 20 C AUX



Conclusions

It was successfully verified that the use of agro-industrial waste, such as molasses waste from Laredo Agroindustrial Company, managed to generate bioelectricity with maximum voltage and current peaks of 0.389 ± 0.021 V and 1.179 ± 0.079 mA, respectively. PH levels were always within the limits of the acidic level, while conductivity values were approximately within the values of 100 mS/cm during the period of greatest

electricity generation, and the degrees Brix always went down from day one to the last day. PD_{MAX} was 3.76 ± 0.62 W/cm² for a CD of 247.55 mA/cm², showing a peak voltage of 0.459 ± 0.52 V. In the yeast count, a logarithmic phase was observed until day 25 reflecting an increase in cell growth. This research work provides an eco-friendly solution for agro-industrial companies to generate their own electricity and reduce costs.

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