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Determination of Electrogenic Potential and Removal of Organic Matter from Industrial Coffee Wastewater Using a Native Community in a Non-Conventional Microbial Fuel Cell

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Abstract: Microbial fuel cells (MFCs) are an alternative to conventional wastewater treatments that allow for the removal of organic matter and cogeneration of electrical energy, taking advantage of the oxidation–reduction metabolism of organic compounds conducted by microorganisms. In this study, the electrogenic potential and the capacity for the reduction of the organic matter of native microbial communities in wastewater from the wet processing of coffee were evaluated using open-cathode MFCs. To determine the electrogenic potential, a factorial experimental design was proposed in which the origin of the residual water and the source of the inoculum were evaluated as factors. The MFCs operated for 21 days in both open-circuit and closed-circuit operation modes. Voltage records, current determinations, and chemical oxygen demand (COD) analyses were used to establish the power reached in the electrochemical system and the degree of the decontamination of the wastewater. During the MFC operation, voltages from 200–400 mV and power and current densities from 300–900 mW·m^{-2} and 10–22 mA·m^{-2}, respectively, were reached. The inoculum used, with a statistical significance of $\alpha < 0.05$, influenced the electrogenic performance of the microbial fuel cell. The previous process of adaptation to the operational conditions of the MFCs of the native microbial community positively influenced the current generation in the system. The degradation rates reached 500–600 mg·L^{-1}·day^{-1}, indicating the metabolic capacity of the microbial community in the MFCs to achieve the decontamination of wastewater from the coffee agroindustry. It was shown the implementation of bioelectrochemical systems constituted a viable option for the treatment of agricultural waste in Colombia. In addition, it was observed the capacity to cogenerate electrical energy from the biotransformation of the polluting organic matter in the effluents of the coffee industry.

Keywords: microbial fuel cell; MFC; electroactive; native microbial community; coffee waste; agro-industrial wastewater

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1. Introduction

In Colombia, agriculture constitutes a fundamental pillar of the regional economy, contributing an estimated USD 6116 million during the first half of 2022 [1]. One of the outstanding Colombian agricultural products is coffee, which is recognized worldwide for its quality. This attribute comes from the cultivated species and the post-harvest process used, known as wet coffee processing [2–4]. This process consists of a series of washing, pulping, and mucilage removal stages that require high pure water consumption, estimated at up to 20 L for each kg of processed coffee beans [4]. In Colombia, between 13 and 14 million 60-kg bags of dry parchment coffee are produced annually [5], and pure water consumption of more than 1.7×10^7 m^3 is estimated for the different stages of wet processing of coffee [4]. Consequently, a large amount of liquid waste, called agro-industrial wastewater (AWW), is generated. In general, agricultural activities intensify in proportion to a population increase to meet the growing food demand, and consequently, the consumption of resources and the generation of waste also increase.

For the removal of contaminants in liquid waste, systems known as wastewater treatment plants (WWTP) are designed and installed [6]. However, these installations are generally found in urban areas or cities, and they are not distributed in agricultural areas. Only 20% of WW treatment is conducted in these plants, mainly domestic WW [7]. Considering that nearly 95% of the harvest and post-harvest of coffee takes place in the artisanal facilities of small farmers, there is no effective treatment and control of AWW discharges [3,8–10]. The difficulties of accessibility and the limitation in the collection of liquid waste from rural areas generate environmental and public health problems due to the dumping of AWW into surface water sources [7].

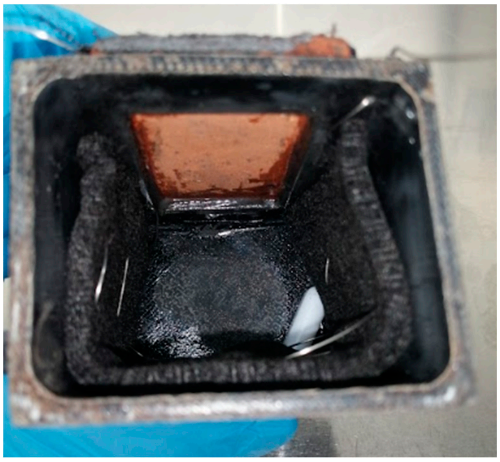
As an alternative to conventional wastewater treatment processes in WWTP, bioelectrochemical systems (BESs), has been proposed [11,12]. BESs are systems that, through the microbial oxidation–reduction metabolism of organic compounds, remove the contaminant load of WW by cogenerating chemical or electrical energy or biofuels, depending on the type of system implemented or treated waste [13]. BESs can be classified depending on the type of cogenerated energy, and the most widely distributed are (i) microbial electrolysis cells (MECs), from which biofuels, such as methane (CH_4) and hydrogen (H_2), are recovered [14–16], and (ii) microbial fuel cells (MFCs) that generate usable energy in the form of electricity [17–20]. The implementation of a specific BES depends largely on the composition of the organic matter that is used as a substrate. The implementation of BES has been reported to have the potential to decontaminate agro-industrial effluents, such as, for example, as mentioned in various studies, the AR treatment of coconut crops, food derivatives, and, in general, various residues from the production of raw materials, raw and processed foods, or even from the agricultural inputs industry [19,21–25]. Different microorganisms, consortia, and microbial communities involved in the generation of energy from the degradation of organic matter have been reported; these are known as electrogenic microorganisms [26–30]. MFCs use microbial metabolism, mainly anaerobes, which release electrons derived from the catabolism of the organic molecules required for their growth, reproduction, or cell maintenance [31,32]. In some stages of wet coffee processing, such as mucilage removal, the native microbial communities of the same coffee load are used in a fermentative process to remove the organic film (mucilage) of the fruit to obtain the grain [8,33]. These native microbial communities in WW have an advantage in terms of adaptation to the environment and do not require conditioning or biostimulus to consume organic matter. The purpose of this study was to determine the capability of native microorganisms used as the inoculum in non-conventional MFCs to generate energy and remove organic matter from industrial coffee wastewater.

2. Materials and Methods

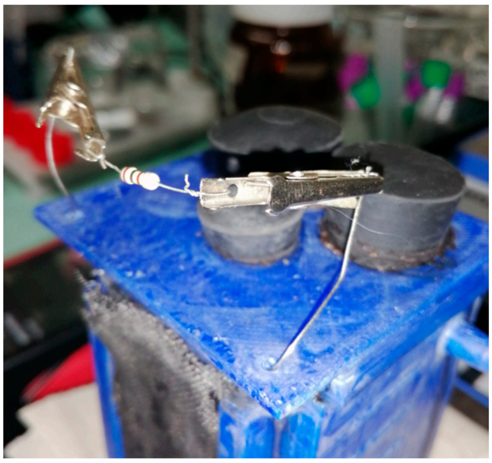
Substrate and inoculum. Industrial coffee wastewater (CWW) from a wet coffee processing plant located in the municipality of Andes in Antioquia, Colombia, was used as the substrate for the MFCs. In the experiment, two different wastewaters were used: WW from a fermentation stage (*S1*) and leachate from a coffee-pulping stage (*S2*). The inocula used were a mixed native microbial community (*I1*) obtained from a 1:1 mixture of both CWW substrates and a mature native microbial community *(I2)* [34] obtained from a previous experiment with non-conventional MFCs. To conserve the inocula, each sample was mixed with a proportion of 15% pure glycerol in 15 mL conical tubes and stored at −80 °C. The substrate samples were stored in plastic containers at 4 °C.

Experimental setup. The open-cathode MFC used in the experiment was fabricated with unconventional materials [34]. The chamber was fabricated with ABS polymeric material. The anode was made of carbon felt (G475, AvCarb®) (0.3 × 6.0 × 12.0 cm). The ion exchange membrane was fabricated with traditional Colombian ceramic materials (Medellín, Colombia) (brick mesh No. 30) (0.5 × 4.0 × 7.0 cm). A carbon cloth (1.77 $g \cdot cm^{-3}$, CCP-30S, Fuel Cell Earth®), 4.0 × 6.0 cm, was used as a cathode, and it was joined to the ceramic membrane with 99% activated carbon with 1:4 sodium chloride binder. Conventional titanium wire (Gr1, CrazyWire® Warrington, UK) was used as a collector in the

MFCs' circuits. The anodic chamber has an effective volume of 200 mL. Figure 1 shows the experimental assemblies.



(**a**)



(**b**)

Figure 1. MFC assembly: (**a**) anode and cathode (internal view); (**b**) closed-circuit resistance connection.

Experimental design and operation mode. To determine the electrogenic potential and organic-matter-removal capability of the native microbial communities found in CWW, a 2^2 factorial experimental design was developed. The sources of substrate (*S*) and inoculum (*I*) were evaluated as factors. The levels of inocula were mixed inoculum (*I1*) and maturated inoculum (*I2*), and the substrate levels were fermentation CWW (*S1*) and leached CWW (*S2*); all these were previously described. The experiment was made in duplicate and with two controls, using an electrogenic medium of reference adapted from Bagchi et al. [35]. The experimental design's set of conditions are presented in Table 1. For each setup, 185 mL of substrate was poured into the anodic chamber, and 15 mL of inoculum was added. The MFCs were operated at room temperature (approx. 24 °C) in open-circuit and closed-circuit operation modes for 21 days during each stage. The substrate in the anodic chamber was maintained in suspension with magnetic stirring equipment (INTLLAB®) operated at 150 rpm. The MFCs were operated in the fed-batch mode with the addition of 10 mL of substrate per day.

Table 1. Experimental design.

Experiment	Inoculum	Culture Media/Substrate
M1 and M5	Mixed inoculum (*I1*)	Fermentation CWW (*S1*)
M2 and M6	Mixed inoculum (*I1*)	Leached CWW (*S2*)
M3 and M7	Maturated inoculum (*I2*)	Leached CWW (*S2*)
M4 and M8	Maturated inoculum (*I2*)	Fermentation CWW (*S1*)
Control 1 (C1)	Mixed inoculum (*I1*)	Reference electrogenic medium
Control 2 (C2)	Maturated inoculum (*I2*)	Reference electrogenic medium

Results register and analysis. Response variables of the experiment were established: the voltage for open-circuit operation, the current determination for closed-circuit operation, and the COD determination for the organic-matter-removal capability. Voltage and current potentials were determined every 24 h with a digital multimeter (UT61C, UNIT® Dongguan City, China). The power and current densities of the MFCs were calculated considering Ohm's law, as shown in Equations (1)–(4). At the end of each open-circuit and closed-circuit stage, polarization curves were made with resistors between 100 and 15,000 kΩ to establish the internal resistance of the system. A cyclic voltammetry analysis was made vs. Ag.AgCl (KCl 3M, Metrohm Autolab® Utrecht, The Netherlands), the reference electrolyte $Fe(CN)_6$ 5×10^{-3} M (in KCl 0.1 M), and reference Au and Pt electrodes (CHI102 CH Instruments® Austin, TX, USA) to evaluate the electrochemical performance of each MFC in this study; for the analysis, an M204 Metrohm Autolab® (Utrecht, The Netherlands) was used. COD analysis was completed in samples taken every 72 h, following the method proposed in SM 5220 [36]. The statistical analysis for the experimental design was made by an analysis of variance (ANOVA) and comparison tests (LSD–Tukey's) with the statistical software R Studio (V. 2022.02.0 + 443 Boston, MA, USA).

3. Results

3.1. Determination of Electrogenic Potential and Removal of Organic Matter

The cyclic voltammetry analyses of the reference electrodes and the experimental electrodes were evidence of the system's ability to transfer electrons (energy generation) in the non-conventional MFCs used in this study. The behavior exhibited in the tests conducted through CV analyses showed the same current peaks for the oxidation of 0.3 mA and −0.3 mA for the reduction of the reference electrolyte ($Fe(CN)_6$) in the tests conducted on the reference electrodes and the carbon electrodes used in the non-conventional MFCs. These results showed a similar magnitude for the redox current, indicating that the MFCs' electrodes fabricated with carbon felt had electric charge transfer properties suitable for energy recovery without limitations in this BES. The voltammograms are presented in Figures 2 and 3.

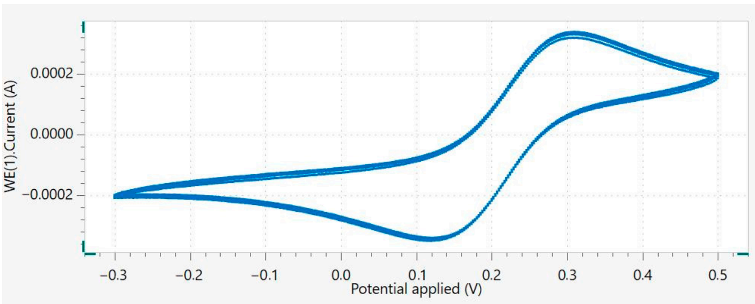


Figure 2. CV voltammogram vs. Ag.AgCl de $Fe(CN)_6$ 5×10^{-3} M (in KCl 0.1 M) with commercial Pt and Au as counter electrode and working electrode, respectively.

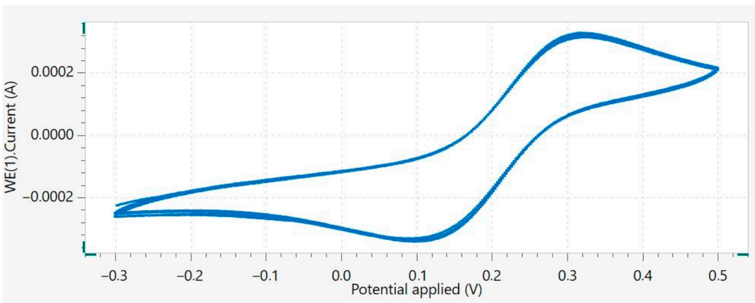


Figure 3. CV voltammogram vs. Ag.AgCl de $Fe(CN)_6$ 5×10^{-3} M (in KCl 0.1 M) with electrodes used in non-conventional MFC assemblies.

The positive voltage measures recorded during the open-circuit operation and positive-current measures obtained during the closed-circuit operation of the MFCs were an indication of the electrogenic potential of the CWW's native microbial communities. Voltage values between 74 and 350 mV were recorded during open-circuit operation. The highest voltages were present in MFCs M4 and M8, which corresponded to the maturated inoculum

(*I2*). These results suggest an influence by the type of inoculum on the performance of the cells. Even with C2, it was verified in a reference culture medium inoculated with *I2* that the electrogenic potential during open-circuit operation was considerably higher. The current in the MFCs during closed-circuit operation was higher in the same experiments for M4, M8, and C2. In general, currents between 0.01 and 0.06 mA were recorded. The MFCs with the mixed inoculum without previous biostimulus (*I1*) showed lower potentials compared with those with maturated inoculum. However, both the voltage and the current measured directly in these MFCs were positive. The summary of the results of the maximum values reached for *V* and *I* at the end of each stage are presented in Figures 4 and 5.

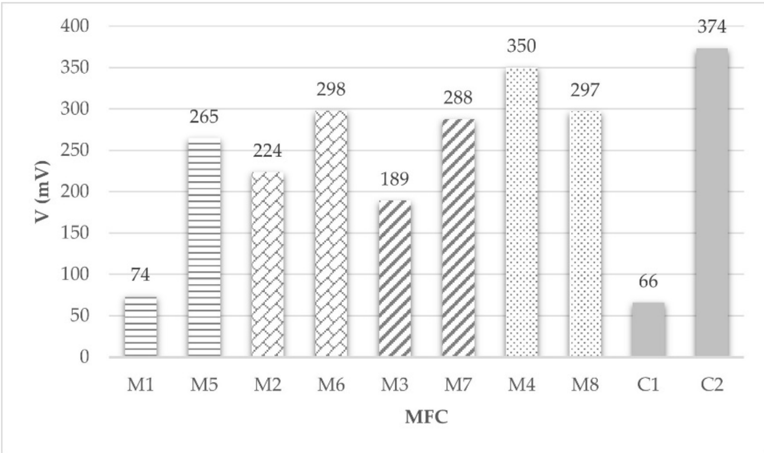


Figure 4. Maximum voltage recorded in each MFC during open-circuit operation; each bar corresponds to an experiment using the experimental design.

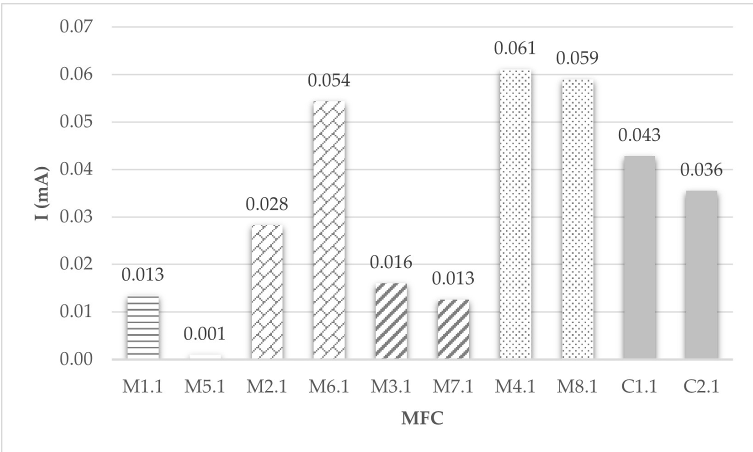


Figure 5. Maximum current recorded in each MFC during closed-circuit operation; each bar corresponds to an experiment using the experimental design.

During closed-circuit operation, there were polarization curves between 100 and 15,000 Ω. The electrical resistance for which the MFC registered the highest potential was considered the minimum resistance to overcome the internal resistance of each system. The summary of the results is shown in Table A1 in Appendix A. The power density (DP) and current density (DI) per unit area of the anode electrode, determined using Ohm's law, are summarized in Figures 6 and 7. In general, the internal resistances of the systems oscillated in the range from 1000–3800 Ω. The resistances of MFCs M3–M7 were superior to 10 kΩ. These results are related to the electrochemical performance of the cells, considering the influence of the culture medium and the microbial biofilms. In Figure A1, presented in the Appendix A, it is possible observe the biofilm formed in the anode. This conformation is a common microbial growth on the surface that could generate transfer mass limitations and affect an MFC's performance. However, the results of the maximum potential determined by means of the polarization curves for each MFC could be an indication of the specific behavior of the microbial community and the origin of the culture medium used in each experiment. These results show that the native microorganisms have an electrogenic potential associated with the oxidation–reduction metabolism of the organic matter.

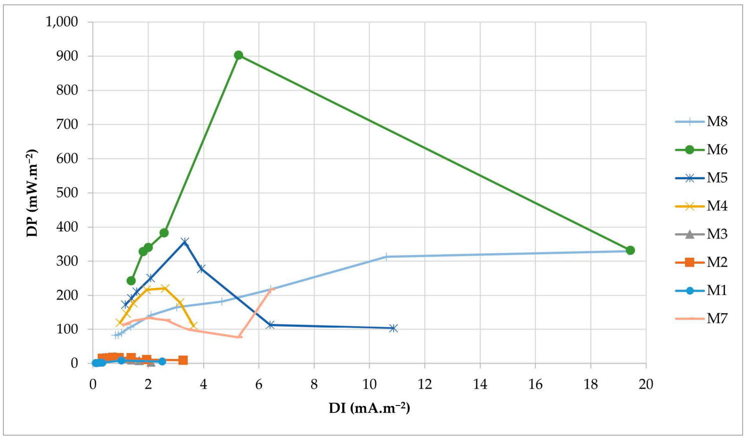
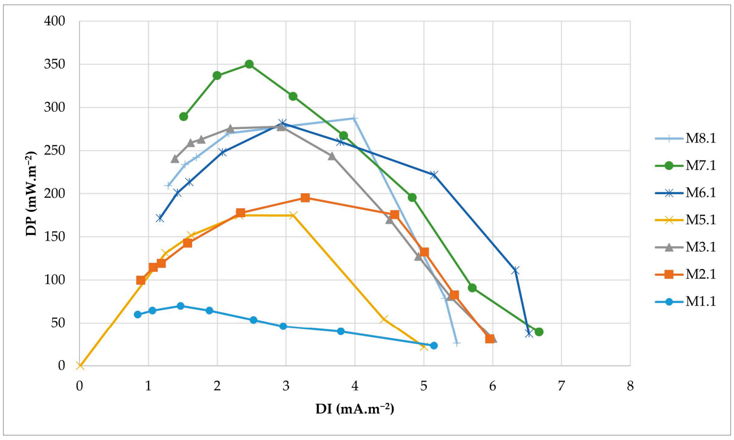
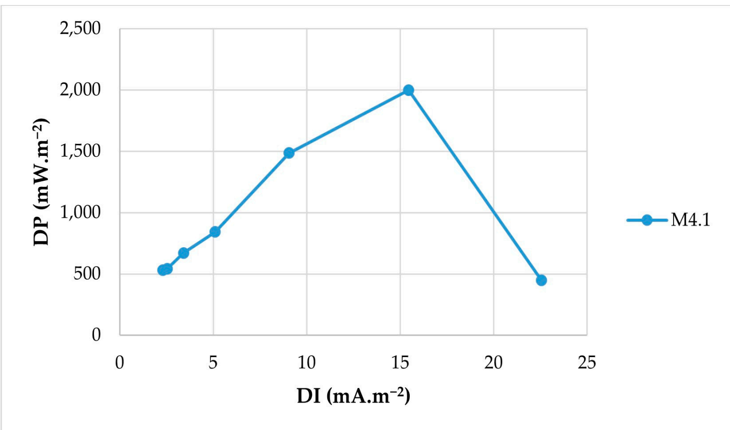


Figure 6. Polarization curves of each MFC at the end of open-circuit operation.



(**a**)



(**b**)

Figure 7. Polarization curves of each MFC at the end of closed-circuit operation: (**a**) experiments M1.1, M2.1, M3.1, M5.1, M6.1, M7.1, and M8.1; (**b**) experiment M4.1.

The electrogenic potential of MFCs is directly related to the ability of the microorganisms in the anodic chamber to metabolize the organic load. The ability of native CWW microbial communities to reduce water contamination was determined by changes in the COD concentration of the industrial coffee wastewater used as the substrate. A significative reduction in the contained organic matter was registered for each experiment. The rates of COD degradation in the MFCs were approximately similar for the open-circuit and closed-circuit operation modes. COD removal rates between 400 and 600 mg L^{-1} day^{-1} were reached, and the results are shown in Figure 8. These results indicated microbial adaptation in a complex substrate, such as CWW, and the ability of the native microbial community to metabolize the organic material in this type of wastewater. Figures 9 and 10 show the behavior of MFCs M4 and M8 in which the highest potentials were obtained. The voltage, current, and COD concentration were tracked as a function of time. A progressive increase in the voltage and current values was obtained. For M4, shown in Figure 9a, a gradually increasing potential was generated from the sixth day until the end of the open-circuit operation. In Figure 9b, after connecting the electrical resistance of the MFCs for closed-circuit operation, a short period of three days of adaptation was observed; after that period, a continuous potential increase was also observed.

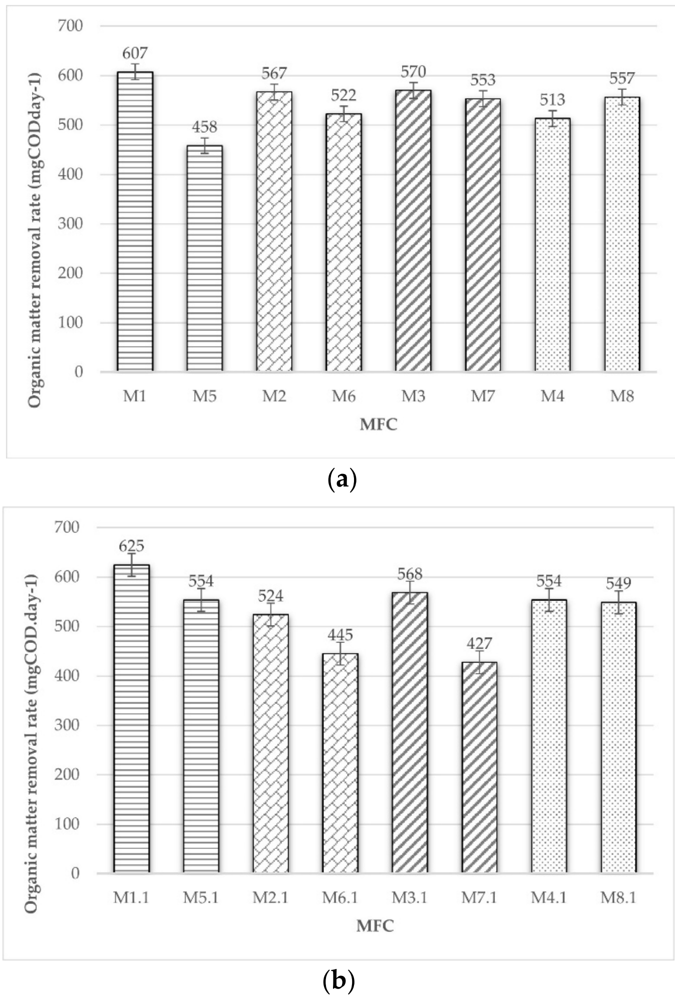
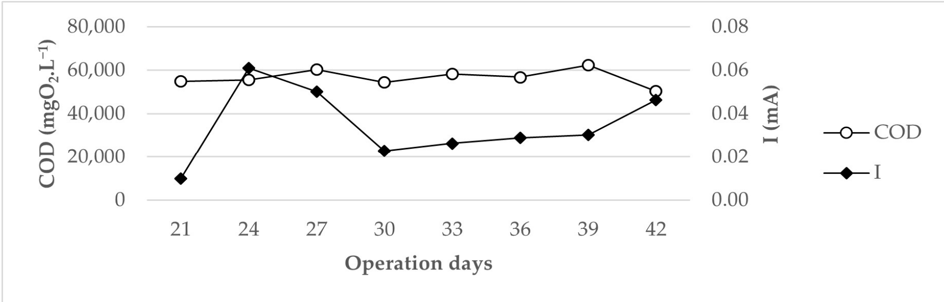


Figure 8. COD rate results for each experiment using the experimental design: (**a**) MFC in open-circuit operation; (**b**) MFC in closed-circuit operation.

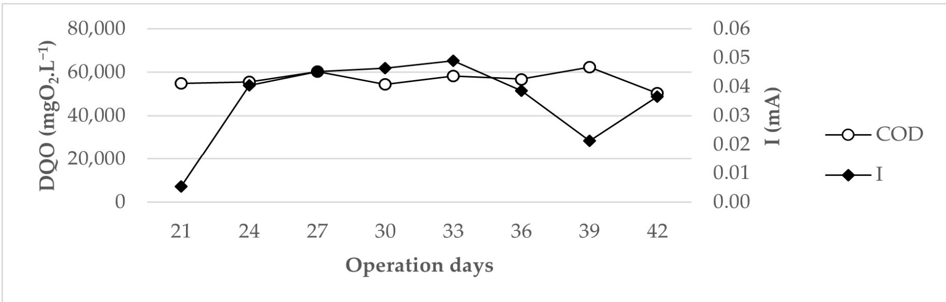
(**a**)



(**b**)

Figure 9. Performance of MFC M4 during 21 days of (**a**) open-circuit operation and (**b**) closed-circuit operation.

(**a**)

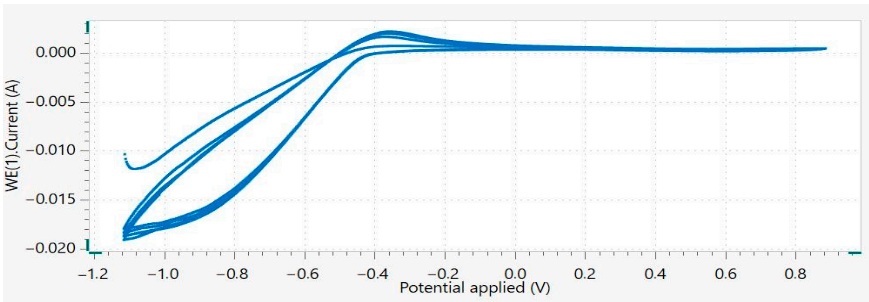


(**b**)

Figure 10. Performance of MFC M8 during 21 days of (**a**) open-circuit operation and (**b**) closed-circuit operation.

For COD concentration, during the first part of open-circuit operation approximately constant values were observed. However, at the end of each operation stage, a reduction in organic matter contained in the substrate occurred. This performance could be explained by the operational mode used in the experiment: the MFCs were operated in feed-batch mode, with a daily substrate addition to maintain a constant volume in the anodic chamber. However, this behavior could be attributed to the microbial specialization of the native microbial community during the MFCs' operation and the substrate's transformation as a consequence of microbial metabolic activity. The relationship between these parameters during the MFCs' performance was inversely proportional, as the organic matter was transformed and consumed by the electrogenic microbial metabolism, while the voltage and current were incremented.

An electrochemical characterization of the wastewater used as the substrate in the anodic chamber was performed by cyclic voltammetry, and the results are presented in Figure 11. The CV voltammograms for *S1* and *S2* are presented in Figure 11a,b, respectively. A negative current is observed in both substrates, indicating that industrial coffee wastewater is mainly composed of species with reduced potential. The presence of oxidation peaks was not detected in the samples, and the current was greater than 0.0 mA. Considering the results and the origin of the initial substrates, their chemical composition is mainly organic molecules with reducing functional groups. The electrochemical characterization by cyclic voltammetry of the substrate at the end of open-circuit operation for MFCs M6 and M8 is shown in Figure 12. In Figure 12a, a positive potential for the CV of MFC M6 was recorded. This result indicates the presence of oxidated organic compounds in the culture medium. On the other hand, in Figure 12b, a slightly negative potential for the CV of MFC M8 was recorded due to the peak having a negative potential. This result suggests the organic compounds were reduced. The low values obtained at the end of open-circuit operation in MFCs M6 and M8 indicate changes in the organic compounds present in the substrates. These results suggest metabolic differences in the substrates' transformation are conducted by the mixed native microbial community in M6 and by the matured native microbial community in M8. Moreover, these results could suggest differences in the intermediated compounds obtained from the leached substrate (*S2*) and from the fermentation substrate (*S1*). In general, all the MFCs with an experimental design exhibited the same electrochemical behavior associated with the physicochemical changes of the medium and the native microbial community that was used.



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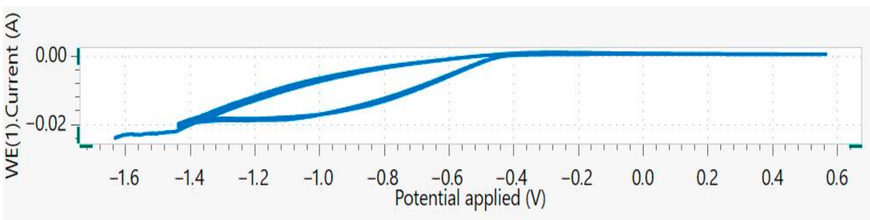
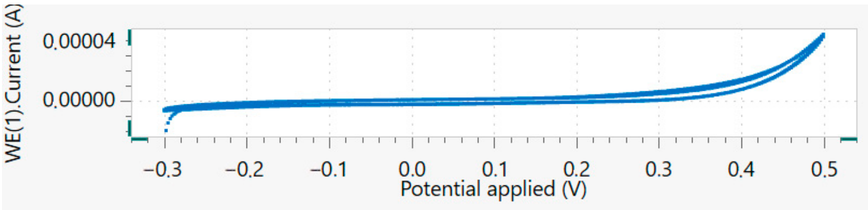
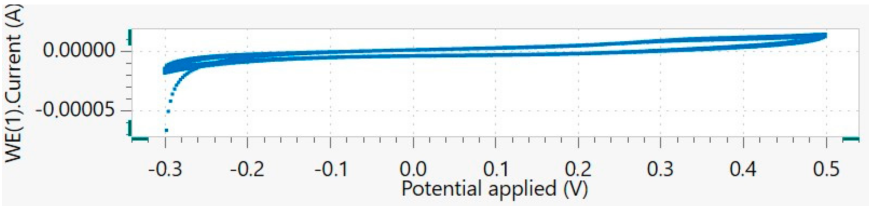


(**b**)

Figure 11. CV voltammograms of CV vs. Ag.AgCl for (**a**) M6 and (**b**) M8 at the end of open-circuit operation.



(**a**)



(**b**)

Figure 12. CV voltammograms of CV vs. Ag.AgCl for (**a**) M6 and (**b**) M8 at the end of closed-circuit operation.

3.2. Statistical Analysis

The results of the multiple variance analysis (ANOVA) shown in Figure 13a indicate that the current obtained using the experimental design, produced by the combination of the inocula and substrates, is statistically significant ($\alpha < 0.05$). However, the current of each factor individually is not statistically significant. After identifying the effect of the interaction between the inocula and substrates on the generation of the current, each level of the inoculum factor was set, and an ANOVA was performed again; the results are presented in Figure 13b. The statistically significant effect corresponded to the matured inoculum (*I2*). However, there was no evidence (results not shown) of the effect of the individual factors or their combination on the voltage-response variable during open-circuit operation. Figure 14 presents the LSD and Tukey's multiple comparison tests. The test was performed to establish which factor majorly influenced on the response variable. The combination of the matured inoculum (*I2*) and CWW from coffee fermentation (*S1*) presented the highest effect. These statistical analyses confirm that the experiments with a better performance were M4 and M8, as they had the highest electrogenic potential. It is possible to attribute a positive effect to the biostimulation of the inoculum before it is used in the MFC. The results show that the maturated native microbial communities in CWW are better adapted to a complex substrate and the operational conditions of the BES.

3.3. Formatting of Mathematical Components

$$I = V/R \tag{1}$$

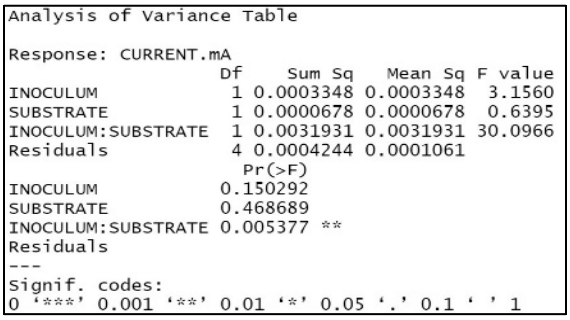
$$DI = I/A \tag{2}$$

Here, I is the current (mA), V is the voltage (mV), R is the resistance (Ω), DI is the current density (mA.m^{-2}), and A is the anode area (m^2).

$$P = I \times V \tag{3}$$

$$DP = P/A \tag{4}$$

Here, P is the power (W), I is the current (mA), V is the voltage (mV), DP is the power density (mW.m^{-2}), and A is the anode area (m^2).



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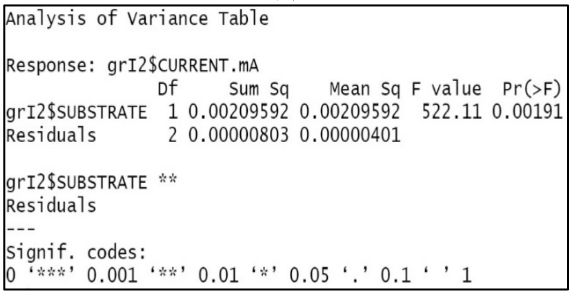
Analysis of Variance Table

Response: CURRENT.mA
                   Df    Sum Sq   Mean Sq F value
INOCULUM            1 0.0003348 0.0003348  3.1560
SUBSTRATE           1 0.0000678 0.0000678  0.6395
INOCULUM:SUBSTRATE  1 0.0031931 0.0031931 30.0966
Residuals           4 0.0004244 0.0001061
                     Pr(>F)
INOCULUM           0.150292
SUBSTRATE          0.468689
INOCULUM:SUBSTRATE 0.005377 **
Residuals
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

(**a**)



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Analysis of Variance Table

Response: grI2$CURRENT.mA
               Df     Sum Sq    Mean Sq F value  Pr(>F)
grI2$SUBSTRATE  1 0.00209592 0.00209592  522.11 0.00191
Residuals       2 0.00000803 0.00000401

grI2$SUBSTRATE **
Residuals
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
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(**b**)

Figure 13. ANOVA analysis of response variable current in experimental design: (**a**) general ANOVA; (**b**) ANOVA fixing the factor maturated inoculum (*I2*) as group (gr*I2*).

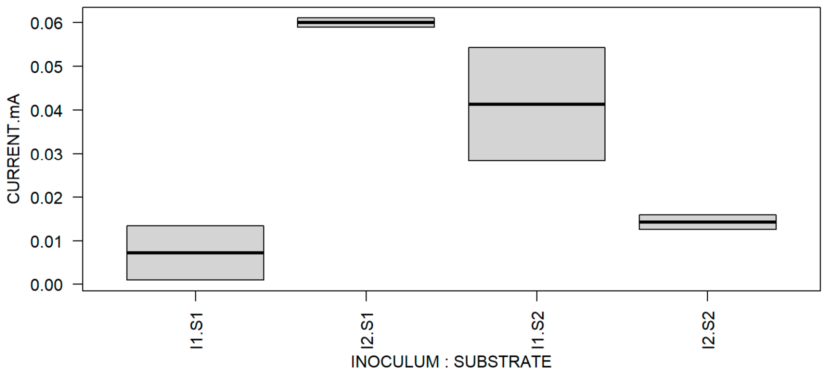

Figure 14. Results of LSD–Tukey's tests.

4. Discussion

As has been reported in some investigations, MFCs have been evaluated mainly in the treatment of domestic wastewater, with potentials between 100 and 3000 mW.m^{-2} reported [13,37–41]. According to the results obtained in this study, microbial fuel cells are promising as an alternative to the conventional treatment of agro-industrial wastewater. Proper functioning by non-conventional microbial fuel cells to obtain electrical potentials and organic matter removal of wastewater from wet coffee processing allows for their application as an alternative wastewater treatment in coffee-growing regions. Conventionally, the microbial communities responsible for efficiency in MFCs arise from the adaptation, maturation, enrichment, or biostimulation of the microorganisms settled in activated sludge or other WW treatments [24,42–44]. However, there are a few reports on the application of these techniques to the native microbial communities obtained directly from the specific wastewater used as the substrate in an anodic chamber. The obtained results indicate the capacity of the native microbial communities in industrial coffee wastewater to remove polluting organic matter. The biostimulus and bioaugmentation of native microbial communities increase the electrogenic potential of non-conventional MFCs, and the native communities are better adapted to a complex substrate composition. The use of an inoculum maturated under the same operational conditions positively influences power generation. These results coincide with other reports, which show that the specialization of microbial communities with electrogenic potential occurs as a function of time [23,45–47] and due to the operational and environmental conditions [42–44,48–50]. The specialization of the native CWW microbial communities in MFCs showed similar potential to those reported in these studies.

The organic matter degradation rates observed in the non-conventional MFCs evidence that the native microbial communities in CWW are adapted for the catabolism of this complex substrate, and they can remove large amounts of organic matter with a high initial COD load in a short period of time. In general, studies of the electrogenic potential derived from the consumption of organic molecules focus on electrogenic metabolism from simple carbon sources, such as glucose, or salts, such as sodium or potassium acetate [51–53]. The organic components of CWW are possibly transformed from large carbohydrates, such as cellulose or starch, to simple sugars, such as glucose and fructose. These compounds are metabolized by microorganisms in routes that release energy in the form of ions. The process is conducted for the native microbial diversity present in the substrate [39,54]. During wet coffee processing, the native microbiota is used during a stage known as the fermentation process to remove the mucilage residues from the surface of the coffee bean [2,3,33]. The mucilage is an organic layer that consists of water, sugars, and pectic substances and has an associated microbiota, mainly yeasts and lactic acid bacteria [8,55]. The native microbiota could be responsible for the early stage of degradation of the complex organic components, transforming them into the intermediates and precursors for the action of microorganisms with electrogenic metabolism and with the potential to release electrons during oxidation–reduction reactions. A diverse microbial community could interact in MFCs and develop a synergistic biological relationship that allows for the specialization of a community with the capability to produce electricity from the degradation of the organic matter in CWW.

Different investigations have addressed the biological diversity associated with the electrogenic potential in BESs, including a large amount of information on electrogenic strains and microbial consortia [27,28,56–59]. Some studies have reported the performance of BES in the treatment of WW with a considerable organic load, in addition to the presence of nutrients derived from nitrogen and phosphorous, for example, in a mixed system known as MFC-CW (MFC coupled to a constructed wetland). Tao et al. (2020) report that the most abundant phyla in an MFC were proteobacteria, cyanobacteria, bacteroidetes, acidobacteria, chloroflexi, and nitrospirae; in this BES, a >90% reduction of nitrates and a power density of 6.09 $mW \cdot m^{-2}$ were achieved using xylose as carbon source compared to 10% and 2.91 $mW \cdot m^{-2}$ using cellulose [60]. In another study using MFC-CW, Ge et al. (2020) recorded a degradation of COD, NO^{3-}-N, total inorganic nitrogen, and total phosphorus of 71.9%, 70.1%, 63.2%, and 91.2%, respectively, as well as current and power densities of 47.77 $mA \cdot m^{-2}$ and 6.74 $mW \cdot m^{-2}$, respectively. The predominant microorganisms found were the phyla proteobacteria, actinobacteria, acidobacteria, bacteroidetes and chloroflexi [61]. On the other hand, in studies on the treatment of hydrolyzed food waste, an open COD productivity of 0.97 $kWh \cdot kg^{-1}$ was found with a cylindrical cathode MFC [37], or, another example, in a residues derived from corn stover treatment, a power density of 296 $mW \cdot m^{-2}$ will be prolonged by purification with a double-chamber MFC [62]. However, to apply BESs for wastewater treatment, it is necessary to know the microbial communities that are responsible for the oxidation–reduction process of organic matter and energy transformation. For example, within a native microbial community of a complex substrate, such as CWW, there could coexist a great diversity of prokaryotic and eukaryotic microorganisms responsible not only for producing usable energy but also for degrading the carbon sources and complex nutrients during different stages [63,64]. In previous studies [34,45,64] the presence of the genera *Acetobacter* sp., *Bacillus* sp., *Clostridium* sp., and *Lactobacillus* sp., among others, were associated with, in addition to an electrogenic potential, the anaerobic or fermentative metabolisms that produce the metabolic intermediates used by different electrogenic microorganisms as substrates.

5. Conclusions

The native microbial communities present in wet coffee processing have an electrogenic potential and the ability to remove organic matter from coffee wastewater using non-conventional MFCs. The results are evidence that BESs could be a promising strategy for the treatment of agro-industrial coffee wastewater. Native microbial communities could be adapted and bio-stimulated to favor electrogenic metabolism. Thus, it is necessary to study the microbial composition of the communities involved in the transformation of organic matter and electricity production to understand their behavior within the system, to propose strategies focused on increasing the efficiency of MFCs for the treatment of coffee wastewater, and on exploring the use of MFCs with different agro-industrial residues. Bio-electrochemical systems are limited to the laboratory level, as their application in the field is a challenge due to the cost of materials; however, with an MFC fabricated with non-conventional materials, it is possible to reduce the material costs and create an alternative to more expensive conventional wastewater treatment systems.

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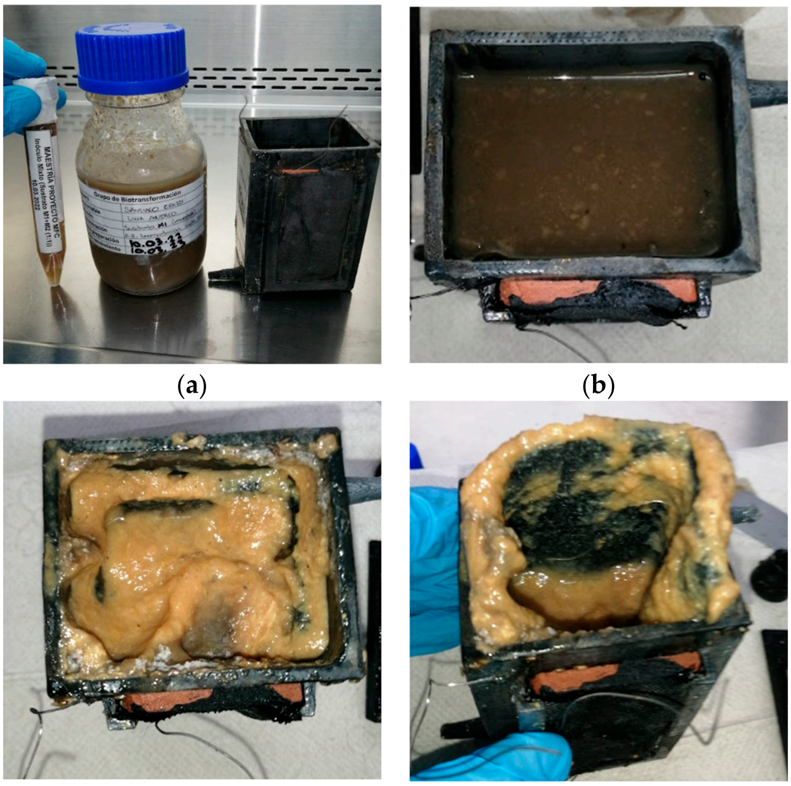
Appendix A

Table A1. Results of polarization curves: maximum potential (DP and DI) for each electrical resistance.

Experiment	Electrical Resistance (Ω)	DP ($mW \cdot m^{-2}$)	DI ($mA \cdot m^{-2}$)
M1	1000	9.2	1.1
M1.1	3800	73.1	1.5
M2	3800	17.8	0.8
M2.1	2200	203.7	3.4
M3	12,000	20.8	0.5
M3.1	3800	289.6	3.1
M4	3800	230.2	2.7
M4.1	1000	286.0	16.1
M5	3800	372.3	3.5
M5.1	1000	205.0	5.1
M6	3800	941.5	5.5
M6.1	3800	293.1	3.1
M7	15,000	155.1	1.1
M7.1	12,000	403.0	2.1

Table A1. *Cont.*

Experiment	Electrical Resistance (Ω)	DP (mW·m^{-2})	DI (mA·m^{-2})
M8	100	343.8	20.1
M8.1	620	337.5	8.2
C1	1000	11.0	0.0
C1.1	10,000	161.0	1.4
C2	1000	868.6	10.4
C2.1	1000	247.7	5.6



(**a**) (**b**) (**c**) (**d**)

Figure A1. Photographs of the interior of the MFC: (**a**) and (**b**) initial or day 0, (**c**) final OC day 21, and (**d**) final CC day 42.

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