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Evaluation of a Low-Cost Device for Monitoring Potential and Enrichment of Microbial Cultures Used in a Biocathode Microbial Fuel Cell

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ABSTRACT. The microbial fuel cell (MFC) constitutes a versatile and sustainable technology which uses the metabolism of microbial cultures for the generation of electric power while simultaneously achieving removal of organic pollutants from effluents. The performance of the MFC is highly related to the microbial activity, thus, a specialized culture could improve the metabolic rates of the reactions and achieve higher power generation. Enrichment in electrochemically active bacteria (EAB) is a technique used to promote the growth of strains able to transfer electrons to solid phase electrodes, i.e., that are able to perform extracellular electron transfer. While there are several examples of bioanode enrichments, studies of EAB enrichment of the biocathode culture to improve cell's performance are limited.

This work aimed at two issues: *(i)* to evaluate a low-cost voltage-measuring device (Arduino UNO) vs a commercial, more expensive multimeter for monitoring the potential delivered by a biocathode a MFC; and *(ii)* to determine the effect of enrichment of cultures on the performance of biocathode MFC. The readings of the Arduino UNO showed an average error of 0.75 mV (with a standard deviation ± 0.66 mV) respect to the multimeter readings. The statistical analysis consisted of the test of hypothesis of means of matched (paired) samples, based on the potentials measured with Arduino and multimeter. The test indicated that there was no significant difference between the potentials.

Maximum enrichment index (ε) of 5.51 mM Fe^{+2} d^{-1} in the second cycle for the biocathode enrichment, similar to ε values reported in literature using similar culture conditions (i.e. 3.61 and 1.95 mM Fe^{+2} d^{-1}) was obtained. For the bioanode enrichment the maximum ε was 24.83 mM Fe^{+2} d^{-1} in the fifth cycle, in previous studies of the work group the ε ranged from 6.5 to 38 mM Fe^{+2} d^{-1} for bioanode enrichments. The batch runs in bioacathode MFC showed a major improvement in cells with enriched bioanode against the one with non-enriched bioanode. Nevertheless, the biocathode enrichment did not lead to any improvement on the cells electrical performance.

INTRODUCTION

The microbial fuel cells constitute a relatively new technology that utilizes microbial cultures as catalysts to transform the chemical energy in organic matter directly into electricity (Logan 2007). Those that use microbial catalysts can be further classified based on their energy source: *(i)* microbial fuel cell MFC, that use a microbial culture to oxidize organic matter, harvest electrons from it, and transfer them to the anode that is externally connected to cathode, generating an electric current. Once in the cathode, the electrons are transferred to a terminal electron acceptor, like oxygen or other oxidized compound; and *(ii)* microbial electrolysis cells (MEC), that utilize an external energy input, allowing half-cell operation and consuming electric power (Liu et al., 2013). Typically, MEC are used for the production of biofuels and value-added products (Hernández-Correa et al., 2017).

MFC has a distinctive advantage: it can use wastewater or other low-grade biomass to produce bioelectricity, which is hardly utilized by other technologies (Zhou et al., 2013).

Microbial cultures seeded to the anodic chamber (often referred to as biocatalysts) anoxically oxidize the substrate or "harvest" electrons from the organic matter, and transfers the electrons to the anode. These electrons are externally conducted to the cathode, where the electrochemical reaction is completed thus generating an electric current. For instance, if O_2 is the electron acceptor in the cathode, when the electrons reach this electrode the O_2 is reduced to water completing the reaction with the protons incoming through the proton exchange membrane separating both chambers (Du et al., 2007), assuming that protons are available. For neutral analytes/catalysts, the O_2 reacts with water to give hydroxyl anions. This reaction has a standard potential very close to the O_2 reduction with protons.

Among the most important advantages of the biocathode are the decreased costs of construction and maintenance of the cell, avoiding the expensive metallic catalyst (typically platinum), and the capability of the microbial metabolism to remove a wide variety of pollutants that could serve as electron acceptors, providing the MFC with a reductive treatment capacity, increasing its applications options and sustainability (He & Angenent, 2006).

Typically, one of the main monitoring variables for cell performance consists of voltage measurements. Cell power P and current intensity I can be easily derived by using the potential and the external resistance (Logan et al., 2006; Poggi-Varaldo et al., 2010) based on Ohm's law for direct current circuits, i.e.

$$I = E / R_{ext} \quad [1]$$

$$P = E^2 / R_{ex} \quad [2]$$

where
E is the potential measured cell potential, R_{ext} is the external resistance.

Bioelectrochemical studies are often limited due to the need of precise and expensive instrumentation for electrochemical measurements. In this work we propose the use of a low-cost microcontroller board "Arduino UNO". The Arduino UNO is an open-source platform with its own compiler that uses simplified *C++* language. Among the features that Arduino UNO offers are the easy to load the code and data acquisition via USB cable, multiple analog ports for multiple voltage readings simultaneously, and the low cost of the board (pricing around 21 $USD in the Mexican market by the time it was bought). However, the Arduino UNO board has only 10 bits resolution for analog to digital conversion. Thus, before using it, its readings must be tested against a commercial voltage measure instrument to evaluate the feasibility of replacing expensive multimeters or potentiometers with Arduino UNO.

Regarding the biocatalysts used in MFCs, cell performance also depends on the microbial activity in both bioanode and biocathode. Microbial activity refers to a special feature that relates to the capability of microbes to transfer electrons to solid surfaces (electrodes). Microbes with this special characteristic are known with a variety of names being electrochemically active bacteria (EAB) on of the most commonly used. So, it is central to use optimum inocula in both chambers (Poggi-Varaldo et al., 2009; 2010).

So far, the enrichment of EAB has been done by two methods: the in-cell electrochemical enrichment, where a voltage or an electric current is applied on the culture for an extended duration (Ortega-Martínez et al., 2010, 2013; Zhang et al., 2011; Doyle & Marsili, 2015;); and the ex-cell enrichments (either chemical or electrochemical, Hernandez-Flores et al., 2015). In the ex-cell chemical enrichment the microbial culture is subjected to selective pressures via incubation with an electron acceptor or donor

depending on the purpose of the culture. Previous studies with ex-cell chemical enrichment of anodic biocatalysts have reported the improvement of MFC performance using Fe^{+3} as electron acceptor (Sathish-Kumar et al., 2013; Hernández-Flores et al., 2015; Vázquez-Larios et al., 2015).

Recently, the biocathode performance has become a bottleneck of biocathode MFC (Poggi-Varaldo et al., 2009; Ortega-Martínez et al., 2010). Moreover, studies on cathodic cultures enrichment are almost non-existent. Gregoire et al. (2014) reported an improvement of the performance of a cathodic inoculum using two consecutive stages: first an ex-cell chemical enrichment incubation with $FeCO_3$ as electron donor followed by electrochemical enrichment in an electrochemical device. Nevertheless, the first stage of the enrichment process was not monitored or quantified. In the open literature, the performance of an enriched biocathode implemented in a MFC has not been reported.

This work aimed at two issues: *(i)* to evaluate a low-cost voltage-measuring device (Arduino UNO) versus a commercial, more expensive multimeter for monitoring the potential delivered by a biocathode a MFC; and *(ii)* to determine the effect of enrichment of cultures for on the performance of biocathode MFC. To the best of our knowledge, this is the first time tha cultures for both bioanode and biocathode are enriched and applied to a biocathode MFC. Also, it is the first time that a low-cost device was tested for cell voltage monitoring and shown that can replace the more expensive multimeter.

METHODOLOGY

Evaluation of Arduino UNO for monitoring cell potential. A double chamber MFC was inoculated with mixed cultures. Each chamber volume was 27 cm^3, and contained 8 cm^3 of graphite flakes totaling an electrode area of 193 cm^2. The external resistance used in the test was a 1000 Ω resistor. The chambers were separated with a Nafion® 117 protonic exchange membrane (PEM). The anode chamber was inoculated with sulfur-reducing culture (SR) and the cathode chamber was inoculated with a denitrifying (DN) culture. The medium used in the anode chamber contained the follow composition, in g/L: $(NH_4)_2HPO_4$ 0.6, KH_2PO_4 0.6, $NaHCO_3$ 0.2, $MgCl_2$ 2 and sodium acetate 6. The catholyte had a similar composition, except that $NaNO_3$ 11.6 g/L was supplemented and sodium acetate was not added. The medium initial pHs were 6.91 and 6.73 for the anolyte and catholyte, respectively.

The cell voltage was simultaneously monitored with both an Arduino UNO and a multimeter (ESCORT 3136A); the readings were set every 15 min. In order to improve the accuracy of the Arduino UNO, we improved the resolution of the Arduino board and decreased the noise. Arduino UNO has a 10 bits analog to digital converter (ADC) resolution, meaning that when using the default voltage reference of 5 V, every digital unit represents 4.88 mV, being a low resolution. To overcome this issue voltage reference of 0.83 V was set to improve the ADC resolution. This needed a constant DC power source, it was used an AA battery as a DC source and was implemented a voltage divider with a 33 kΩ resistor and the internal resistance of 32 kΩ of the Arduino board. According to the voltage divider equation $V_f = V_i * 32 / (32+33)$, the final voltage should be around 0.73 mV, but after reading it with a multimeter, the true voltage (0.83 mV) was added to the code.

Program code for the computer connected to the Arduino UNO. The code was also written to take readings every 100 milliseconds and calculate the average every 20 readings, and print only the results every 15 min. Based on previous tests an adjustment factor was added for better fitting at low voltages, the printing command of the code was modified to work with the public interface PLX-DAQ in Microsoft Excel. The final code is shown in Table 1.

Data analysis. The collected data (potential readings) during the operation of the cell was evaluated with different statistical parameters. Absolute and relative errors were estimated with Eq 3 and 4:

TABLE 1. Program code of Arduino UNO.

```
const int NUM_LEC = 20;
const int NUM_PRINT = 450;
float voltage0 = 0;
float voltagePrint0 = 0;
float voltageAV0 = 0;
float voltage01 = 0;
float TOTAL_LEC = 0;
int Lec = 0;
int i;
int j = 0;
int Index = 0;
float totalLecturas = 0;
float promedioLecturas = 0;
int Index2 = 0;
int inputPin0 = A0;
void setup() {
  Serial.begin(9600);
  analogReference(EXTERNAL);
  Serial.println("CLEARDATA");
  Serial.println("LABEL,TIME,CELL 1 (V)");
  analogReference(EXTERNAL);
}
void loop() {
  while (j < NUM_PRINT){
        for (int i = 0; i < NUM_LEC; i++){
        Lec = analogRead(inputPin0);
        float voltageLEC0 = Lec * (0.83 / 1023.000);
        totalLecturas = totalLecturas + voltageLEC0;
        delay(100);
         }
        TOTAL_LEC = totalLecturas;
        totalLecturas = 0;
     i = 0;
     j++;
  }
     voltageAV0 = TOTAL_LEC/NUM_LEC;
     if (voltageAV0 <= 0.100){
     voltagePrint0 = voltageAV0 + 0.00245;}
     else if (voltageAV0 > 0.100){
     voltagePrint0 = voltageAV0 + 0.0018;}
     Serial.print("DATA,TIME");
     Serial.print(",");Serial.print(voltagePrint0,5); Serial.print(",");
     Serial.println("ROW,SET,2");
     TOTAL_LEC = 0;
     voltageAV0 = 0;
     voltagePrint0= 0;
     j = 0;
     voltage01 = 0;
}
```

Absolute error:

$$e_{Abs} = |E_{Arduino} - E_{mult}| \quad [3]$$

Relative error:

$$e_{rel}(\%) = \frac{|(E_{Arduino} - E_{multo})|}{E_{mult}} x\ 100 \quad [4]$$

where E_{mult} is the voltage reading of the multimeter at any specific time and $E_{Arduino}$ is the potential reading with the Arduino UNO at the same time.

The average absolute error was estimated with Eq 5 below:

$$\bar{e}_{Abs} = \frac{1}{N}\sum|(E_{Arduino} - E_{mult})| \quad [5]$$

Average voltage:

$$\overline{E_{Arduino}} = \frac{1}{N}\sum E_{Arduino} \quad [6]$$

and similarly for the average of the potential measured with the multimeter

The Norm, a common fitting parameter, was defined as:

$$Norm = \sum(E_{Arduino} - E_{mult})^2 \quad [7]$$

where *N* is the total number of readings for each instrument. The error variance, standard deviation and variation coefficient were calculated as follows:

Variance:

$$\sigma_e^2 = \frac{1}{N-1}\sum(E_{Arduino} - E_{mult})^2 \quad [8]$$

Standard deviation:

$$\sigma_e = \sqrt{\sigma_e^2} \quad [9]$$

Variation coefficient:

$$CVar_e = \left(\frac{\sigma}{\bar{E}}\right) x\ 100 \quad [10]$$

A magnitude called “divergence” *d* (to be used for the test of means of matched or paired samples) was defined by Eq. 11 below:

$$d = E_{Arduino} - E_{multimeter} \quad [11]$$

Divergence average:

$$\bar{d} = \frac{1}{N}\sum(E_{Arduino} - E_{mult}) \quad [12]$$

Apart from the descriptive statistics, an hypothesis test of means (for matched or paired samples (Kreyszig, 1972) was performed, using α = 0.999

Null hypothesis, H_0: $\bar{d} = 0$ [13 a]

Alternative hypothesis, H_1: $\bar{d} \neq 0$ [13 b]

We used the statistic Z_{exp}(standardized Gaussian statistic) estimated with the following equation:

$$Z_{exp} = \frac{\bar{d}-0}{\sigma_d/\sqrt{N}} \quad [14]$$

Enrichment of cultures used in the biocathode microbial fuel cell. The purpose of this activity was to enrich biocatalysts in *EAB*. Both anodic and cathodic enrichment cultures departed from previously adapted cultures. The inoculum for the anodic enrichment was taken from a sulfate-reducing reactor, and the cathodic culture from a denitrifying reactor. The anodic enrichment used the technique described by Lovley & Phillips (1986, 1987) where serum bottles containing and insoluble electron acceptor, FeOOH, were inoculated with a sulfate reducing culture and the appearance of Fe^{+2} was determined (as Fe^{+3} was reduced) with a spectrophotometric technique using FerroZine as indicator (Stookey, 1970).

The cathodic enrichment was performed according the first stage of the procedure reported by Gregoire et al. (2014). This technique, also uses consecutive (transfers) serum bottles incubation, and utilizes an insoluble electron donor, in this case, $FeCO_3$. 12 mL of the denitrifying culture was inoculated into serum bottles, completing a final volume of 75 mL with liquid medium. The composition of the medium was (in g/L): $NaHCO_3$ (2.52), NH_4Cl $7H_2O$ (0.3), $MgCl_2$ $6H_2O$ (0.4), KH_2PO_4 (0.6), $CaCl_2$ $2H_2O$ (0.1). Fe^{+2} ion was added from a stock solution of $FeCl_2$ for a final concentration of 6 mM, and 0.606 g KNO_3 was supplemented as electron acceptor for a final concentration of NO^{-}_3 of 10 mM. Fe^{+2} disappearance was monitored with the FerroZine technique, since the microorganisms oxidize the Fe^{+2} to Fe^{+3}.

Every new inoculation into a fresh serum bottle was denominated a pass; the enrichment factor was evaluated at each pass. The enrichment of the anodic culture was evaluated using the enrichment index (Vázquez-Larios et al., 2015) calculated with Equation 15 below:

$$\varepsilon = \frac{\Delta[Fe^{+2}]}{t_{lag}} \quad [15]$$

where

ε is the enrichment index, t_{lag} the time before the activity starts, and Δ [Fe^{+2}] was the concentration difference calculated as the difference between the maximum iron (II) concentration and its initial concentration in a given pass (incubation cycle).

Bioelectrochemical performance evaluation of enriched cultures. To evaluate the enrichment on the cell performance, three cells were inoculated with different inocula. Their configurations are shown in Table 2. The cells used were biocathode MFCs that

consisted in two-chamber cells divided by a Nafion 117 membrane with an area of 9 cm^2. Graphite granules were used as solid electrodes (both in the anode and cathode) for increasing the electrode area, and a graphite rods acted as electron collectors. Each chamber had a volume of 27 cm^3 and the graphite granules occupied 8 cm^3, leaving a working volume of 19 cm^3.

After inoculation, the MFCs we run two weeks of adaptation in the cells with decreasing external resistance, called stages. The biomass concentrations for each culture was reported as volatile suspended solids (in mg/L): *BA* (125), BC (110), SR (450), and DN (1035). For the first inoculation, the cell was left overnight at open circuit voltage (OCV), stage I started the next day closing the circuit with a 5600 Ω resistor. After a week, stage II started with 1000 Ω. The cells were refilled with new medium at the beginning of each stage, the medium composition was (in g/L): $(NH_4)_2HPO_4$ (0.6), KH_2PO_4 (0.6), $NaHCO_3$ (0.2), $MgCl_2$ (0.2), 5 g of sodium acetate for the anolyte and 7.5 g of KNO_3 for the catholyte, plus 10 %v/v of mineral solution and 5 %v/v of vitamin solution.

TABLE 2. Inoculum selection in each cell configuration.

		Inoculum		
	Cell configuration	**Bioanode**	**Biocathode**	
	E1	Enriched anodic culture (*BA*)	Denitrifying culture (*DN*)	
	E2	Enriched anodic culture (*BA*)	Enriched cathodic culture (*BC*)	
	E3	Sulfate reducing culture (*SR*)	Denitrifying culture (*DN*)	

For the main run (stage III), the cell characterization was carried out after refilling the anode with fresh organic substrate and the cathode with nitrate salt. The characterization techniques used were polarization curve (by varying the external resistance) and electrochemical impedance spectroscopy (EIS) with a potentiostat (PARSTAT® 2273) at 10 kHz to 100 mHz with 100 cycles per decade for the internal resistance of the cells by component. The polarization regression line *E-I* and power curve were fitted to experimantal data. The voltage monitoring started after the cell characterization using an Arduino UNO board.

The cell performance was evaluated in terms of several parameters, for the electrochemical performance was calculated the maximum volumetric power ($P_{v,max}$), and maximum power density per unit of surface area of the electrode ($P_{s,max}$).

$$P_{s,max} = \frac{(I*E)}{TES} \quad [16]$$

$$P_{v,max} = \frac{(I*E)}{NVC} \quad [17]$$

where *I* is current intensity, *E* cell potential, *NVC* net volume of the cell, and *TES* the total electrode surface. Current density calculations were carried out with Eq. 18 and 19.

$$j_{max} = \frac{I}{TES} \quad [18]$$

$$j_{v,max} = \frac{I}{NVC} \quad [19]$$

RESULTS AND DISCUSSION

Evaluation of Arduino UNO for monitoring cell potential. The MFC run for the Arduino evaluation lasted 4 days. Figure 1 shows the voltage measurement of both instruments and that of the Arduino UNO follows closely the multimeter line, showing only some observable noise in the readings.

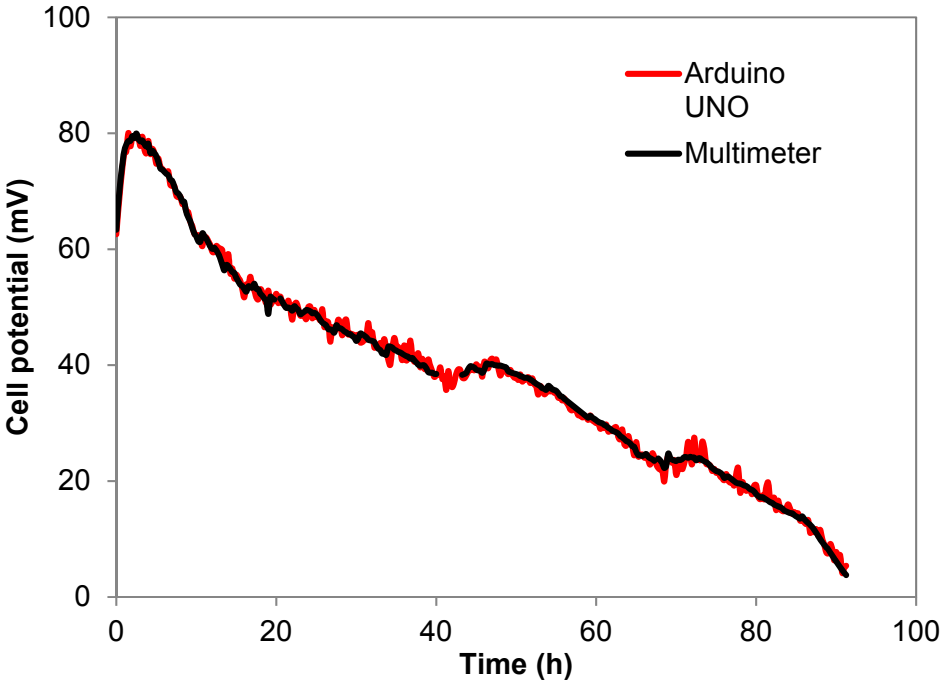

FIGURE 1. Potential readings of a bioacthode microbial fuel cell using Arduino UNO and Escort multimeter.

Table 3 displays the main statistical results of the evaluation. The average cell voltages of the batch operation were 38.84 ± 18.26 mV and 38.87 ± 18.21 mV for the multimeter and Arduino, respectively. Differences between standard deviations was also insignificant. The average absolute error of the Arduino UNO was 0.75 ± 0.66 mV, this represents a relative error under 1% when working with potentials over 80 mV, easily achieved in a MFC.

TABLE 3. Statistical parameters of Arduino UNO evaluation experiment.

Parameter	Arduino	Multimeter	Hypothesis test of means (matched or paired samples)
N[a]	350	350	NA[j]
$\bar{E}$ [b](mV)	38.87	38.84	NA
S_E [c](mV)	18.21	18.26	NA
$\bar{e}_{abs}$ [d](mv)	NA	NA	0.75
$\bar{e}_{rel}$ [e] (%)	NA	NA	2.87
Norm ((mV)^2)	NA	NA	357
Divergence mean squares (mV)	NA	NA	0.054
Variation coefficient (%)	46.86	47.01	NA
$\bar{d}$ [f](mV)	NA	NA	0.026
S_d [g](mV)	NA	NA	1.01
Z_c [h](-)	NA	NA	$-Z_{c1}$ = -3.29; Z_{c2} = +3.29 for α = 0.001
Z_{exp} [i](-)	NA	NA	0.486

Notes: [a] sample size; [b] average voltage; [c] voltage standard deviation; [d] average absolute error; [e] average relative error; [f] average divergence; [g] Divergence standard deviation; [h] critical *Z*-value; [i] experimental value of *Z*; [j] not applicable.

For the hypothesis test the value parameters were: $\bar{d}$ = 0.026 ± 1.01 mV, and the sample size N was 350. The Z_{exp} value was

$$Z_{exp} = \frac{0.026-0}{1/\sqrt{350}} \quad [20]$$

$$Z_{exp} = 0.486 \quad [21]$$

According to Z-score tables, the Z_{c1} and Z_{c2} limits of the acceptance region were -3.29 and +3.29, respectively (Table 3). Since Z_{exp} = 0.486, it belongs to the acceptance region of the null hypothesis (H_o: $\bar{d}$ = 0), that is, -3.29 < 0.486 < 3.29. Therefore, there is no evidence to reject the null hypothesis so $\bar{d}$ = 0. With this result, we can confirm that we did not find significant difference between the voltage measurements of both instruments.

Enrichment of cultures used in the biocathode microbial fuel cell. Table 4 exhibits the summary of the enrichment passes. The anodic enrichment showed increases of maximum Fe^{+2} concentration and progressive decreases of lag time with each pass, reaching its maximum enrichment index with 24.83 mm Fe^{+2} d^{-1} at pass 5, and maintaining a high activity afterwards.

On the other hand, the cathodic enrichment showed a different pattern when incubated with an carbon source sodium acetate (*BC + FC*) against the incubation without carbon source (*BC*). In the enrichment with no carbon source, the Fe^{+2} disappearance decreased with each pass; in contrast, the bottles with carbon source showed a slight decrease of the

enrichment index in the first pass, but maintained a steady index afterwards, reaching its maximum at pass 1 with a enrichment index of 5.51 mM Fe^{+2} d^{-1}. This pattern shown by the cathodic enrichment could possibly be due to the maintenance of the biomass. Indeed, chemolithoautotrophic microbes, likely to be present in the flasks, could not produce enough biomass in the incubation time to counter the biomass wash out with each pass. In contrast, bottles with iron carbonate and sodium acetate could have higher microbial diversity with some heterotrophic microbes sustained by the organic carbon source. In previous studies, Neubauer et al. (2002) and Sobolev & Roden (2004) reported biomass yields of 0.58 and 0.6 g of biomass/ mol Fe^{+2} using oxygen as electron acceptor. Hafenbradl et al. (1996) reported a very low biomass yield of 0.00278 g biomass/mol Fe^{+2} working with nitrate as electron acceptor. The heterotrophic metabolism of the acetate consumption could maintain the biomass after passes, as reported previously by Sobolev & Roden (2004); they found a 3 times increase of the biomass yield when adding sodium acetate as auxiliary carbon source.

TABLE 4. Evolution of the enrichment indices of microbial cultures in the three enrichment processes tested in this work.

	ε (mM Fe^{+2} d^{-1})				
	Pass 0	Pass 1	Pass 2	Pass 3	Pass 4
BC	0.19	6.44	2.69	0.25	0.15
BC+FC	0.02	5.51	4.47	4.62	4.85

	ε (mM $Fe^{+2}d^{-1}$)						
	Pass 0	Pass 1	Pass 2	Pass 3	Pass 4	Pass 5	Pass 6
BA	1.29	0.79	0.38	8.28	24.11	24.83	10.99

Notes: *BC*: enriched biocathode culture without sodium acetate; *BC+FC*: enriched biocathode culture with sodium acetate; *BA*: enriched anodic culture.

Bioelectrochemical performance evaluation of enriched cultures. The voltage monitoring of the experiment is shown in Figure 2, along with the duration of every stage. The cell operation had 13 days adaptation previous the experiment corresponding to stage I and II. After that, the cells were refilled and characterized, starting the stage III monitoring shortly after.

The cell *E1* showed in general a higher cell potential at stage I (when connected to a higher external resistance); afterwards, the potential decreased as the external resistance decreased. Inversely, potentials of cells *E1* and *E2* increased as the external resistance diminished. This could be attributed to the enrichment of the cultures: cells *E1* and *E2* both had enriched cultures in the anode, whereas in *E3* neither of the cultures was enriched, preventing the cell to work with high current intensities associated to lower external resistances.

At the beginning of Stage III, a cell characterization was carried out with different techniques (polarization curves and electrochemical impedance spectroscopy, EIS). Figure 3 displays the polarization curves and power curves of the three cells at the beginning of stage III. The R_{int} determined with both techniques are shown in Table 5.

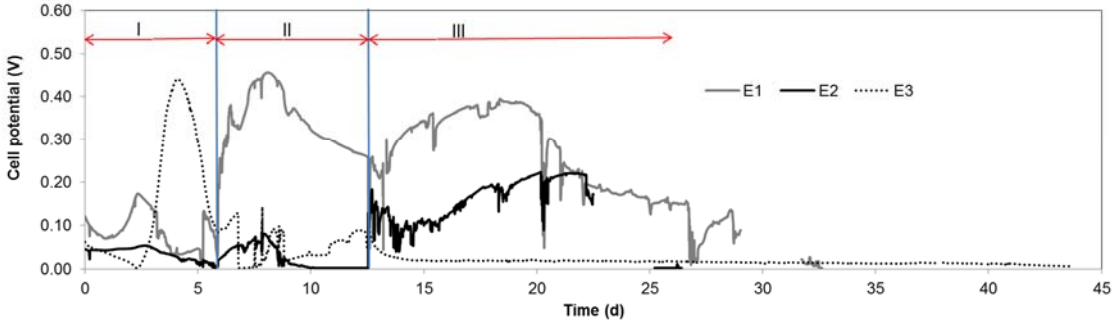

FIGURE 2. Potential time courses of cells loaded with enriched cultures and control cell.

Keys: I, II, and III, stages or periods of operation; *E1*, *E2*, *E3* biocathode fuel cells loaded with enriched inocula. *E1*: continuous gray line, *E2*: black gray line; *E3*: dotted line.

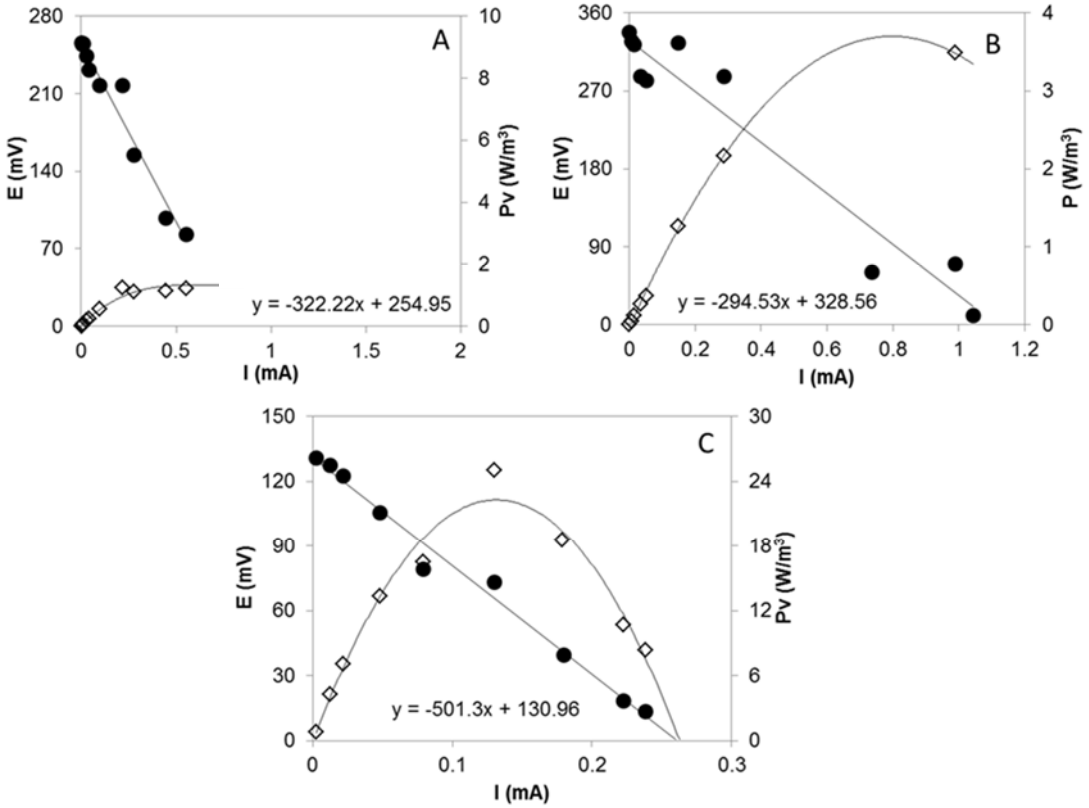

FIGURE 3. Polarization curves (●) and power curves (◇) of the three cells: (A) *E1*; (B) *E2*; (C) *E3*.

The cells *E1* and *E2* showed relatively similar R_{int} with both techniques, with a difference of 50 Ω for *E1* and 73 Ω for *E2*; differences of this magnitude were reported previously by Sathish-Kumar et al. (2012). They observed a difference of 82 Ω between values of internal resistances when characterizing a single-chamber MFC with abiotic cathode.

On the other hand, cell *E3* showed a greater difference between values of R_{int} obtained with different techniques, *ca.* 221 Ω. Vázquez-Larios et al. (2010) reported a similar difference of 180 Ω for a single-chamber MFC with extended electrode, and a 585 Ω difference for a single-chamber MFC with sandwiched cathode PEM. These studies were carried out in single chamber, atmospheric air cathode MFCs whereas our present work used a double-chamber MFC with denitrifying biocathodes. Our configuration could add resistive components not considered in the equivalent circuit for the simulation model (Hidalgo et al., 2015), this extra resistance may be shown with the polarization curve thus explaining the differences in the R_{int} values obtained with the two techniques.

Table 5. Internal resistance determined with both polarization curve and *EIS* at the beginning of stage III

	Internal resistance (Ω)	
Cell	**Polarization Curve**	***EIS*[a]**
***E1*[b]**	322.2 ± 15.3	372.9 ± 16.7
***E2*[c]**	294.5 ± 13.4	377.1 ± 15.8
***E3*[d]**	501.3 ± 17.9	280.2 ± 14.7

Notes: [a] electrochemical impedance spectroscopy; [b] cell configuration *E1*; [c] cell configuration *E2*; [d] cell configuration *E3*.

The external resistances for the stage III operation were 100 Ω for cells *E1* and *E2*, and 560 Ω for *E3*. The summary of the cells' performance is reported in Table 6. It was noticeable how the electrochemical parameters such as volumetric power and current densities increased when progressing stages in time. The results of both cells *E1* and *E2* were remarkable for biocathode MFCs. The maximum volumetric power achieved by the cell *E1* was the highest, with 41.13 W m^{-3}, followed by the cell *E2* with 13.1 W m^{-3}, and cell *E3* reaching only 4.78 W m^{-3}. Oon et al. (2016) worked with a double-chamber cell with 1000 Ω R_{ext} and nitrate as electron acceptor and achieved volumetric power of 669 mW m^{-3} and current density of 3.48 A m^{-3}. Compared to our results in stage II with a similar R_{ext} we observe that our cell *E1* reached higher results, with 5.48 W m^{-3} and 12.01 A m^{-3}. However, cell *E2* and *E3* fell behind in performance. Zhang et al. (2011) reported 99 W m^{-3} working with an aerobic biocathode utilizing an R_{ext} of 100 Ω; the electrode used was a combination of graphite fiber brush and graphite granules. When graphite granule was used as the only electrode material, as the current work, they reported a lower power of 72.8 W m^{-3}.

Electrodes with enriched inoculum exhibited slightly higher resistances than their non-enriched counterparts. This could be attributed to deposits of the iron species (Fe^{+2}/Fe^{+3}) present in the enriched inoculum, increasing its resistance. However, the R_{int} of the three cells were similar, so the internal resistance argument alone cannot fully explain the difference in the performance of the cells. Another factor that could explain the behavior of the cells is the biomass concentrations. The biomass concentration of each culture was (in mg/L): *BA*, 12; *BC*, 110; *SR*, 450; and *DN*, 1035. Cells inoculated with *BA*-seed howed a better performance than that seeded with *SR* inoculum, even if the biomass load was 260 % higher for *SR* inoculum. This confirms the benefits of the bioanode enrichment improving the culture efficiency.

In spite of the inoculum in the biocathode of cell *E1* was non-enriched, it resulted in higher power generation than cell *E2*, whose biocathode was loaded with enriched culture *BC*. This was unexpected. Yet, it could indicate that the denitrifying culture was able, to

some point, to handle the current generated with the enriched bioanode. The average power generation during the experiment was 18.41 W m^{-3} for *E1*, that is more than double the average volumetric power of cell *E2*, with 7.36 W m^{-3}. However, the biomass load at inoculation time was almost ten times greater in cell *E1* than the one in cell *E2*. This non-proportionality between power generation and biomass load could indicate a higher activity by the enriched inoculum.

TABLE 6. Electrochemical parameters of the experiment.

Stage	E_{av} [a] (mV)	$P_{s,max}$ [b] (mW/m^2)	$P_{v,max}$ [c] (mW/m^3)	$P_{v,av}$ [d] (mW/m^3)	j_{max} [e] (mA/m^2)	$j_{v,max}$ [f] (mA/m^3)
I. R_{ext} [g]= 5600 Ω						
E1[h]	87.1 ± 41.6	0.139	142	43.9 ± 38.3	0.80	815
E2[i]	35.7 ± 14.1	0.013	13.7	6.9 ± 4.2	0.25	254
E3[j]	159 ± 153	0.900	914	230 ± 315	2.04	2073
II. R_{ext} = 1000 Ω						
E1	341 ± 65	5.40	5487	3178 ± 1200	11.83	12016
E2	24.7 ± 25.4	0.19	192	33.1 ± 46.2	2.21	2247
E3	52.3 ± 34.5	0.53	540	103 ± 107	3.71	3771
III. R_{ext} = 100 Ω						
E1	244 ± 105	40.5	41132	18415 ± 13512	104.0	105682
E2	160 ± 48	12.9	13104	7376 ± 3845	57.8	58715
*E3**	21.1 ± 7.4	0.47	479	25 ± 38	4.7	4743

Notes: * the external resistance was 560 ohms; [a] average cell potential; [b] maximum power density; [c] maximum volumetric power; [d] averace volumetric power; [e] maximum current density; f maximum volumetric power; [g] external resistance; [h] cell configuration *E1*; [i] cell configuration *E2*; [j] cell configuration *E3*.

CONCLUSION

The evaluation of the Arduino UNO device for MFC voltage measurement showed that potentials from Arduino and multimeter were not significantly different. General statistical analysis of data as well as the test of hypothesis of means (for matched or paired samples) showed that potentials measured with Arduino and multimeter Escort were equivalent. Therefore, replacing the multimeter by Arduingo UNO is feasible and economic: Arduino UNO costs represent less than a 1% of the price of the commercial multimeter. In addition, Arduino UNO has up to 5 channels (allowing the simultaneous monitoring of five cells) whereas the multimeter Escort has only one.

The ex-cell enrichment of the cultures lead to an improvement of the electrochemical performance in some cases. The cells with enriched cultures (*E1* and *E2*) showed higher electrochemical performance than the cell with non-enriched cultures (*E3*), confirming the

effectiveness of bioanode enrichment and its implementation in double chamber *MFC* with biocathode.

Interestingly, cell *E1* (with enriched bioanode and non-enriched biocathode) was the cell with best performance, and not cell *E2* (with both enriched cultures in bioanode and biocathode), as it could have been expected. The power generation of *E2* was acceptable for MFC with anaerobic biocathodes even with a biomass inoculum 10 times lower than cell *E1*. This could indicate that the biocathode enrichment possibly increased the microbial activity. Nevertheless, this experiment could not confirm the benefits of biocathode enrichment with the technique used in this work. More studies considering factors like biomass load are needed to confirm if the technique used contributes to the improvement of MFC performance with enriched biocathode.

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NOTATION

ADC	Analog to digital converter
BA	Enriched anodic culture
BC	Enriched cathodic culture
d	Difference between potentials (Arduino minus multimeter), also called divergence in this paper
$\bar{d}$	Average divergence
DC	Direct current
DN	Denitrifying culture
E	Cell potential
$\bar{E}$	Average potential
$E_{Arduino}$	Potential reading of the Arduino UNO
E_{mult}	Potential reading of multimeter Escort
EAB	Electrochemically active bacteria
e_{abs}	Absolute error
$\bar{e}_{Abs}$	Average absolute error
e_{rel}	Relative error
$\bar{e}_{rel}$	Average relative error
E1, E2, E3	Biocathodic *MFC* configurations
EIS	Electrochemical impedance spectroscopy
FC	Carbon source
H_o	Null hypothesis
H_1	Alternative hypothesis
I	Current intensity
j	Current density per surface area of the electrode
j_v	Volumetric current
MEC	Microbial electrolysis cell
MFC	Microbial fuel cell
N	Number of pair of data of potentials
NVC	Net volume of the cell
OCV	Open circuit voltage
P	Power
PEM	Protonic exchange membrane
P_s	Power density
$P_{s,max}$	Maximum power density per unit of surface area of the electrode
P_v	Volumetric power
$P_{v,av}$	Average volumetric power
$P_{v,max}$	Maximum volumetric power
R_{ext}	External resistance
R_{int}	Internal resistance
SR	Sulfate-reducing culture
TES	Total electrode surface
t_{lag}	Lag time, or time before the activity starts
Z_c	Critical value of the standardized gaussian statistic

Z_{exp}	Value of the standardized gaussian statistic
Greek characters	
α	Probability of Type 1 error in the test of hypothesis of means (matched samples)
Δ [Fe^{+2}]	Net increase of concentration of iron (II) calculated as the difference between the maximum iron (II) concentration and its initial concentration in a given pass
ε	enrichment index