
# El Colegio de la Frontera Sur

## Generación de electricidad y tratamiento de aguas residuales de café por medio de celdas microbianas de combustible

**TESIS**

presentada como requisito parcial para optar al grado de

Maestría en Ciencias en Recursos Naturales y Desarrollo Rural

Por:

Verónica Martínez Quevedo
vemartinez@ecosur.edu.mx

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**DEDICADO A:**

A mi esposo Raúl, a mis padres Jesusita y Jorge por su amor y apoyo, por sus enseñanzas de vida y por impulsarme siempre a alcanzar mis metas.

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## ÍNDICE

## 1. INTRODUCCIÓN

El cultivo del café es de gran importancia en México. El país ocupa el primer lugar de producción de café orgánico a nivel mundial y Chiapas es el estado con mayor producción, con 24% de la producción nacional de café (USDA, 2010). La extracción del grano se puede llevar a cabo por dos métodos: beneficiado húmedo y seco. En México el 84% de la producción de café se procesa por beneficiado húmedo (USDA, 2010). Este proceso consiste en la remoción mecánica de la pulpa y el mucílago con ayuda de grandes cantidades de agua (Bello-Mendoza *et al*., 1995; Orozco *et al*., 2005). Por lo tanto se generan aguas residuales las cuales se caracterizan por tener altas demandas química y bioquímia de oxígeno (DQO y DBO), y altas concentraciones de solidos totales (ST), nutrientes como fósforo y nitrógeno, pH ácido, olor fétido y color café claro. Estas características se deben a que contienen compuestos como pectina, taninos, azúcares, lignina, proteínas, polifenoles, ácidos grasos (Braham y Bressani, 1978; Brezan, 1972), y algunas sustancias neurotóxicas como la cafeína o la sustancia nefrotóxica conocida como ocratoxina A (Batista *et al*., 2009).

Debido a sus características fisicoquímicas y su alto contenido de materia orgánica biodegradable y de nutrientes, las aguas residuales de café (ARC) son consideradas una fuente importante de energía para microorganismos heterótrofos por lo que se han empleado distintas tecnologías biológicas para su tratamiento (Bello-Mendoza *et al*., 1998; Orozco *et al*., 2005; Orozco *et al*., 2006; Kondo *et al*., 2010).

Las celdas microbianas de combustible (MFC, por sus siglas en ingles) son dispositivos que utilizan el metabolismo bacteriano para producir una corriente eléctrica a partir de la biodegradación de sustratos orgánicos. Las bacterias producen electrones a partir del

sustrato, estos son transferidos al ánodo y de ahí fluyen hacia el cátodo a través de un material conductor y una resistencia externa. Se ha descrito que los electrones pueden ser transferidos al ánodo por mediadores de electrones o transportadores, por asociación directa con la membrana (Rabaey y Verstraete, 2005) o por nanocables producidos por las bacterias (Gorby *et al*., 2006), aunque se reconoce que puede haber algún otro mecanismo aún no elucidado. Las MFCs son construidas utilizando una gran variedad de materiales y con una diversidad de configuraciones. Estos sistemas son operados bajo diferentes condiciones de temperatura, pH, aceptor de electrones, superficie del electrodo, tamaño del reactor y tiempo de operación (Logan *et al*., 2006; Rabaey y Verstraete, 2005; Logan y Regan, 2006). Los estudios experimentales con este tipo de tecnologías son de gran importancia, ya que contribuyen al desarrollo de nuevas fuentes de energía renovable que pudieran contribuir a contrarrestar el consumo de combustibles fósiles.

En este trabajo se empleó por primera vez agua residual de café como sustrato en celdas microbianas de combustible de una sola cámara y celdas de dos cámaras tipo H, para evaluar el desempeño de estas para la generación de energía y para la remoción de la materia orgánica presente en las ARC.

## 1.2 OBJETIVOS

### Objetivo General:

Evaluar el desempeño de MFC para el tratamiento de las aguas residuales de café y la generación de potencial eléctrico.

**Objetivos específicos:**

- Determinar el tiempo de retención y la resistencia que favorecen el mejor voltaje y densidad de corriente.
- Determinar la capacidad de remocion de DQO, nitrógeno total, fósforo total, y ST de las ARC en las MFC.
- Identificar los diferentes morfotipos de microorganismos presentes en las biopelicula del ánodo de las MFCs.

## 1.3 HIPÓTESIS

Las MFC permiten la remoción de materia orgánica presente en las ARC y la generación de energía eléctrica.

## 2. ARTÍCULO: Evaluation of the evolution of H type microbial fuel cell using coffee wastewater.

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**Evaluation of the evolution of H type microbial fuel cell using coffee wastewater**

Martínez-Quevedo Verónica, Herrera-López David, Bello-Mendoza Ricardo, Mejia-González Gamaliel, y Calixto-Romo Angeles.

El Colegio de la Frontera Sur Unidad Tapachula, Grupo Académico de Biotecnología Ambiental. Carretera antiguo aeropuerto km 2.5, C.P. 30700.Tapachula, Chiapas, México.

Corresponding author. mcalixto@ecosur.mx,

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**Summary**

Extraction of coffee beans by wet processing uses large quantities of water to transport the coffee bean causing high concentrations of organic material representing pollution source for aquatic systems. For this reason wastewater from this process was characterized. In this study, H type double chambered microbial fuel cells were tested to evaluate energy generated and the removal of organic material contained in wastewater from coffee production (CWW), which still has not been studied in these types of cells.

Chemical removal capacity was evaluated as follow: chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN), total solids (TS) and power generation under three hydraulic retention times (HRT) 72, 144 and 288 h, observing highest removal capacity of COD (30%), TP (30%), TN (37%), TS (28%) was obtained with the HRT of 72 h. Power density was 0.98-1.82 mW/m$^2$ and coulombic efficiency was 0.012 – 0.062%. Biofilm formed in the anode of the MFCs was also observed using a scanning electron microscope (SEM) obtaining 55 morphotypes of microorganisms.

**Key words:** bioenergy, biofilm, coffee, MFC, organic material removal.

## 1. Introduction

Mexico holds first place in production of organic coffee worldwide, the majority of coffee is exported to Europe and it is cultivated in the state of Chiapas which is the main coffee producer in Mexico, counting for 24% of the total production in the country. Extraction of coffee grounds can be achieved by two methods: dry and wet processing, last method is the most used (84%), USDA [1]. Wet processing uses large quantities of water in different steps: to transport the coffee bean, in mechanical removal of pulp, in fermentation and degradation of mucilage and washing step [2]. For that reason, there is a large quantity of wastewater from this process that flows into adjacent rivers. CWW have high concentrations of organic material, it must be: chemical oxygen demand (COD), up to 20,000 mg/L, biochemical demand (BOD) up to 9,000 mg/L, nutrients like phosphorus and nitrogen, acidic pH, fetid odor and dark coloration [3]. All of these characteristic are generated by compounds like: pectins, tannins, sugars, lignin, proteins, polyphenols and fatty acids [4,5], some neurotoxic substances like caffeine or the nephrotoxic substance known as ochratoxin A [6]. Even though the WWC represents

a source of contamination, these are discarded into rivers without previous treatments [7,8]. For that reason it is necessary to apply adequate treatments before releasing wastewater into these waterbodies [9-12]. Due to the high content of organic biodegradable material and nutrients in the CWW, it contains substances that are important sources of energy for heterotrophic microorganisms and for that reason biological treatment systems have been employed [9,13].

Microbial fuel cells (MFCs) uses bacterial metabolism to produce electric current stemming from organic material (as substrate) by anaerobic oxidation. Electrons and protons produced in the anodic chamber are driven as follows: the electrons travel through a conductive material to the cathode, while the protons travel through a protonic exchange membrane (PEM) or a saline source to finally form $CO_2$ and $H_2O$ in the cathode [14-16]. There have been many studies done on a large variety of domestic types of wastewater [17,18] and agroindustrial wastewater, as well as molasses wastewater [19], beer wastewater [20] and pig farm wastewater [21,22], among others. It has been demonstrated that the treatment of wastewater and the power density vary according architecture of the MFCs, chemical characteristics of the substrate and the microorganisms involved. To evaluate other types of wastewater, CWW were employed as substrate, to analyze the organic material removal capacity and the generation of electricity realized in H type microbial fuel cells.

## 2. Materials and Methods

### 2.1 Wastewater

Aquapulpping was used (this comes from skin and pulp removed step) and water from washing step (which is from mucilage elimination) from Arabic coffee, both were mixed

at a ratio of 1:1 and was conserved at -20°C until its use and characterization. Wastewater was used in inoculation period to obtain polarization data. In HRT optimization, wastewater was filtered through a membrane with 200 μm pore size. Characteristics of the CWW are represented in Table 1, all of the determinations were made in conformation with APHA methods [23].

Table 1. Physical chemistry characteristics of coffee wastewater

| Parameters | Non filtered wastewater | Filtered wastewater |
|---|---|---|
| **COD (mg/L)** | 40,000 - 50,000 | 30,000 – 39,000 |
| **$BOD_5$ (mg/L)** | 19,000-30,000 | 11,700 – 22,700 |
| **TP (mg/L)** | 26 | 22 |
| **TN (mg/L)** | 88 | 58 |
| **TS (mg/L)** | 3.22 | 0.85 |
| **pH** | 3.8-3.9 | 3.8-3.9 |
| **Conductivity (mS/cm)** | 1.5-1.7 | 1.5-1.7 |

2.2 Microbial Fuel Cells

Two microbial fuel cells H type double-chambered were used with a nominal volume of 250 mL. Anode used was a carbon fiber brush with a diameter of 14 mm and 20 mm in length (Mill-Rose Company). Cathode was composed of flexible carbon fiber measuring 3 x 5 cm, covered with a Pt catalyst on one of its sides (0.5 mg Pt/$cm^2$) [24]. Both chambers were joined with a glass tube and a cationic exchange membrane (Membranes international Inc. CMI-7000S) previously activated following manufacturer's

instructions, distance between electrodes was 12 cm. Anode was connected to an electrical resistor with copper wire, and cathode was connected through a titanium wire (Figure 1). Anodic chamber was provided with a magnetic agitator and cathodic chamber with an air disperser connected to a peristaltic pump.

2.3 Inoculation, stabilization and polarization of the MFCs.

Anodic chamber was fed with CWW to colonize the anode, as CWW contains biodegradable compounds like sugars and fatty acids [4,5] no other substrate was used. Cathodic chamber was filled with a buffer solution composed of sodium phosphate (SBF, 0.5mM, pH 7) [25]. Inoculation period of the MFCs was 15 days and during this time the voltage was monitored every 15 minutes using a multimeter (Steren Mul-600) with a data acquisition system and used a resistance of 1000 Ω.

CWW was substituted with synthetic wastewater, which was fed into the chamber periodically to ensure the MFCs were able to function properly and especially in the generation of energy through the biofilm formed on the anode. Composition of synthetic wastewater was prepared as follow (g/L): $MgSO_4$ (22.5 g/L), $CaCl_2$ (0.24 g/L), $FeCl_3$ (0.25 g/L), $CH_3COONa$ (1.2) in phosphate buffer, pH 3.8. 24 hour cycles were used with the same resistance of 1000 Ω. Voltage registered the same frequency as the inoculation period.

Polarization data was obtained by varying the external resistance (120, 1000, 3300, 5600, 8200 y 10000 Ω) in the circuit and measuring the voltage. Every cycle had a hydraulic retention time (HRT) of 72 hours, all experiments were made in triplicate. Current (I), potency (P) and the coulombic efficiency were calculated according to Oh *et*

*al*. [26]. Aside from the voltage (V) and the resistance (R) the current intensity was also calculated (I = V/R). Power density and current density were obtained by normalizing the cathode area (15 $cm^2$) [26].

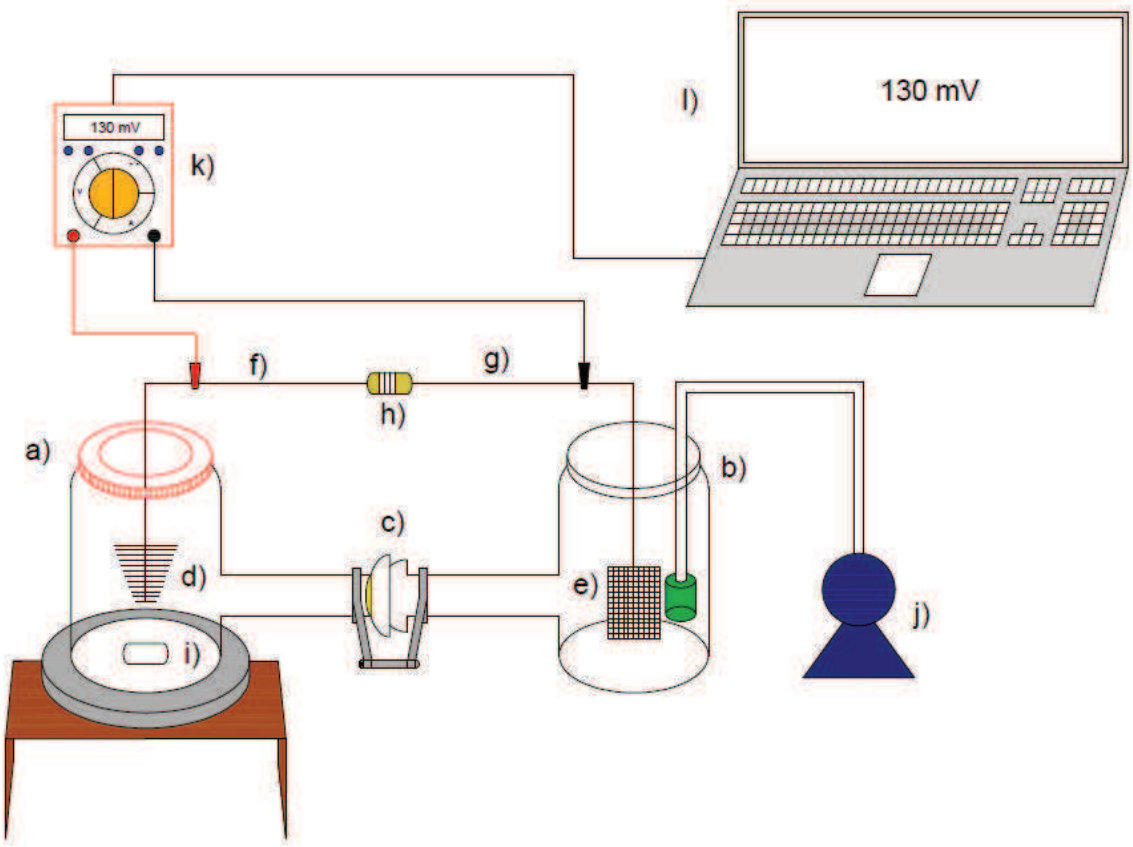


Figure 1. Configuration of microbial fuel cell. a) anodic chamber b) cathodic chamber, c) cationic exchange membrane, d) anode, e) cathode, f) copper wire, g) platinum wire, h) electric resistance, i) magnetic agitator, j) air pump, k) multimeter, l) computer equipment.

2.4 Hydraulic retention time assay

Three different HRTs were evaluated (72, 144 y 288 h) each one in triplicate with each condition using filtered CWW. At the beginning and end of each experiment the pH was measured (OAKTON 1100 series) and the conductivity (Thermo Scientific Model Orion 4 stars). Also the concentration of following parameters were determined: COD, $BOD_5$, TP, TN, and TS according to the APHA methods [23] and the removal percentage was calculated for each one.

2.5 Scanning electron microscopy

0.2 g of biofilm adhered to the anode was collected from each MFCs and each samples was separately placed in a 1.5 mL polypropylene tube. In each tube a glass pearl was introduced with 0.5 mL of solution consisting of SBF, pH 4.0 and was agitated in vortex. Afterwards, the pearl was removed and each tube was submerged in an ultrasonic bath three times for 30 seconds each time. It was brought to 1.5 mL as total volume with SBF 0.5 mM, pH 4.0, solution was filtered through a membrane with the pore size of 50 μm and collected in a polypropylene tube then it was centrifuged for 30 seconds at 6,000 rpm to eliminate precipitated material. The supernatant was extracted with a syringe and the rest of the 1.5 mL volume was filled using distilled water. All of the syringe's contents were passed through a 0.2 μm pore sized filter. The bacteria dispersed and adhered to the filter were fixed in 3% glutaraldehyde in SBF, pH 7.0, for 30 minutes, they were washed two times with distilled water and incubated 5 minutes at room temperature each wash. After, they were dehydrated with ethanol solutions of (30, 50, 70, 90, and 100%) incubating 10 minutes each time and dried two times with hexamethyldisilazane incubating 5 minutes each ones. Filtered was removed from the solution and left in a drying unit with silicon gel. Each sample was placed in an aluminum cylinder using

carbon conductive tape with adhesive on both sides and recovered with a layer of gold-palladium with a thickness of approximately 20 nm (DENTON VACCUM, model DESK II.). Observations were made with a scanning electron microscope (TOPCON, model SM-510) at a high vacuum at 8 KV of acceleration.

## 3. Results and Discussion

### 3.1 Inoculation, start up and polarization data

Anodic chamber was fed with CWW until formation of biofilm could be made on the anode. During inoculation period, an initial voltage of 31.4 mV and 38.1 mV (1000 Ω) was generated in the MFCs 1 and 2, respectively. The voltage increased in the first 5 hours and after that time it gradually diminished until 150 hours had passed, approximately, possibly due to the consumption of substrate present in the CWW. After this period, a more stable behavior was observed in MFC1, while voltage decreased in MFC2, suggesting a different consumption of substrate in each MFC. Work conditions were the same for both reactors.

In the first 5 hours of operation of the two MFCs (start time period) fed with synthetic water (1.2 g/L of acetate, pH 3.8) as substrate, an increment in the voltage was observed, approximately 60 mV in both MFCs. In second cycle, a sudden drop in voltage was observed compared with the first cycle. This also happened in MFC2 but not until the third cycle (Figure 2). However, every time solution of synthetic water was changed in the anode chamber, voltage increased to 42 mV, confirming electron generation microbial while substrate was inoculated. Previously, Kim *et al*. [25] y Liu *et al*. [27] reported the same phenomenon of the increased voltage every time a new substrate was feed into MFCs and suggested that probably, transfer of electrons is

principally due to bacterial activity suspended on electrode, but it is not due to suspended bacteria in solution.

On the other hand, there have been studies using synthetic water containing 20 mM of sodium acetate as substrate for generation of electricity in MFCs. Oh *et al*. [26] used activated mud to colonize the anode in doubled-chamber microbial cells, using a nutritive solution with acetate (20 mM) as substrate obtaining voltage values between 320–340 mV. In this work, generated voltage was lower compared with Oh *et al*. [26] but the characteristics of the wastewater used to colonize the anode were different and different factors could affected electricity generation such as: biofilm characteristics, conductivity of solution, pH, and substrate concentration.

When MFCs was operated was also observed a lost of biofilm from surface of the anode while replacing MFCs' synthetic water in the anode chamber, so the quality of the DQO seemed higher since it presented an initial measurement of 277 mg/L and at the end of the cycle it increased considerably to 1580 mg/L. It is probable that this increased in the DQO affected the voltage generation in the second cycle of MFC1 and in the third cycle of MFC2. For that reason, the inoculation process was repeated before making the polarization curves, until a stability in voltage generation was achieved during the first three cycles and obtained a voltage between 33 y 38 mV.

Polarization data was obtained after feeding the reactors three times and observing a stable voltage behavior. Figure 3 shows the maximum voltage generated with each external resistance. Maximum power density and current density were different with each external resistance tried. The highest power density reached was 0.43 $mW/m^2$ with a current density of 0.007 $A/m^2$ using a resistance of 5,600 Ω (Figure 4).

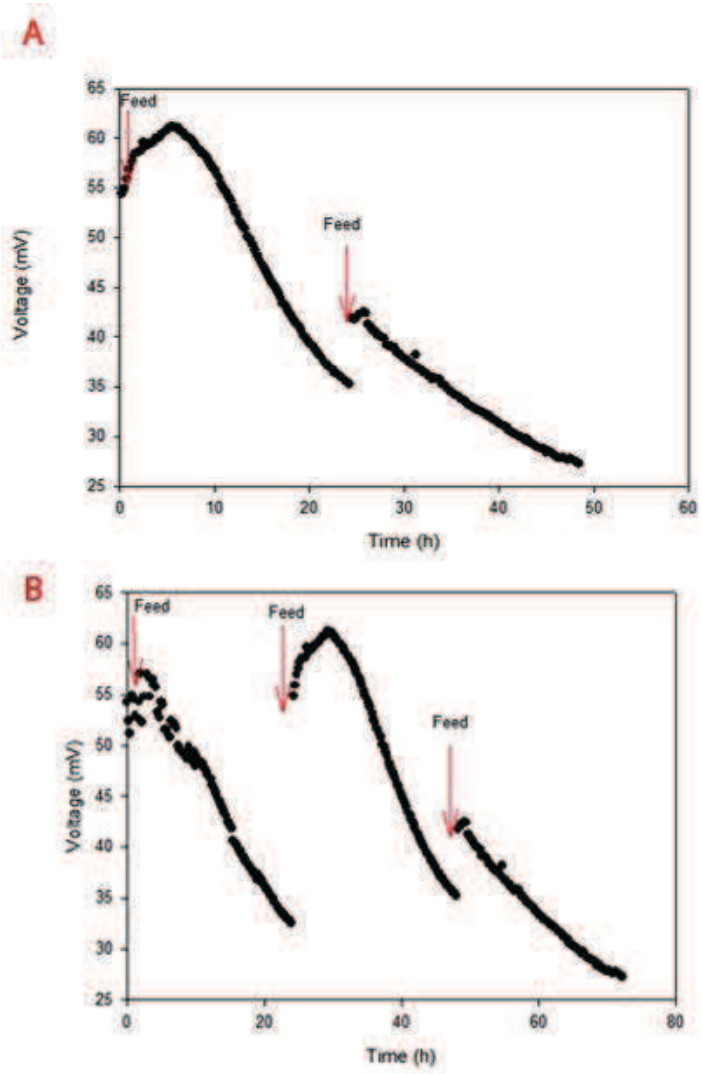


Figure 2. Voltage dynamic during the acclimatization period with synthetic water. The arrows indicate the replacement of substrate. A) Voltage generated by the MFC1 in cycle 1 and 2, B) Voltage generated by the MFC2 in cycle 1, 2 and 3.

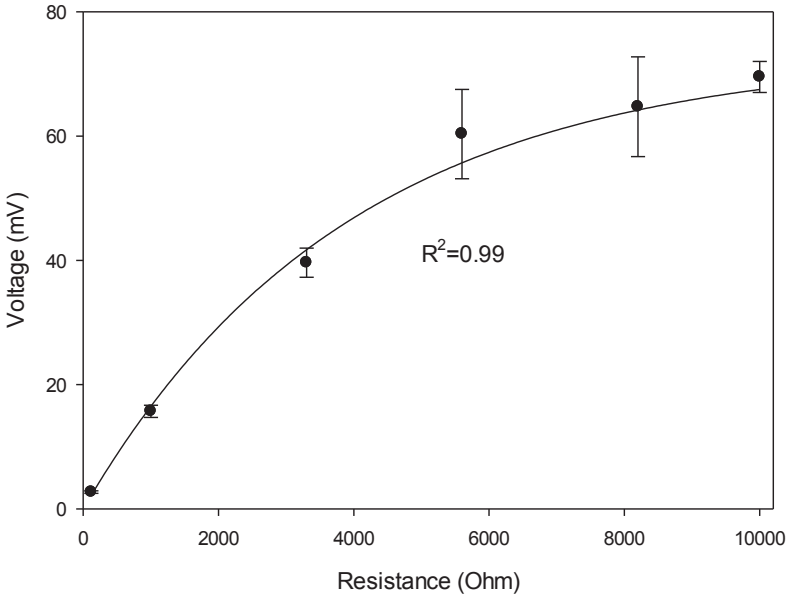


Figure 3. Effect of the external resistance over voltage.

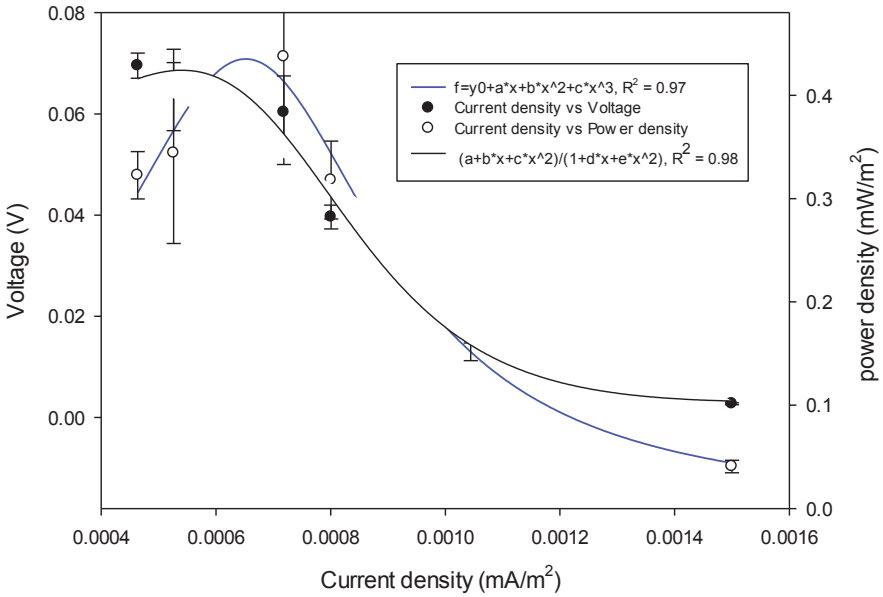


Figure 4.Power density and polarization curve.

3.2 Power generation and coulombic efficiency.

During the HRT tests, an increase was observed in the power density using filtered water (0.98 – 1.81 mW/$m^2$) compared to the power density obtained using unfiltered water (0.43 mW/$m^2$) during the polarization tests. It is probable that increased power density using filtered water was more favorable due to the decrease in the amount of total solids of 3.2 mg/L that contained raw water at 0.85 mg/L after filtration, possibly due to the fact that in the smaller particles in the substrate are more easily degraded by microorganisms [28] and/or to diminished internal resistance while larger sized particles were removed. Power density was greater in the HRT of 72 h, followed by the HRT 288 h and the lowest amount was obtained by the HRT of 144 h, However, no significant difference was observed between them (Table 2).

Power density values (0.98-1.81 mW/$m^2$) and coulombic efficiency (CE) (0.012-0.062 %) obtained in this experiment were less than previously reported by other authors employing other types of wastewater with the same reactor design, such as: swine wastewater where maximum values obtained for power density were 45 mW/$m^2$ and 8% of CE [21], synthetic wastewater with substrate like acetate (43 mW/$m^2$, CE 71% ± 7 [26]; 40 mW/$m^2$, CE 40% [25]) or cysteine (39 mW/$m^2$, CE 10%[29]). Power generation and CE can be affected by distinct factors like the configuration of the MFCs: nature and concentration of substrate, conductivity of solution and pH. Conductivity of the CWW was from 1.5 -1.7 mS/cm, these values are lower in comparison with other reports, like brewery wastewater which showed a value of 3.23 mS/cm, and maximum values generated for power density were 205 mW/$m^2$ [20]. In other tests, SBF was added into substrate to increment conductivity and improve power generation and coulombic efficiency, Huang and Logan [30] used SBF to increment the conductivity of paper

recycling wastewater getting a value of 10.2 mS/cm and achieved an increase in power density by 245%, while Feng *et al*. [20] increased the conductivity of brewery wastewater to 14.6 mS/cm and the power density was 158% it is due to increased conductivity because it permits an easier flow of protons through the PEM and the capture of electrons on the anode [31].

pH can also be a factor that can affect power generation in the MFCs. The CWW presented a pH of 3.8 ± 0.1 and it was used without modification with all the MFCs. Gil et al. [31] used domestic wastewater diluted with distilled water in the anode chamber and evaluated different pH levels from 5.0-9.0. The amount of current was best at a pH 7.0, obtaining values at approximately 0.22 mA, while that with a pH 5.0, the current was 0.18 mA, approximately. The same authors also did tests adding SBF and NaCl to the feeding water with a pH of 7.0 and observed a better stability of the pH. However, in the experiments where no SBF and NaCl were added the pH changed to 9.5 in the cathodic chamber and 5.4 in the anodic chamber, this phenomenon could be due to a lower proton permeability through the membrane, in addition to the lower microbial activity and the limited electron transfer to the anode due to the pH.

In this experiment, the difference in pH between the anodic chamber (pH 3.8) and cathodic chamber (pH 7.0) could have affected the development of electricity generation.

Table 2. Electrochemical development at different hydraulic retention times (HRT)

| Hydraulic Retention Time (h) | Power Density ($mW/m^2$) | Standard Deviation | Coulombic Efficiency (%) | Standard Deviation |
|---|---|---|---|---|
| **72** | 1.81 | 0.3 | 0.012 | 0.0014 |
| **144** | 0.98 | 0.25 | 0.026 | 0.0048 |
| **288** | 1.22 | 0.31 | 0.062 | 0.132 |

3.3 COD, TN, TP and TS removal.

Removal of COD, TN, TP and TS was determined for each cycle, COD removal was higher at 72 h, followed by HRT of 144 h and then 288 h (Table 3). Biofilm growth was increased on the anode when retention time was increased causing thicker biofilm and a subsequent detachment. This factor influenced in final determination of COD, reducing the removal percentage in higher HRT. Biofilm development could be caused by the oxygen diffusion towards the anodic chamber, since the experimental time was longer as described by Min *et al*., (2005) [21]. It was also observed that the TP removal, which showed values of 30, 28 and 9% for the three different retention times (Table 3), were reported in other studies up to a 58% TP removal in domestic wastewater using MFCs with the anode-cathode sequential configuration [18]. It is probable that the TP removal was biological, converting biomass as part of the microbial growth. Also, it has been reported that phosphorus removal due to excessive accumulation in form of polyphosphates by phosphorus accumulators microorganisms [18, 32].

There was no TN removal in HRT of 144 and 288 h; but at HRT of 72 h there was a removal rate of 37%. Removal mechanism of nitrogen is unknown in this study, however different ways of microbial metabolism have been reported such as: denitrification and anaerobic oxidation of ammonia, among others [32]. It has also been reported, nitrogen removal as ammonia (61.8 ± 0.6%) in domestic wastewater [18], while removal of ammonia from pig farm wastewater using double chamber MFCs was 83 ± 4%; but the concentration of nitrates and nitrites increased [21] probably due to the oxygen diffusion from one chamber to another, favoring the nitrification process. This could be the reasons that no TN removal was observed during the longer retention times. However, in this study ammonia, nitrates and nitrites were not determined in order to be able to verify the occurrence of this phenomenon.

TS removal was 28% when HRT was 72 h, 47% at HRT 144 h and 46% at 288 h. Higher removal amount was in large retention times since the microorganisms had more time to hydrolyze bigger compounds and use them in their metabolic functions. Oxygen diffusion from one chamber to another due to exposure time could have also helped in TS removal. Even though removal percentage increased with a high HRT, COD removal was less, probably due to the fact that soluble COD was bigger than TS and for that reason was obtained this behavior. It must be noted that at the end of each cycle, a slight increase in the conductivity and a decrease in pH was also observed, which indicates a higher ion concentration at the end of each cycle.

Table 3. Effect of the HRT on the development of the MFCs

| HRT(h) | % of removal | | | |
|---|---|---|---|---|
| | COD | TF | TN | TS |
| **72** | 32 | 30 | 37 | 28 |
| **144** | 27 | 28 | WR | 47 |
| **288** | 16 | 9 | WR | 46 |

WR- Without Removal

3.4 Morphotypes present on the anode biofilm

A sample of the MFC biofilm from the anode was observed using a SEM. The biofilm from the two MFCs presented different forms of microorganisms, and similar morphotypes were observed even though the composition of the morphotypes was different in each one (Figure 5). Forms found in samples from MFC2 were classified according to the 11 predominant morphotypes described in Bergey´s manual of determinative bacteriology [33]. 55 distinct types of microorganisms were identified according to their shape and dimensions (length and width), they are classified in table 4. It was reported that the bacteria that colonize the anode are also a determining factor for the power generation, since there can be different types of exoelectrogenic microorganisms that are also involved in the removal of organic matter such as methanogens and/or fermentatives, which can start a competition between electron donors [34,35]. Likewise, the low density of exoelectrogenic microorganisms present in the microbial community on the anode's biofilm could cause a lower concentration of electron mediator molecules [36].

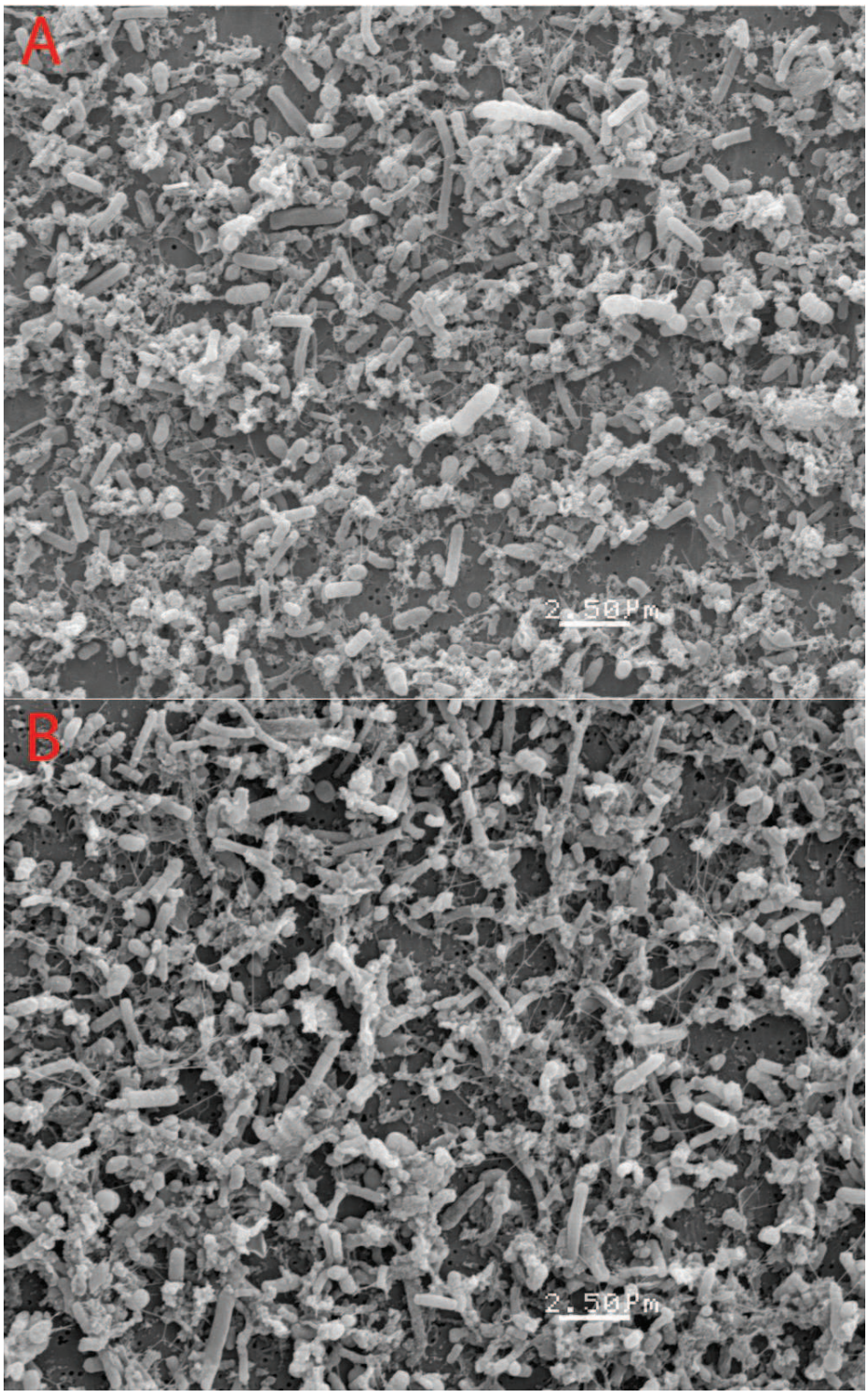


Figure 5. A) Biofilm on anode of MFC1, SEM micrography. B) Biofilm on anode of the MFC2, SEM micrography.

Table 4. Microorganism morphotypes present in the biofilm on the anode of R2 reactor.

| Morphotype | # of Sample | Dimensions(μm) |
|---|---|---|
| Cocci | 22 | From 0.24 μm to 0.80 μm in diameter |
| Curved rod | 13 | Length from 0.5 μm to 5 μm<br>Width from 0.2 μm to 0.8 μm |
| U-shape rod | 3 | Length from 0.8 μm to 3 μm<br>Width from 0.3 μm to 0.4μm |
| Regular rod | 9 | Length from 0.5 μm to 2 μm<br>Width from 0.3 μm to 0.8 μm |
| Unbranched filament | 3 | Length from 1 μm to 5.2 μm<br>Width from 0.3 μm to 0.5 μm |
| Ellipsoid | 5 | Length from 0.5 μm to 3 μm<br>Width from 0.2 μm to 2 μm |

## 4. Conclusions

According to the results obtained, treatment of CWW and generation of energy using MFCs is possible. However, the efficiency of this technology is still not competitive enough up against other more conventional technologies like artificial wetlands and anaerobic reactors. To achieve better electrical energy recuperation and optimize the CWW treatment using this type of MFCs, it is suggested that some modifications need to be made to the reactor as parameters assayed in this work. To avoid the removal of the biofilm from the anode each time the MFCs were feed, it is necessary to add a feeding nozzle and another one to discharge the overflow. On the other hand, CWW could be used with a lower organic load or using coordinated systems like a UASB reactor and MFCs to obtain a better organic material removal capacity. These experiments can also be done to prove other types of microbial relationships that have better exoelectrogenic activity, such as modifying the physical chemistry parameters like the pH and the conductivity could help to better transport the protons to the cathodic chamber.

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## 3. CONCLUSIONES

El primer diseño de MFC de una sola cámara no presentó buenos resultados debido a: 1) la presencia de fugas de agua; 2) que no se pudo proporcionar un estado de anaerobiosis cuando estas MFCs se alimentaron con el ARC ya que había burbujas de aire; 3) Se registraron falsos contactos al conectar el circuito, por lo tanto no fue posible registrar voltaje a pesar de los esfuerzos por localizar la parte errónea del circuito. 4) a la formación de biopelícula en el cátodo.

Sin embargo, el tratamiento de ARC y la generación de energía es posible utilizando MFC´s de doble cámara. La mayor densidad de poder se obtuvo con el TRH de 72h y resistencia de 5600 Ω aunque no se observo diferencia significativa entre los tratamientos. La capacidad de remoción de DQO, nitrógeno total, fósforo total y sólidos totales fue de 32%, 30%, 37% y 46% respectivamente. Además, se identificaron 55 tipos de microrganismos diferentes en la biopelícula formada en el ánodo de la celda microbiana MFC2.

A pesar de los resultados obtenidos cabe resaltar que esta tecnología aún no es competitiva frente a otras tecnologías alternas, tales como los humedales artificiales o los reactores UASB con respecto a la eficiencia en el tratamiento de las ARC (Bello-Mendoza *et al*., 1998, Orozco *et al*., 2006). Para lograr una mejor recuperación de energía eléctrica y optimizar el tratamiento de ARC utilizando MFC´s tipo H se sugiere mejorar el diseño del reactor agregando una boquilla de alimentación y otra de descarga de efluente, para evitar el desprendimiento de biopelícula de ánodo cada vez que se cambia el sustrato. También sería conveniente trabajar con ARC de menor carga orgánica o utilizar sistemas acoplados tales como reactor UASB y MFC. Además se podría inocular con un solo microorganismo o probar otro tipo de consorcio

microbiano que tengan mayor actividad exoelectrogénica, y modificar algunos parámetros físico químicos de las ARC como el efecto el pH y la conductividad, para evaluar su efecto en la generación de energía ya que podrían ayudar a mejorar el transporte de protones a la cámara catódica.

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## ANEXOS

### Anexo 1. Recolección de aguas residuales de café

Se recolectaron (ARC) en la finca la Concepción ubicada en el ejido Carillo Puerto en el Estado de Chiapas. Las muestras corresponden a la etapa de despulpado (eliminación de cáscara y mucílago) y de lavado de café. Esta muestras se mezclaron en proporción 1:1 y se determinó su contenido de DBO, DQO y Nitrógeno total, así como su pH y conductividad (Tabla 1). Las aguas residuales de café presentaron baja conductividad y baja concentración de DQO comparada con ARC antes reportadas (Rossman *et al.,* 2012). Asimismo algunos parámetros (nitrógeno total) se encontraron dentro de los establecidos por la NOM-001- ECOL-1996, por lo tanto se consideró que esta agua no era adecuada para el estudio y se procedió a buscar otra muestra.

**Tabla 1**. Características fisicoquímicas del agua residual de café.

| Parámetro | ARC Finca Concepción | ARC Siltepec | NOM-001-ECOL-1996 |
|---|---|---|---|
| **DQO (mg/L)** | 1,946 - 2500 | 40,000 – 50,000 | NE |
| **DBO (mg/L)** | 946 | 19,000 – 30,000 | 60 |
| **Nitrógeno total (mg/L)** | 18.55 | 88 | 25 |
| **pH** | 5.2 | 3.9 | 5-10 |
| **Conductividad (µS/cm)** | 324 | 1673 | NE |

NE-No especificado

Se obtuvo otra muestra de ARC en el municipio de Siltepec Chiapas. Se recolectó agua de lavado y de despulpado las cuales se mezclaron en una relación 1:1. A esta muestra

se le determinó su concentración de DQO, el pH y su conductividad de la mezcla fresca (Tabla 1) dado que se encontraron valores adecuados de estos parámetros, se decidió trabajar con esta agua, y se conservó a -20°C para utilizarla en los estudios posteriores.

## Anexo 2. Construcción de celdas microbianas (MFC)

### 2.1 MFC de una sola cámara diseño 1

Se construyeron tres celdas microbianas de una sola cámara con el cátodo expuesto al aire a partir de botellas de vidrio de 25 ml marca Schott. Como ánodo se utilizó un cepillo con fibras de carbono de 14 mm de diámetro y 20 mm de largo. El cátodo fue hecho con tela de carbono flexible y presentó un diámetro de 2.5 cm. Este se recubrió por la cara interna con 0.5 mg/cm$^2$ de catalizador de platino, y PTFE en la cara externa (Liu y Logan, 2004) (Figura 1). El ánodo se conectó con una cable de cobre a una resistencia eléctrica de 1000 Ω, mientras que el cátodo se conectó a la misma resistencia con un cable de titanio para cerrar el circuito (Figura 2).

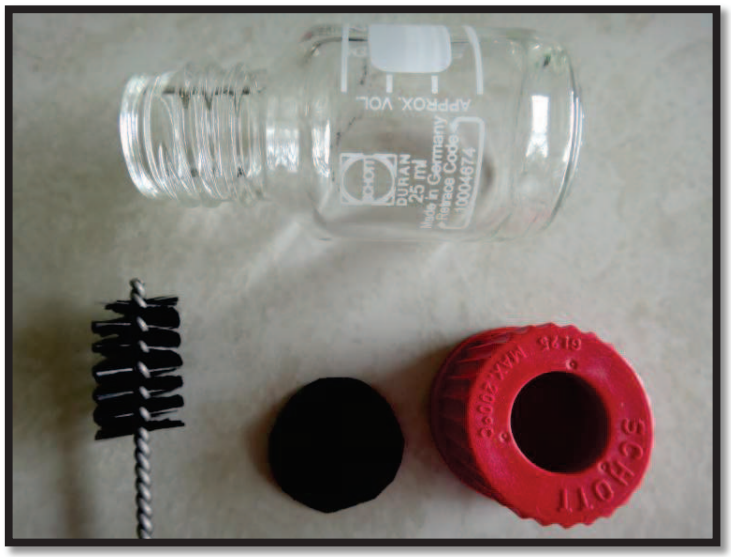


**Figura 1.** Materiales de fabricación de celdas microbianas.

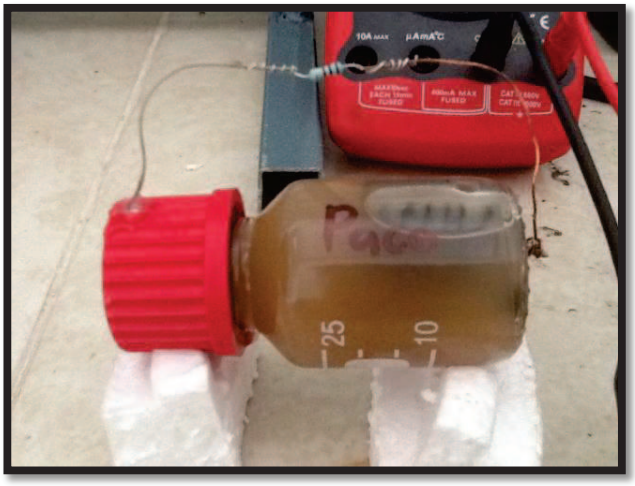

**Figura 2.** MFC alimentada con ARC

### 2.2 MFC de una sola cámara diseño 2

Se construyeron dos MFCs de una sola cámara con el cátodo expuesto al aire, a partir de un cilindro de Nylacero con un interior de 4 cm de largo, 3 cm de diámetro y volumen de 30 ml. El ánodo y cátodo fueron hechos a partir de tela de carbono flexible con un diámetro de 3 cm cada uno (Figura 3). El cátodo fue recubierto por la cara interna con 0.5 mg/cm$^2$ de catalizador de platino, y PTFE en la cara externa (Liu y Logan, 2004). El ánodo se conectó con una cable de cobre a una resistencia eléctrica de 1000 Ω y el cátodo por medio de un cable de titanio (Figura 4).

**Figura 3.** Materiales de construcción de los reactores.

**Figura 4.** Celda microbiana de Nylacero

### 2.3 Inoculación de la MFC de una sola cámara

Para la inoculación se utilizó el ARC con una concentración de DQO de 27,288 mg/L, pH 3.9 y conductividad 1220 µS/cm. Se agregaron 30 ml del ARC a cada una de las celdas para usarlas como inóculo. Se midió el voltaje con un multímetro marca STEREN modelo M-600. El voltaje inicial de la MFC1 fue de 125 mV y en la MFC2 de 115.8 mV. El periodo de inoculación fue de 15 días, con alimentación de ARC cada dos

días. Se observó un decremento del voltaje conforme transcurrían los días a pesar de reemplazar el sustrato. La MFC1 tuvo un voltaje de 23.1 mV en el último día de alimentación y después de 8 días el voltaje descendió a 9.4 mV; para la MFC2 no fue posible registrar estos datos. Al término de los 15 días se desmontaron las MFCs y se retiraron los cátodos, estos estaban cubiertos con una biopelícula (Figura 5) de aspecto filamentoso blancuzco (nata blanca) con algunas aglomeraciones rojizas y verdes, además se observaron abundantes puntos negros que parecían ser restos de catalizador atrapado en la biopelícula. Se tomó una muestra de estos cátodos y se procesaron para observarlos al microscopio electrónico de barrido (SEM).

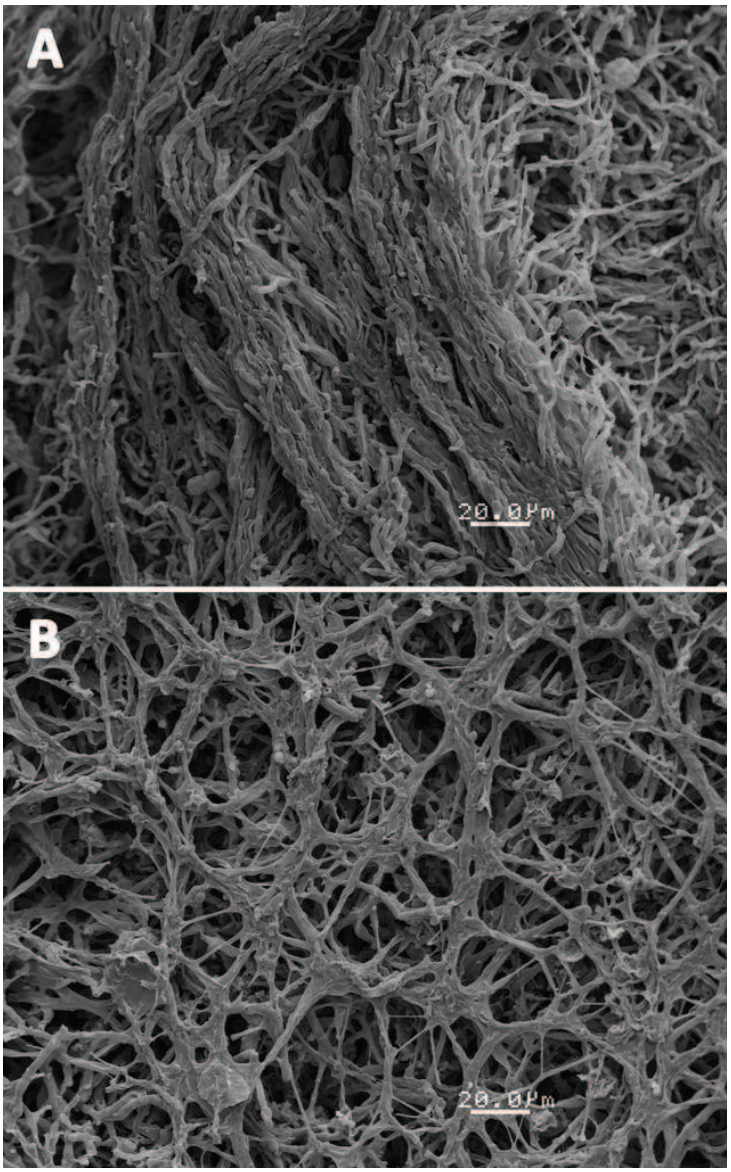


**Figura 5. A**. Imagen SEM de la biopelícula en el cátodo de la MFC1. **5B**. Imagen SEM de la biopelícula en el cátodo de la MFC2.

Se procedió a sustituir los cátodos por unos nuevos y se preparó una solución sintética con sustrato puro a partir de $CH_3COONa$. Para alimentar las celdas y asegurar su buen funcionamiento y en especial la generación de energía por la biopelícula formada en el

ánodo. Este desecho sintético se preparó con 1 L de agua destilada, 3 mL de disolución de $MgSO_4$ (22.5 g/L), 3 mL de solución de $CaCl_2$ (0.24g/L), 3 mL de solución de $FeCl_3$ (0.25g/L), 1 mL de disolución amortiguadora de fosfatos (0.92 g de $NaH_2PO_4$ y 1.1621g de $NaHPO_4$), 1.2 g de $CH_3COONa$ y el pH se ajusto a 3.8 con HCl. En cada ensayo se observó un decremento del voltaje, cada vez que se sustituía la solución sintética (Tabla 2). Por lo que se removieron los cátodos nuevamente y se observó la formación de una biopelícula delgada.

**Tabla 2**. Voltaje generado a partir de solución de sustrato puro.

| MFC | mV Ciclo 1 | mV Ciclo 2 | mV Ciclo 3 |
|---|---|---|---|
| **MFC1** | 128.3 | 42.6 | 20 |
| **MFC2** | 132.0 | 35.4 | 16.2 |

Para evitar la formación de la biopelícula en el cátodo se utilizó una membrana de intercambio catiónico, esta fue activada previamente con una solución de NaCl al 30% a 40°C durante 24 h. Se alimento nuevamente con ARC, las celdas fueron provistas de cátodos nuevos y la membrana de intercambio catiónico se coloco en el extremo aerobio de la celda antes del cátodo en ambas celdas. La MFC1 presentó un voltaje inicial de 268 mV pero a las 24 h descendió hasta 3.2 mV. A pesar del uso de la membrana de intercambio catiónico el cátodo fue nuevamente colonizado por microorganismo y no se pudo mantener un voltaje constante con estos reactores y se decidió utilizar los reactores de doble cámara.

**Anexo 3. MFC de doble cámara**

Se utilizaron dos celdas de doble cámara tipo H con un volumen nominal de 250 ml. Como ánodo se utilizó un cepillo de fibra de carbono con diámetro de 14 mm y 2 mm de largo. El cátodo consistió en tela de carbono de dimensiones 3 x 5 cm y área 15 $cm^2$, recubierta con catalizador de Pt en una de sus caras. También se utilizó una membrana de intercambio catiónico previamente activada. El ánodo se conectó a una resistencia eléctrica con un alambre de cobre, mientras que el cátodo a través de un alambre de titanio.

Se llevó a cabo la inoculación (colonización de ánodo) de las celdas MFCs con CWW durante 15 días y se registraron los voltajes obtenidos. Los datos de polarización fueron obtenidos variando la resistencia externa (120, 1000, 3300, 5600, 8200 y 10000 Ω) en el circuito y midiendo el voltaje. La mayor densidad de poder alcanzada fue de 0.43 $mW/m^2$ con una densidad de corriente de 0.007 $A/m^2$ utilizando una resistencia de 5600 Ω.

Las pruebas de tiempo de retención hidráulica se realizaron con tres diferentes tiempos 72, 144, y 288 h . El TRH de 72 h presentó mayor densidad de poder de 1.22 $mW/m^2$ y mejores porcentajes de remoción de DQO (32%), fósforo total (30%), ST (28%) y nitrógeno total (37%). También se observó en el SEM una muestra de la biopelícula formada en el ánodo de las MFCs, la biopelícula de la MFC2 se observó con mayor detalle y se identificaron 55 morfotipos distintos de microorganismos.