MICROBIAL ELECTROSYNTHESIS FROM CO_2 IS RESILIENT TO FLUCTUATIONS IN RENEWABLE ENERGY SUPPLY

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Abstract

Microbial electrosynthesis (MES) allow CO_2 capture and utilization for the electricity-driven bioproduction of organics such as acetic acid. Such systems can be coupled to any renewable electricity supply, especially those derived from solar and wind energy. However, fluctuations or even absence of electricity may cause damages or changes in the microbial community, and/or affect the performance and robustness of MES. Therefore, the transformation of gaseous CO_2 into organic products in a MES was assessed continuously during 120 days of operation. Time-increasing power outages, from 4 h to 64 h, were applied in order to evaluate the effects of electric energy (current) absence on microbial community, organics formation, production rates and product accumulation. Acetic acid was the main product observed before and after the power outages. A maximum titer and production rate of 6965 mg L^{-1} and 516.2 mg L^{-1} d^{-1} (35.8 g m^{-2} d^{-1}) of acetic acid were observed, respectively. During the absence of power supply, it was observed that acetic acid is oxidized back to CO_2 which suggests microbial activity and/or pathway reversal. However, the electro-autotrophic activity recovered after the power gaps, and acetic acid production was restored after reconnecting the energy supply, reaching a current density of -25 A m^{-2}. The microbial community of the biofilm responsible for this behavior was characterized by means of high-throughput sequencing, revealing that *Clostridium*, *Desulfovibrio* and *Sporomusa* accounted for 93% of the total community attached onto the cathodic biofilm. Such resilience of electrotrophic microorganisms reinforces the opportunity to couple bioelectrochemical systems to renewable energy, overcoming the eventual electrical power fluctuations.

Keywords: Acetate production, CO_2 valorization, Microbial electrosynthesis, Renewable energy, Microbial community, Cathodic biofilm.

Highlights

- Power supply interruption affects the performance of the system for a reduced period of time.
- MES showed to be recovered after power supply interruption
- Longer off periods did not result in longer recovery times.
- MES system proved to be ready for real-field application powered with renewable energy.
- The cathodic biofilm was dominated by bioelectrochemically active acetogenic bacteria.

1. Introduction

In the past few years, renewable energy production has sharply increased together with public concerns for the environment in the developed world [1]. This increasing amount of installed renewable power usually produces energy surplus that can be used, stored or lost. Some alternatives such as batteries, water pumping storage or hydrogen production by water splitting have been proposed for the surplus electricity exploitation [2]. Recently, using excess electricity to convert CO_2 into chemical energy in the form of storable fuels and chemicals has come up as a novel alternative for off-peak electrical overproduction [3,4]. Microbial electrosynthesis (MES) is a recent technology, an offshoot of conventional bioelectrochemical systems (BESs) used for wastewater treatment and energy recovery, proposed in 2010 [5]. This technology is capable of producing chemicals such as volatile fatty acids (VFAs) and/or alcohols from the bioelectrochemical reduction of CO_2 [6]. In this conversion approach, certain kinds of microorganisms can reduce CO_2 using a solid electrode (cathode), which besides to be electron donor for their electroautotrophic metabolism also serves as growth surface for the biofilm [3]. This systems offer a dual advantage, since excess of electrical energy can be stored into chemicals while CO_2 can be removed from the atmosphere or directly captured from heavy CO_2 sources [7]. This fact makes MES an environmental-friendly technology helping to mitigate greenhouse gas emissions in bulk atmosphere or generation source.

MES technologies seem to be an ideal option for the combined purpose of energy storage and CO_2 utilization, which has been therefore purposed as a promising novel alternative for this issue [5,8,9]. However, renewable energy is intrinsically unpredictable due to fluctuations or lack of electrical supply which may affect the MES performance. As electrical electron supply plays the role of electron donor for the electroactive biofilm in MES, a lack of supply could drive the system to an unpredictable stand-by state in which the performance might be compromised or the biofilm altered.

To the best of our knowledge, some studies have indeed proposed MES technologies to take advantage of renewable energy surplus [5,8,9]; however, no studies have been made to test the influence of inconsistent nature of this kind of energy in CO_2 fed MES systems. In another study we firstly tested this effect on a bicarbonate fed system [10], however, as MES cells present different behavior while fed with dissolved bicarbonate or gas CO_2 [11], it is expected that the effect of power interruptions is also different in this case. The aim of this study is therefore investigating the effects of power supply interruptions on an acetogenic MES cell fed with gaseous CO_2, and comparing these results with the behavior found in a previously bicarbonate fed system. For this purpose, a MES system has been operated at fixed applied potential with a scheduled and increasing set of current interruptions from 4 to 64 hours. The system recovery in terms of current consumption and product generation was assessed. Furthermore, a study of the microbial biofilm before starting the interruptions and after the last one allowed checking which microorganisms were affected by power cuts or responsible of system performance changes along the experiment.

2. Material and methods

2.1 Microbial electrosynthesis reactor

Microbial electrosynthesis (MES) for producing organics from CO_2 was performed in a two-chambered H-cell reactor type previously described in [10]. H-cell reactor consisted of a cathodic and an anodic chamber with a volume of 250 mL each, separated by a Cation Exchange Membrane (CEM) Ion Power Nafion membrane N117, Germany (Figure 1). Cathode, used as working electrode, was made of a graphite stick placed between two graphite felts (Mast Carbon, UK) with an effective surface area of 33 cm^2. Anode electrode was a dynamically stable anode (DSA, Magneto Anodes, Netherlands), and reference electrode was an Ag/AgCl - 3M KCl electrode (Radiometer analytical, France), installed in the cathodic chamber in close proximity to the cathode.

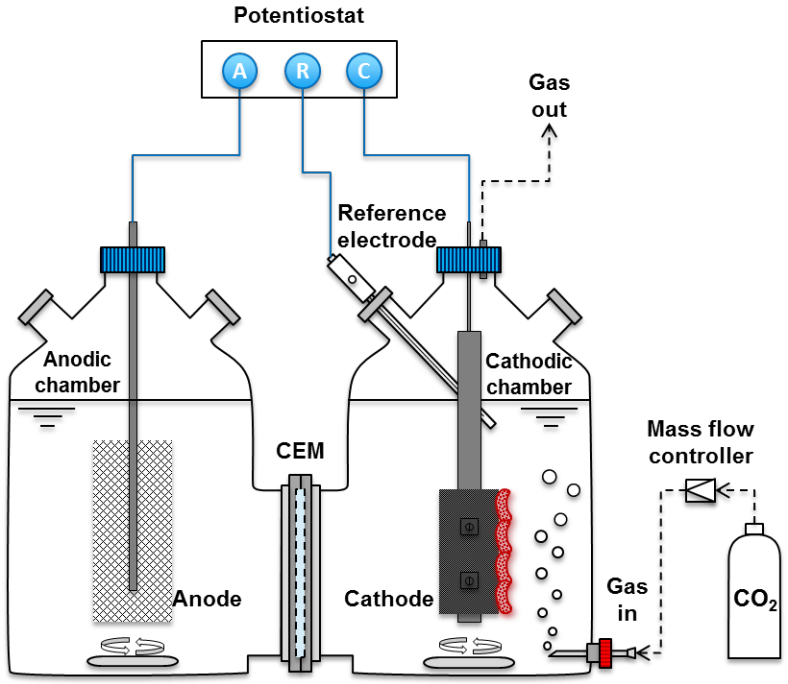

Figure 1 – Microbial electrosynthesis reactor (H-Cell MES)

2.2 Carbon source, electrolyte and inoculum,

Pure CO_2 was provided as the sole carbon source. CO_2 gas was bubbled into the mineral medium by a needle placed in cathodic chamber (Figure 1). A mass flow controller (Brooks instrument GF series) kept the CO_2 inflow at 10 mL min^{-1}. The composition of mineral medium of 2 mS/cm conductivity was: KH_2PO_4 monobasic (0.33 g L^{-1}); K_2HPO_4 dibasic (0.45 g L^{-1}); NH_4Cl (1 g L^{-1}); KCl (0.1 g L^{-1}); NaCl (0.8 g L^{-1}); $MgSO_4 \cdot 7H_2O$ (0.2 g L^{-1}); vitamin solution DSMZ 141 (1 mL L^{-1}), and trace solution DSMZ 141 (10 mL L^{-1}) [12].

The biocathode was taken from an H-Cell MES reactor producing acetate from sodium bicarbonate (0.05 M), which was operated for approximately 210 days [10]. Such a biocathode was subjected to different current supply interruptions from 4 h to 64 h. Original electro-autotrophic microorganism culture was taken from the supernatant of a running acetogenic MES which was enriched from an anaerobic sludge following the protocol reported in [13].

2.3 Set-up

H-Cell MES reactor was operated during 116 days divided in two batches (54 and 62 days), and continuously fed with pure CO_2. Each batch was referred to change of half of the electrolyte in order to dilute acetate concentration and avoid any possible product inhibition. H-Cell MES was subjected of energy supply interruptions of 4 h, 6 h, and 8 h during first batch, and 16 h, 32 h, and 64 h during second batch. Liquid sampling consisted of retrieving 5 mL of electrolyte from cathode chamber using a plastic syringe. Immediately after sampling, the same volume of fresh electrolyte was added in order to maintain constant effective volume. Gas samples of 1 mL were collected from headspace of cathode chamber before the liquid sampling.

2.4 Bioelectrochemical analyses

Volatile fatted acids (VFA) and ethanol were measured by high-performance liquid chromatography (HPLC) (Agilent 1200), equipped with an Agilent Hi-Plex H column and an Agilent 1260 infinity refractive index detector. Inorganic carbon in the liquid was measured in a total inorganic carbon analyzer (TOC 5050A – Shimadzu). Gas composition, i.e. hydrogen (H_2), carbon dioxide (CO_2), oxygen (O_2), nitrogen (N_2) and methane (CH_4), were determined by a gas chromatographic (CTC Analytics model HXT Pal), equipped with a thermal conductivity detector (TCD).

Using a Biologic multichannel potentiostat (software EC Lab vs. 10.23), H-Cell MES reactor was poised at -1.0 V vs. Ag/AgCl - 3M KCl reference electrode on a three-electrode setup. The reduction current was recorded each 600 seconds by means of chronoamperometry.

Production rates, based on volumetric or effective surface area of the cathode, and Coulombic efficiencies, based on acetate and hydrogen, were estimated by the equations previously described in [10].

2.5 Scanning electron microscopy (SEM)

SEM images were taken to verify microorganism attachment on the biocathode. Towards this end, approximately 0.25 cm^2 of biocathode from H-Cell MES and control clean carbon felt were sampled at the end of the experiment. A comparison between images of both the H-Cell reactor and the control graphite felt was believed to confirm the microorganism attachment in the biocathode. Preparation of samples was done as described previously [12] by fixing the microorganisms in 4% glutaraldehyde in sterile

phosphate buffer solution for 1 hour at room temperature; samples were rinsed and stored at 4 ºC overnight. Afterward, samples were dehydrated by series of 10 minutes with alcohol 20%, 30%, 50%, 70%, 90% and 100% and, then dried at CO_2 critical point for three hours, and gold coated.

2.6 Microbial community analysis

In order to analyze the microbial community present on the surface of the electrode, from supernatant at the end of the experiment and, from inoculum before starting power supply interruptions, genomic DNA was extracted using the Soil DNA Isolation Plus Kit® (Norgen Biotek Corp.), following the manufacturer's instructions. The entire DNA extracted was used for the pyrosequencing of eubacteria *16S-rRNA* gene based massive library. The primer set used was 27Fmod (5`-AGRGTTTGATCMTGGCTCAG-3`) /519R modBio (5`-GTNTTACNGCGGCKGCTG-3`) [14]. The obtained DNA reads were compiled in FASTq files for further bioinformatics processing. The following steps were performed using QIIME: Denoising, using a Denoiser [15]. Operational Taxonomic Units (OTUs) were then taxonomically classified using the Ribosomal Database Project (RDP) Bayesian Classifier (http://rdp.cme.msu.edu). Raw pyrosequencing data obtained from this analysis were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI).
Microbial richness estimators (S_{obs} and *Chao1*) and diversity index estimator (*Shannon*) were calculated with the defined OTUs table using MOTHUR software [16], version 1.35.1 at 3% distance level.

3. Results and discussion

3.1 Current supply interruptions on H-Cell MES reactor

Figure 2 shows that the production of acetate from CO_2 by MES was measurable from 5th day of operation, concomitantly with the increase in the reduction current. A continuous and uniform increase was observed until 26th day. During undisturbed period, maximum value of acetate concentration and rate before power interruptions reached 5656 mg L^{-1} and 516 mg L^{-1} d^{-1} (36 g m^{-2} d^{-1}) respectively, with reductive current density of around –27 A m^{-2}. At the beginning of the experiment while acetate was rapidly accumulating, the concentration of inorganic carbon fell from 324 mg L^{-1} to less than 30 mg L^{-1}, and it was maintained on this value. The low concentration of inorganic carbon in the culture medium while the acetate concentration was increasing can be an indication of microorganisms directly using the CO_2 in gaseous form as CO_2 was continuously fed, or immediate utilization of dissolved IC. It can also point out one of the main issues to solve in this kind of systems, which is the poor solubility of CO_2 in comparison with the organics production potential of this technology. This behavior was persistent during the whole experiment.

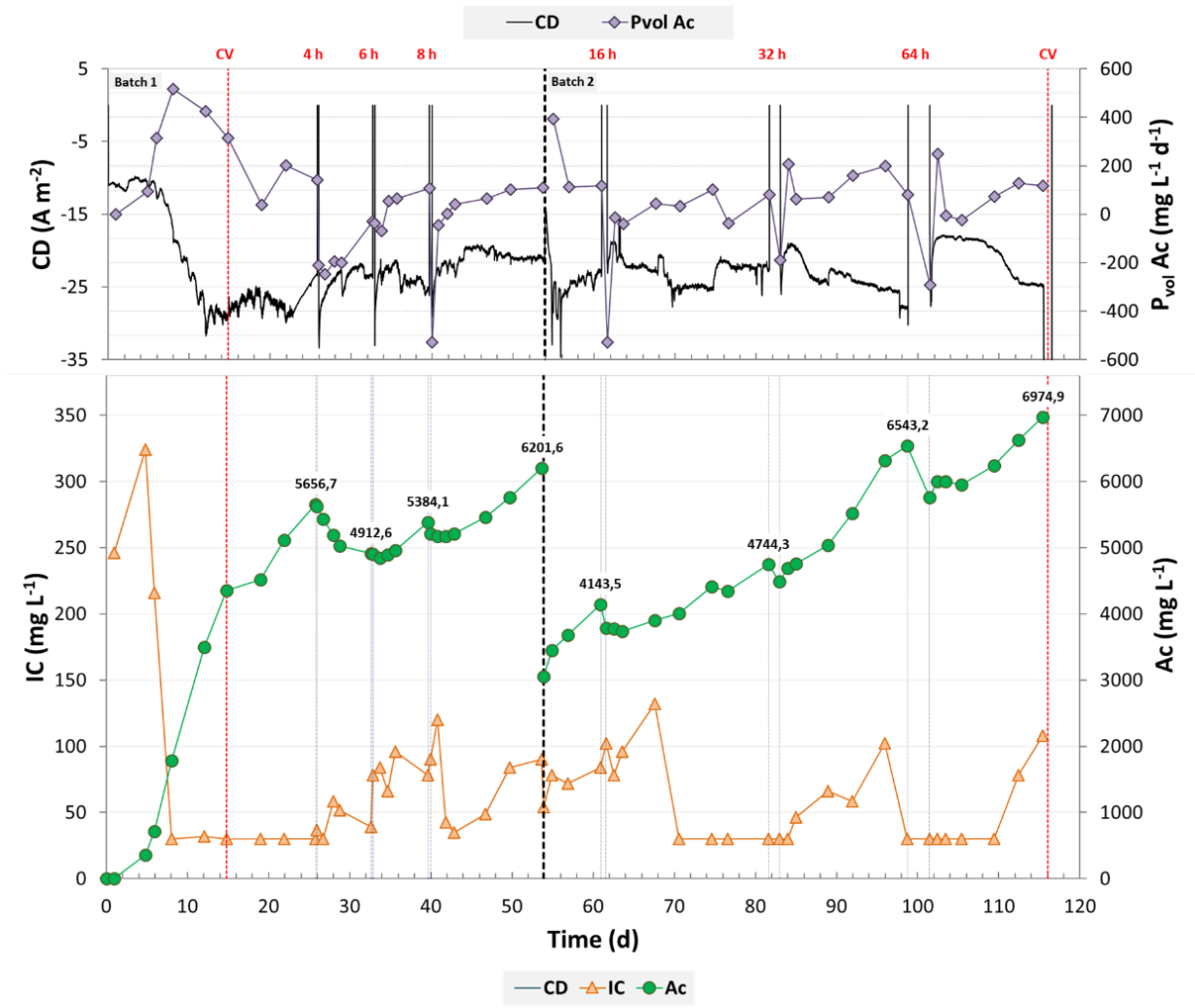

Figure 2 – H-Cell MES reactor performance under different current supply interruptions: Current density (CD), Inorganic carbon (IC) and Acetate (Ac) concentration

The experimental power interruptions period was divided in two different batches replacing half of the culture medium in order to avoid any possible product inhibition during this study and assuring comparability between gaps at the beginning and end. After 4 h of power supply interruption, acetate started to be consumed with a decrease of the concentration up to 4912 mg L^{-1} in seven days. However, the acetate consumption rate, represented by negative acetate production rate, was decreasing after electricity reconnection from –212 to –29 mg L^{-1} d^{-1} (–14.7 to –2.0 g m^{-2} d^{-1}) during the same period. Such a behavior was an indication that, although the interruption of current supply affected the microbial community, microorganisms were able to gradually restore their electroautotrophic activity. After 6 h gap, the initial response was similar to the previous one; the concentration of acetate was reduced from 4912 to 4845 mg L^{-1} in one day, but after that, it increased to 5384 mg L^{-1}. The acetate production rate fell just to –68 mg L^{-1} d^{-1} (–4.7 g m^{-2} d^{-1}), and it recovered and reached 107 mg L^{-1} d^{-1} (7.4 g m^{-2} d^{-1}) in five days. A similar behavior was found at the interruption of 8 h reaching a maximum acetate concentration of 6201 mg L^{-1} after 14 days of reconnection. This

means that after 40 days of continuously feeding pure CO_2 and without any addition of bicarbonate, the mixed culture biofilm was well stablished despite the three increasing interruption durations. Microorganisms could recover their electroautotrophic activity even when the interruption of electricity implied the consumption of a part of acetate during a short period of time.

During the second batch, in which 50% of the mineral medium was replaced in order to avoid any product inhibition caused by high acetate concentration, the system was firstly left connected without interruption for 5 days. After this period, acetate concentration achieved was 4143 mg L^{-1} at 115 mg L^{-1} d^{-1} (8.1 g m^{-2} d^{-1}). During the interruptions of 16, 32 and 64 h, acetate concentration had a decrease of approximately 500 mg L^{-1} immediately after each disconnection. However, after each interruption the production rate was recovered, reaching an average of 128 mg L^{-1} d^{-1} (8.8 g m^{-2} d^{-1}) (negative rates not taken into account) in all three cases, and achieving a maximum acetate concentration of 6975 mg L^{-1}. Such negative rates were observed just on the first day after electricity reconnection, time that microorganisms used for their recovery.

In a previous study, the microbial community producing acetate from bicarbonate (0.05 M) was showed to suffer some effects under different interruption regimes of electricity supply [10]. In those experimental batches, during the time off, electroautotrophic active microorganism seemed to go through a lag phase or a lethargy state, changing to fermentation and optimizing the energy gain. In some cases, acetate was re-oxidized, using the organic carbon for microorganism survivability and, then releasing CO_2 gas. Table 1 shows average current density and recovery time observed after the interruptions, in both cases, fed with bicarbonate and CO_2 gas. It is noticeable that the current density in MES fed with CO_2 gas was around 10-fold higher compared with the MES fed with bicarbonate. As expected, this increase on the use of electrons by microorganisms resulted in acetate production improvement.

Table 1 – Average current density and recovery time comparison between MES fed with bicarbonate and fed with CO_2 gas

Off period (h)	Fed with bicarbonate [10]			Fed with CO_2 gas		
	Average Current Density (A m^{-2})		Time recovery (h)	Average Current Density (A m^{-2})		Time recovery (h)
	Before off period	After off period		Before off period	After off period	
0	-1.78	-	-	-21.66	-	
4	-2.39	-2.09	1.4	-26.79*	-23.99	5.17
6	-2.15	-1.40	7.2		-23.75	5.33
8	-2.35	-1.62	11.7		-21.02	9.67
16	-2.38	-1.71	12.5	-24.03**	-22.81	6.67
32	-2.44	-1.11	15.6		-23.71	7.50
64	-2.52	-1.49	15.6		-22.22	8.17

* After Cyclic Voltammetry (CV), from 15th to 26th day of operation.
**Start of the second batch; from 54th to 61st day of operation.

On the other hand, Table 1 show that the recovery time after the interruptions was increasing in the first three gaps together with longer off periods similarly to MES fed with bicarbonate. However, in MES with CO_2 gas such an increase was not maintained between both batches. Although the first interruption in the second batch was 16 h, the recovery time was 3 h less than the recovery time observed after 8 h off. Therefore, recovery time could have been affected not only by the interruption period, as well as by the accumulation of acetic acid.

3.2 SEM

SEM images were used to visualize both the clean graphite electrode (Figure 3: A and B) and the inoculated electrode (Figure 3: C, D, E and F). These SEM images show irregular coverage of bacteria but confirmed clear biofilm formation on the electrode surface in the biofilm samples. In the control samples a smooth carbon material can be seen with a limited amount of impurities or dust. An overview of a graphite fiber can be seen in Figure 3C flanked by other two fibers and covered by a biofilm. Interestingly, a bacterial accumulation can be seen in the center of the image from which a bacterial bridge is formed to connect this fiber with the next one. A detail of the microbial accumulation (D) and the bridge (E) can also be seen in this figure. Last image in this figure (F) corresponds to the detail of a rod-shaped cell that is physically connected to the surface of the graphite fiber via pilus-like appendages. This kind of pilli has been reported to play an important role in the bioelectrochemical mechanism for product generation [3,17]. These studies state that this type of connections are nanowires which favor electron transfer, however, in our study more specific analytical techniques would be necessary to ensure this fact [18]. These pilus-like appendages can also be seen in between microorganisms facilitating interspecies electron transfer [17]. Similar extracellular appendage (pili or flagella)-like structures as those described in *Geobacter* spp., have been also identified in some species of *Desulfovibrio* (identified as one of the main genus present in our biofilm (Figure 4) and might also be involved in adherence to electrode [19]. In addition, some salt deposits can be seen over the carbon surface.

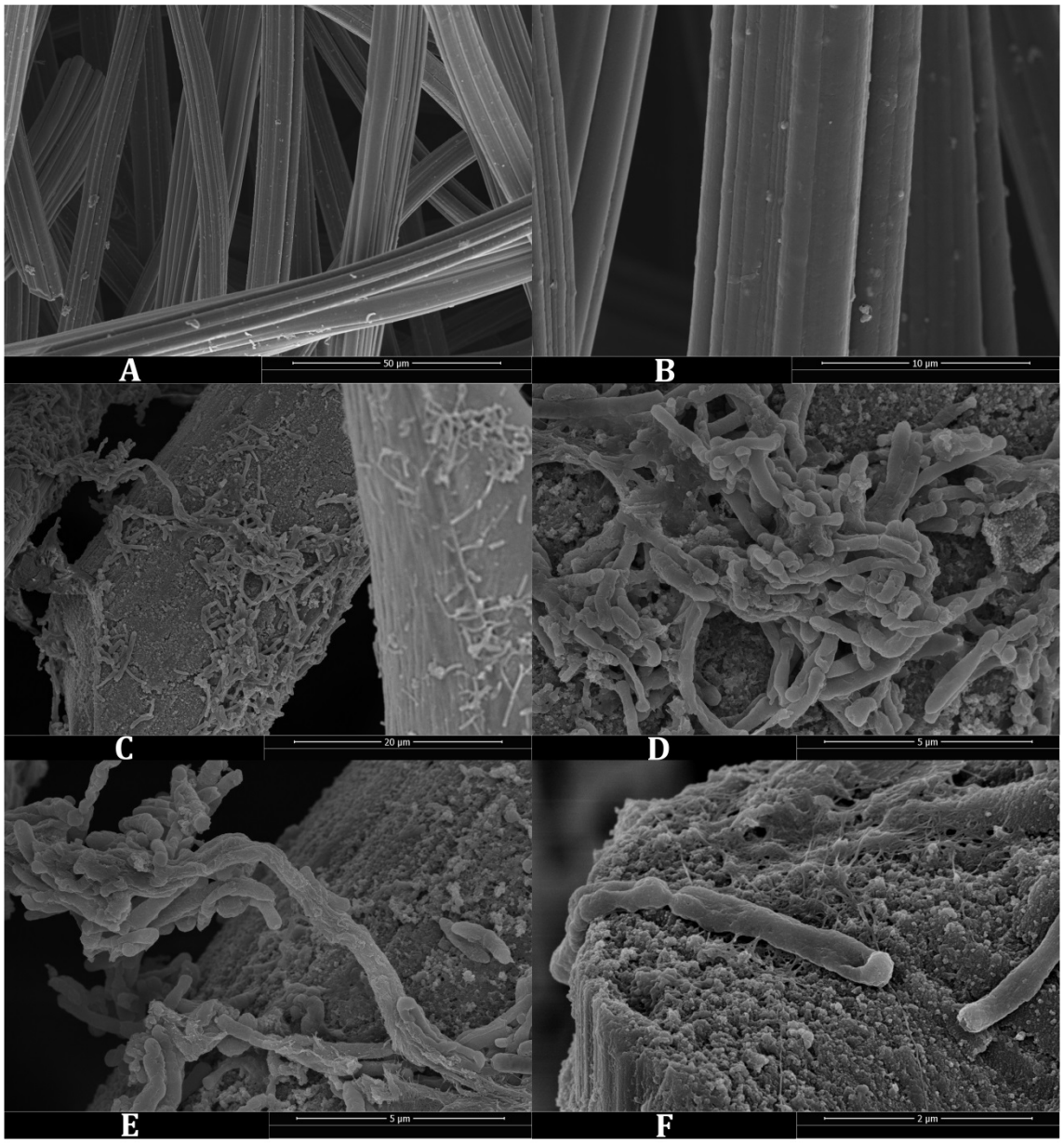

Figure 3 – SEM at different magnification of control clean graphite felt (A and B) and enriched biofilm covering the electrode (C, D, E and F).

3.3 Microbial community analysis

3.3.1 *Microbial diversity assessment*

High-throughput sequencing based on *16S rRNA* gene massive libraries was carried out in order to analyze the microbial community and structure both in the initial inoculum and in the final biofilm and supernatant. The alpha and beta-diversity analysis were performed in the three analyzed samples. The highest difference between the samples was the number of quality reads found for each one (Table 2). The number of sequences detected in the cathodic biofilm was 5-fold higher than in the supernatant, which means that the concentration of planktonic eubacteria with respect to those attached on the electrode was low. Despite the difference between the number of

sequences on each sample, the coverage values range was close to 100%, and therefore all diversity is represented on each sample. In general, these values show a great specialization in terms of eubacterial populations due to the high number of sequences analyzed corresponding to low species richness, as it has been observed by the OTUs and Chao1 index (Table 2). This could be due to the inoculum used, which came from an original electroautotrophic community previously enriched in another MES, as it has been described in material and methods section. On the other hand, the 1/Simpson index shows how the biofilm diversity index was reduced to less than half compared to the diversity represented in the inoculum. Moreover, the low bacterial concentration existing in the supernatant was, however, highly diverse approximately at the level of the biofilm (Table 2).

Table 2 - Nº of sequences and OTUs, estimated richness (Chao1), diversity index (Shannon) and sample coverage values for eubacterial operational taxonomic units (OTUs).

Sample	Nº Seqs.	Sobs OTUs	Chao1		1/Simpson		Coverage (%)
			mean	c.i.*	mean	c.i.*	
Inoculum	63367	295	398	356-466	13.1	12-13	99.8
Biofilm	49899	234	356	295-476	4.9	4.8-5.0	99.8
Supernatant	9521	130	229	176-354	12.8	12.4-13.5	99.5

*c.i.: 95% confidence intervals

3.3.2 *Microbial community composition.*

The evolution of the microbial communities from the inoculum to the eubacterial population established in the biofilm is represented in Figure 4. The phyla representation in the inoculum is 45.3% *Proteobacteria* and 36.4% *Firmicutes*. However, *Proteobacteria* decreased to 13% in the biofilm, while *Firmicutes* increased to 85%. Most part of *Firmicutes* (81%) in the biofilm is represented by two families, *Veillonellaceae* and *Clostridiaceae*. The third most dominant family enriched in the biofilm is *Desulfovibrionaceae*, belonging to *Proteobacteria*. It should be noted that these three families are composed by only one genus, demonstrating again the high specialization of these populations. The family *Veillonellaceae* is strongly represented by *Sporomusa* (56.5%), *Desulfovibrionaceae* by genus *Desulfovibrio* (12%) and *Clostridiaceae* by *Clostridium* (24.5%). These three genera accounted for 93% of the total eubacterial community attached to the electrode.

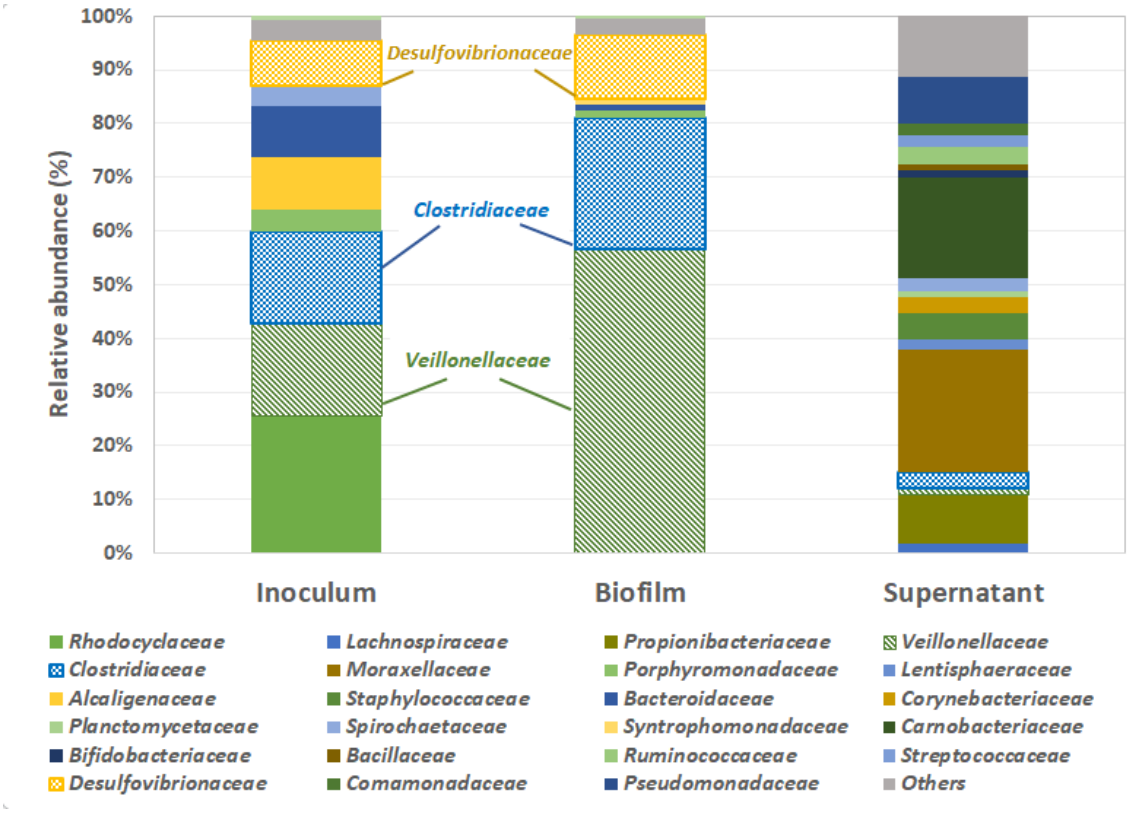

Figure 4 – Taxonomic classification of 16S rRNA gene from eubacterial classification at a family level. Groups making up less than 1% of the total number of sequences per sample were classified as "others".

An analysis of the microbial community composition in the supernatant (Figure 4) revealed a dramatic difference with respect to those identified in the electrode. This population is not specialized and is composed of a high diversity community (Table 1) in a low relative abundance. The highest relative abundance families were *Moraxellaceae* previously described as electrotrophic microorganism in cathodic communities (Semenec and Franks, 2015), *Carnobacteriaceae*, *Propionibacteriaceae* and *Lachnospiraceae*, which may be involved in intermediary metabolic pathways. Moreover, *Pseudomonadacea* was detected, which is usually more abundant in suspension, and it has physiological evidence for hydrogenase activity [21] and is known for producing shuttles in bioelectrochemical systems, and hence plays a role in extracellular electron transfer [22].

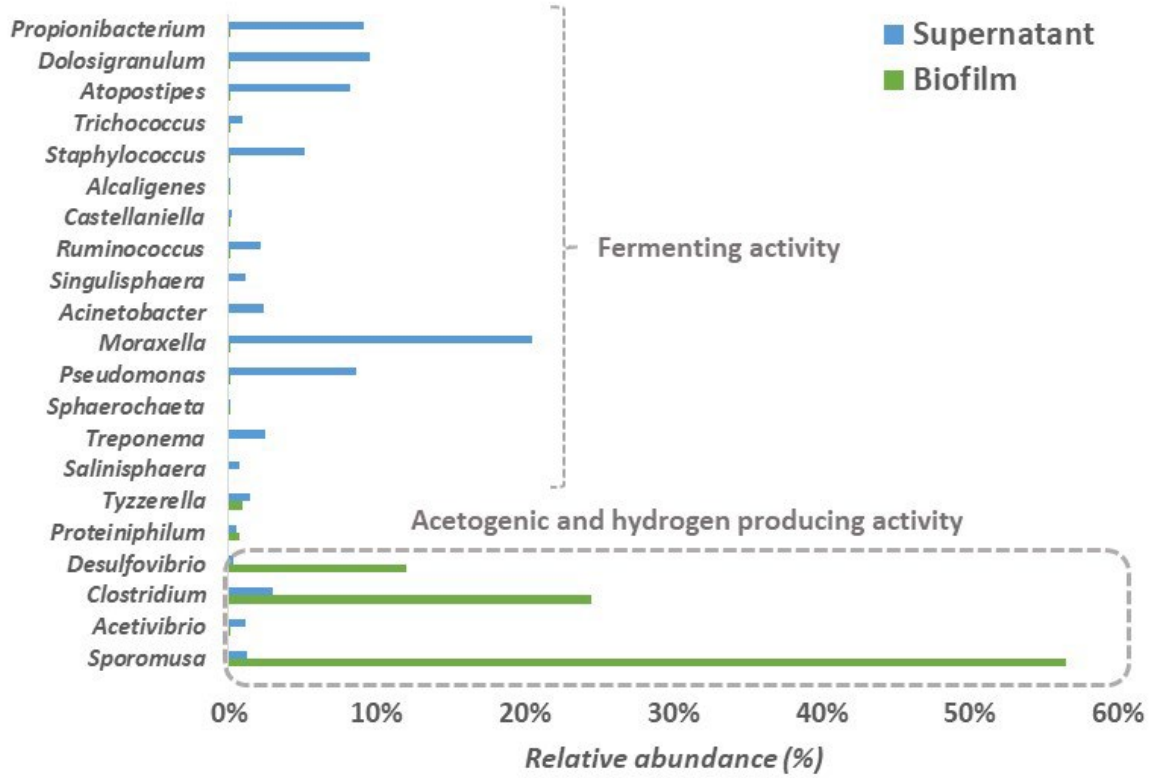

Figure 5 – The most abundant genera identified in the supernatant and biofilm samples.

3.3.3 The role of main identified genera.

The community attached on the electrode was absolutely dominated by microorganisms belonging to three genera namely *Sporomusa*, *Clostridium* and *Desulfovibrio* (Figure 5). The potential function of the dominant genera can be classified as acetogenic and hydrogen producing activity. The OTUs belonging to *Sporomusa* and *Clostridium* genera could be responsible of the maximum production rate of 516 mg L^{-1} d^{-1} acetic acid reached in this experiment. Moreover, contrary to other well-known acetogenic bacteria as *Acetobacterium,* dominant in MES, both *Sporomusa* and *Clostridium* have been identified as acetogenic bacteria with bioelectrochemical activity [23]. In general, acetate is the primary product of these acetogenic bacteria but other intermediates as 2-oxobutyrate and formate are formed [24]. A syntrophic relationship can be stablished in the biofilm as *Desulfovibrio* presents formate dehydrogenase activity to produce CO_2, and this could explain that it was more abundant on the electrode compared to the supernatant [25,26]. Furthermore, the electrochemically active *Desulfovibrio* has been previously shown as being able to catalyze hydrogen production on biocathodes [19], and it also performs a combination of carbon-fixation and acetate utilization [26]. Therefore, *Desulfovibrio* could be responsible for acetic acid oxidation to CO_2 during the absence of power supply (Figure 2).

Regarding the supernatant, the main genus observed is *Moraxella* (20.4%) and the rest of the community is composed by groups below 10% among which

Pseudomonas, *Propionibacterium*, *Acinetobacter* or *Treponema* are present (Figure 5). It is quite evident that both communities (biofilm and supernatant) are completely different (Figure 5), however some bacteria are common to both. Phylotypes identified as *Sporomusa* have been found in the supernatant (1.2%), while this abundance increased up to 56.5% in the biofilm being the clear dominant bacteria. The same happens with *Clostridium* and *Desulfovibrio* which increased their abundance in the biofilm but are also identified in the supernatant although below 3%. Some minor OTUs belonging to *Proteiniphilum*, which has been also shown to generate acetate [26], are also present in both communities.

To sum up, it can be stated that apart from some similarities between biofilm and supernatant communities, the acetogenic activity was represented by members attached onto the biofilm, while the supernatant community was responsible of the fermentative metabolism.

4. Conclusion

This study explores the effect of electrical power interruptions on a MES system fed with gaseous CO_2. The results showed that power supply interruptions affected the behavior of the system for a period below one day, in which the microbial community reverses the acetogenic reaction, consuming a part of the product for survivability. After this disturbance, the system showed to be recovered, due to a robust population formed by bioelectrochemically active acetogenic bacteria, reaching production rates and current consumptions similar to the values found before the interruptions. Highest product titer was found at the end of the experiment (6975 mg L^{-1}) while highest production rate was achieved before power interruptions (516 mg L^{-1} d^{-1}). The maximum recovery time was 9.7h after 8 h off. These high production figures were due to a cathodic biofilm absolutely dominated by well-known electroactive bacteria such as *Sporomusa*, *Clostridium* and *Desulfovibrio*. Therefore this MES system proved to be ready for real-field application, in which the bioelectrochemical system could be powered by energy surpluses from renewable energy.

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