Microbial electrosynthesis (MES) 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.

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 [2], water pumping storage [3] or hydrogen production by water splitting [4] have been proposed for the surplus electricity exploitation [5]. Recently, using excess electricity to convert CO_2 into organic chemicals and fuels has come up as a novel alternative for off-peak electrical overproduction, in which electrical energy is stored in the form of chemical energy [6,7]. MES is a recent

technology, an offshoot of conventional bioelectrochemical systems (BESs) used for wastewater treatment and energy recovery, proposed in 2010 [8]. This technology is able to produce chemicals such as volatile fatty acids (VFAs) and/or alcohols from the bioelectrochemical reduction of CO_2 [9]. In this conversion approach, certain kinds of microorganisms can reduce CO_2 using a solid electrode (cathode), which besides being an electron donor for their electroautotrophic metabolism also serves as growth surface for the biofilm [6,10]. This systems offer a dual advantage, since the 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 [11]. This fact makes MES an environmental-friendly technology helping to mitigate greenhouse gas

emissions in bulk atmosphere or generation source.

Therefore, MES seem to be an ideal option for the combined purpose of energy storage and CO_2 utilization, which has been purposed as a promising novel alternative for this issue [8,12,13]. 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 [8,12,13]; however, no studies have been made to test the influence of inconsistent nature of this kind of energy in CO_2 fed MES systems. Moreover, none of these studies have evaluated the influence of the microbial community present in the reaction chamber and electrode.

A preliminary study, using MES fed with sodium bicarbonate showed that absence of electricity during periods between 4 h and 64 h affected product formation, causing temporary pathway reversal and decrease in production rates [14]. Nevertheless, the same study indicates that the microbial community is able to recover after reconnection. However, as MES cells present different behavior while being fed with dissolved bicarbonate or gaseous CO_2 [15], it is expected that the effect of power interruptions is also different in this case.

In that context, the aim of this study is to assess 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 [14]. For this purpose, a MES system fed with pure CO_2 gas rather than bicarbonate was operated at fixed applied potential in a semi-continuous mode under a scheduled and increasing set of current interruptions from 4 to 64 h. The system recovery was evaluated in terms of product generation and rate, and current consumption before, during and after power interruptions. The microbial biofilm and supernatant was analyzed before starting the interruptions and after the last one, allowing identifying which microorganisms were affected by power cuts or responsible of system performance changes along the experiment. Therefore, to the best of our knowledge, this is the first study presenting the alterations on the microbial community of MES submitted to electricity fluctuations.

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 [14]. 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 (Fig. 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 - 3 M KCl electrode (Radiometer analytical, France), installed in the cathodic chamber in close proximity to the cathode.

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 (Fig. 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
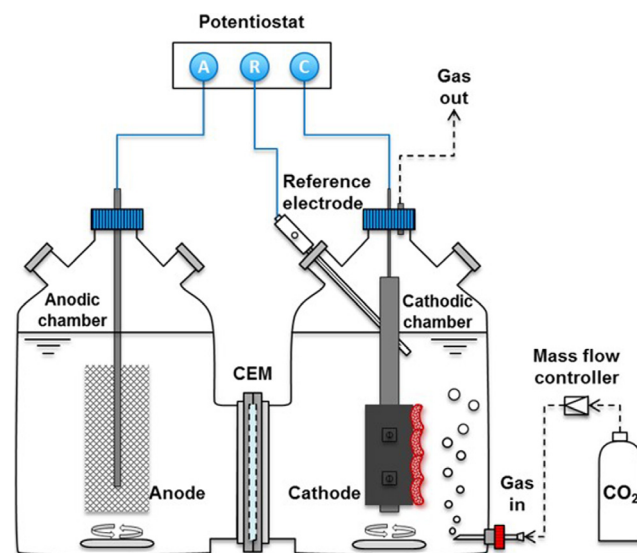

Fig. 1. Microbial electrosynthesis reactor (H-Cell MES).

(1 mL L^{-1}), and trace solution DSMZ 141 (10 mL L^{-1}) [16].

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 [14]. Such a biocathode was subjected to different current supply interruptions from 4 h to 64 h. Original electroautotrophic microorganism culture was taken from the supernatant of a running acetogenic MES which was enriched from an anaerobic sludge following the protocol reported in [17].

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 - 3 M KCl reference electrode on a three-electrode setup. The reduction current was recorded each 600 s 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 [14].

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 [16] by fixing the microorganisms in 4% glutaraldehyde in sterile phosphate buffer solution for 1 h at room temperature; samples were rinsed and stored at 4 °C overnight. Afterward, samples were dehydrated by series of 10 min 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′-GTNTTACNG-CGGCKGCTG-3′) [18]. The obtained DNA reads were compiled in FASTq files for further bioinformatics processing. The following steps were performed using QIIME: Denoising, using a Denoiser [19]. Operational Taxonomic Units (OTUs) were then taxonomically classified using the Ribosomal Database Project (RDP) Bayesian Classifier (http://rdp.cme.msu.edu).

Microbial richness estimators (S_{obs} and Chao1) and diversity index estimator (Shannon) were calculated with the defined OTUs table using MOTHUR software [20], version 1.35.1 at 3% distance level.

3. Results and discussion

3.1. Current supply interruptions on H-Cell MES reactor

Fig. 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.

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 [14]. 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.

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 (Fig. 3: A and B) and the inoculated electrode (Fig. 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 Fig. 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
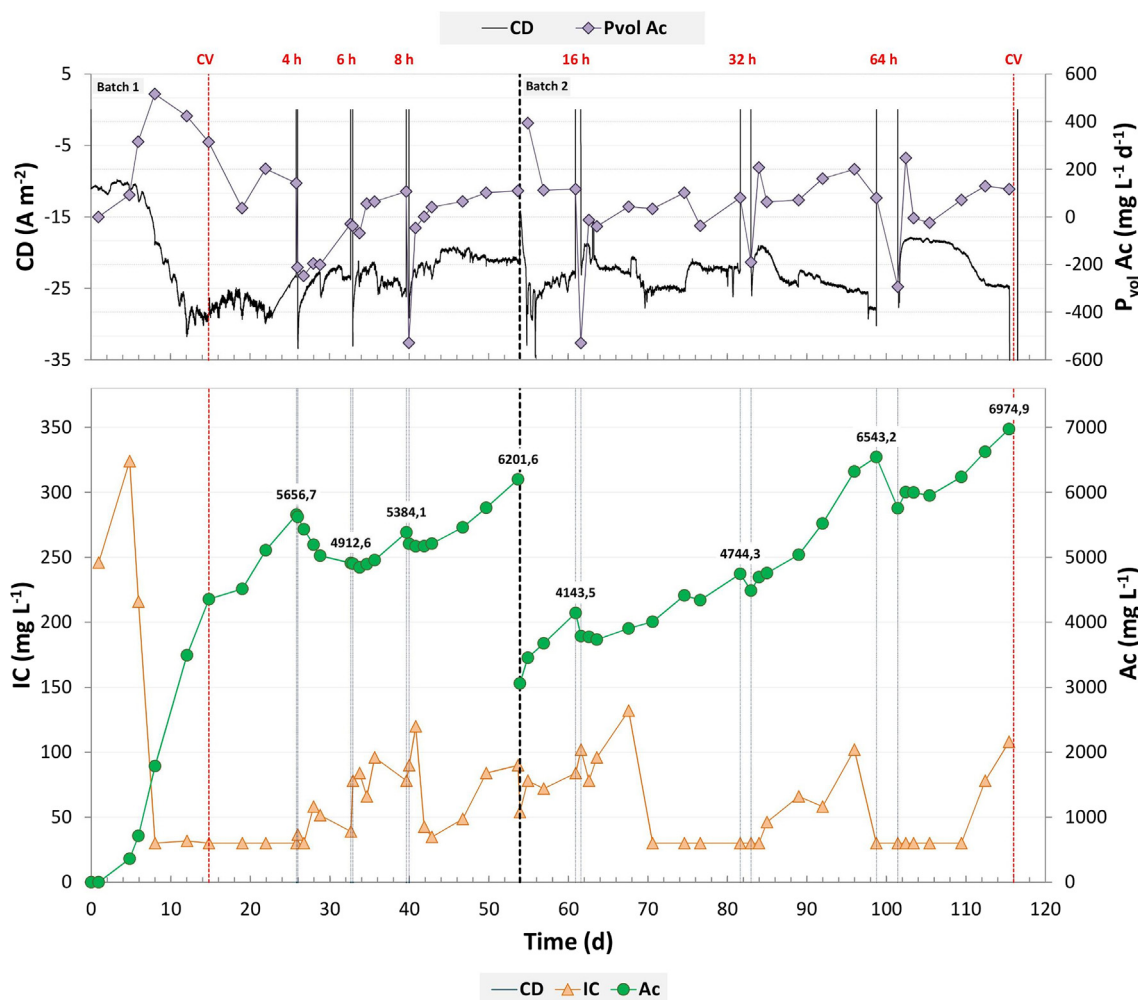
275-279

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

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 [14]			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.

appendages. This kind of pili has been reported to play an important role in the bioelectrochemical mechanism for product generation [6,21]. 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 [22]. These pilus-like appendages can also be seen in between microorganisms facilitating interspecies electron transfer [21]. 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 (Fig. 4) and might also be involved in adherence to electrode [23]. In addition, some salt deposits can be seen over the carbon surface.

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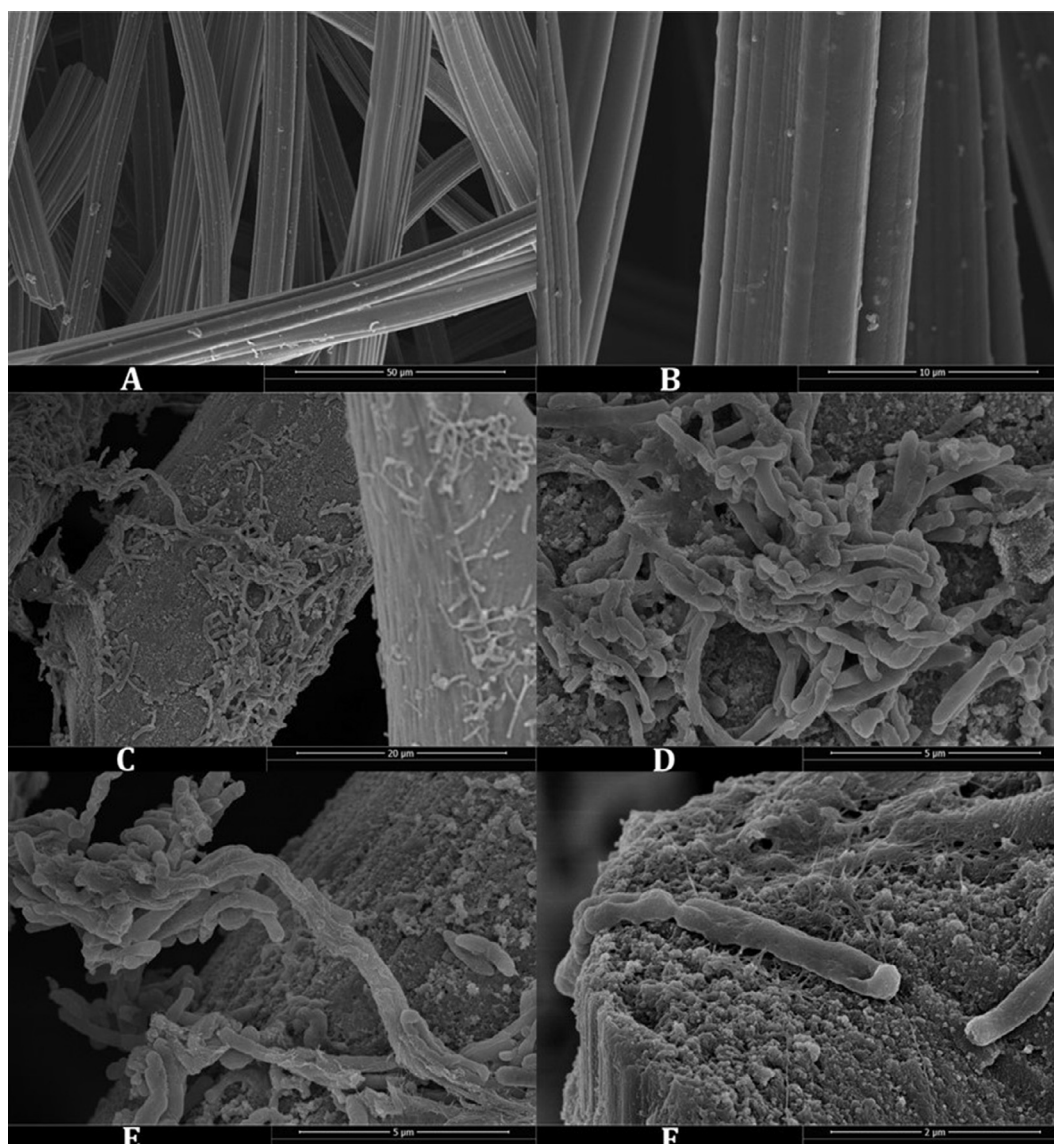
Fig. 3. SEM at different magnification of control clean graphite felt (A and B) and enriched biofilm covering the electrode (C, D, E and F).

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).

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 Fig. 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.

An analysis of the microbial community composition in the

supernatant (Fig. 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 [24], *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 [25] and is known for producing shuttles in bioelectrochemical systems, and hence plays a role in extracellular electron transfer [26].

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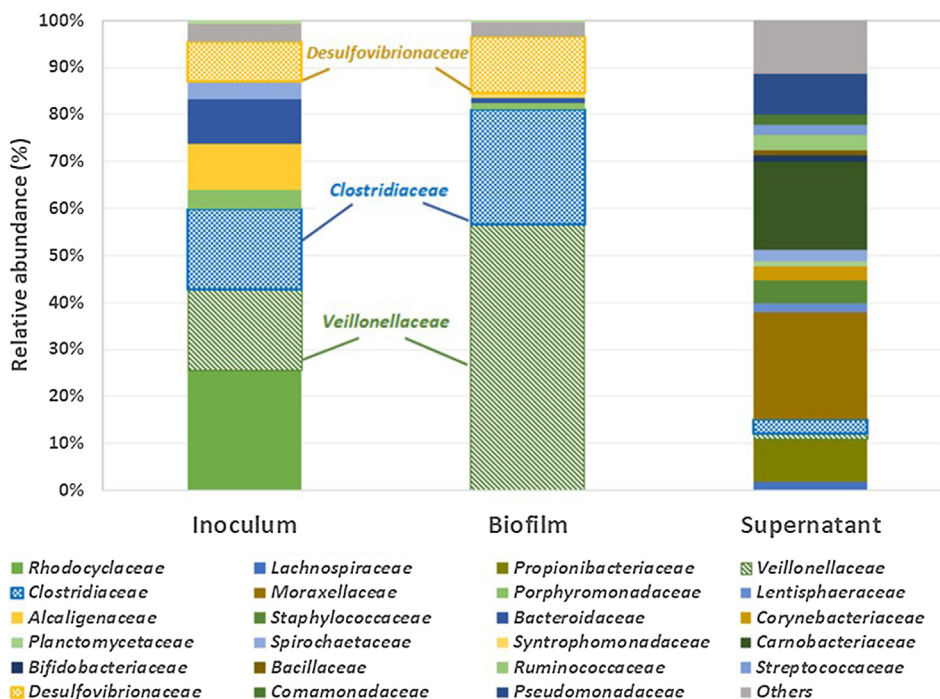
Fig. 4. Taxonomic classification of 16S rRNA gene from eubacterial classification at a family level. Groups making up less than 1% of the total number of sequences per sample were classified as "others".

Table 2
N° of sequences and OTUs, estimated richness (Chao1), diversity index (Shannon) and sample coverage values for eubacterial operational taxonomic units (OTUs).

Sample	N° Seqs.	Sobs OTUs	Chao1		1/Simpson		Coverage
			mean	c.i. [*]	mean	c.i. [*]	(%)
Inoculum	63,367	295	398	356–466	13.1	12–13	99.8
Biofilm	49,899	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.

both *Sporomusa* and *Clostridium* have been identified as acetogenic bacteria with bioelectrochemical activity [27]. In general, acetate is the primary product of these acetogenic bacteria but other intermediates as 2-oxobutyrate and formate are formed [28]. A syntrophic relationship can be stablished in the biofilm as *Desulfovibrio* presents formate de-hydrogenase activity to produce CO_2, and this could explain that it was more abundant on the electrode compared to the supernatant [29,30]. Furthermore, the electrochemically active *Desulfovibrio* has been previously shown as being able to catalyze hydrogen production on bio-cathodes [23], and it also performs a combination of carbon-fixation and acetate utilization [30]. Therefore, *Desulfovibrio* could be
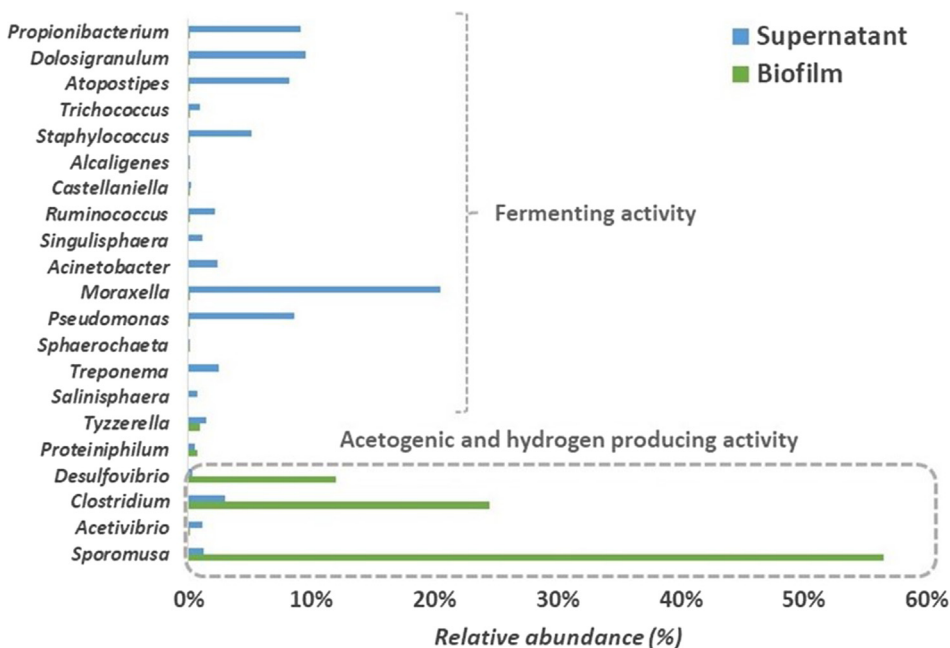

Fig. 5. The most abundant genera identified in the supernatant and biofilm samples.

responsible for acetic acid oxidation to CO_2 during the absence of power supply (Fig. 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 (Fig. 5). It is quite evident that both communities (biofilm and supernatant) are completely different (Fig. 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 [30], 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.

3.4. Technological and commercial perspectives

Here, we have shown how MES systems can withstand power supply interruptions without a significant deterioration in its performance, which allows for moderate optimism about its integration with renewable energy sources. Still, significant challenges lay ahead, making the journey for real-field implementation a demanding one. In a simplified techno-economic evaluation, ElMekawy et al. [31] situated MES, together with microalgae and photosynthetic systems, as equally promising and viable technologies for CO_2 conversion into chemicals, additionally showing a relatively large potential market for acetic acid (~12,900 tonnes per year). However, the production costs of pure acetic acid from CO_2 with a MES system as core reactor can be as high as 3.84–5.74 £/kg (~4.30–6.42 €/kg for a 1000 tonnes per year basis) [32], which is still one order of magnitude above its current market price. Improved titers and productivities are critical to make acetate production through MES competitive.

In addition to market barriers, MES need to face significant technological hurdles, most of which are common to all BES, but some others are intrinsic to MES. On the one hand, as renewable energy production is unpredictable and must be coupled to a CO_2 stream (that could also present fluctuations), suitable energy control systems and strategies will be critical to effectively combine both systems. The perturbation and observation method proposed by Tartakovsky et al. [33] for the optimization of a single microbial electrolysis cell, or the dynamically adaptive control system for stacked BES developed by Andersen et al. [34] can provide the basis for specific control systems for MES. Another obstacle of CO_2-reducing MES systems has to do with the poor substrate availability to microorganisms, as CO_2 solubility kinetics are not fast enough in these culture media. In proof-of-concept and lab scale studies, MES are usually provided with an excess of CO_2 to improve mass transport and production rates. However, an important amount of substrate is lost in the process, making this approach unsuitable for real-field application. Recirculation or stirring systems to achieve higher mass transfer from the bulk to the biofilm could become relevant alternatives. Relatively high energy consumption rates represents another important drawback of MES that impacts directly on its economic feasibility. Water oxidation (Standard reaction potential: +1.23 V vs. SHE) is the reaction that usually takes place in the counter electrode to provide electrons and protons to the biocathode. Although pure oxygen can be recovered as a byproduct in the process, cell potential gets easily over 3 V resulting in large energy usage per gram of acetic acid produced. Using bioanodes as counter electrode could reduce cell potential to half as they can operate at around 0 V vs. SHE [35]. However, this approach is not without difficulties since bioanodes would require an organic substrate (most probably a waste stream),

which can give rise to contamination issues.

4. Conclusion

This study explores for the first time the effect of electrical power interruptions on a MES system fed with gaseous CO_2. Power supply interruptions affected the behavior of MES, causing the microbial community to reverse the acetogenic reaction for a period below one day and consume a part of the product for survivability. However after power interruptions, the system showed to recover with a maximum recovery time of 9.7 h after 8 h of power interruption. Such a recovery was associated with the 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}). Cathodic biofilm was dominated by the well-known electroactive bacteria *Sporomusa*, *Clostridium* and *Desulfovibrio*, which showed to be resilient to frequent interruptions of electricity supply. Therefore, a MES system proved to be ready for renewable energy supply coupling withstanding power fluctuations. This fact opens the pathway to a profitable symbiotic relationship between renewable energy and MES systems, although extensive further work must be carried out to achieve real-field implementation of MES systems together with renewable energy power plants.

References

[1] IEA. World Energy Outlook 2017. Organisation for Economic Co-operation and Development, OECD; 2017.

[2] Hu X, Zou C, Zhang C, Li Y. Technological developments in batteries: a survey of principal roles, types, and management needs. IEEE Power Energ Mag 2017;15:20–31.

[3] Bakelli Y, Hadj Arab A, Azoui B. Optimal sizing of photovoltaic pumping system with water tank storage using LPSP concept. Sol Energy 2011;85:288–94. https://doi.org/10.1016/J.SOLENER.2010.11.023.

[4] Chi J, Yu H. Water electrolysis based on renewable energy for hydrogen production. Chin J Catal 2018;39:390–4. https://doi.org/10.1016/S1872-2067(17)62949-8.

[5] Yekini Suberu M, Wazir Mustafa M, Bashir N. Energy storage systems for renewable energy power sector integration and mitigation of intermittency. Renew Sustain Energy Rev 2014;35:499–514. https://doi.org/10.1016/J.RSER.2014.04.009.

[6] Rabaey K, Rozendal RA. Microbial electrosynthesis—revisiting the electrical route for microbial production. Nat Rev Microbiol 2010;8:706–16.

[7] Desloover J, Arends JBA, Hennebel T, Rabaey K. Operational and technical considerations for microbial electrosynthesis. Biochem Soc Trans 2012;40. 1233 LP-1238.

[8] Nevin KP, Woodard TL, Franks AE, Summers ZM, Lovley DR. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. MBio 2010;1:e00103–10.

[9] Bajracharya S, Srikanth S, Mohanakrishna G, Zacharia R, Strik DP, Pant D. Biotransformation of carbon dioxide in bioelectrochemical systems: state of the art and future prospects. J Power Sources 2017;356:256–73. https://doi.org/10.1016/j.jpowsour.2017.04.024.

[10] Mateos R, Sotres A, Alonso RM, Escapa A, Morán A. Impact of the start-up process on the microbial communities in biocathodes for electrosynthesis. Bioelectrochemistry 2018;121:27–37. https://doi.org/10.1016/j.bioelechem.2018.01.002.

[11] Sato K, Kawaguchi H, Kobayashi H. Bio-electrochemical conversion of carbon dioxide to methane in geological storage reservoirs. Energy Convers Manage 2013;66:343–50. https://doi.org/10.1016/J.ENCONMAN.2012.12.008.

[12] Abate S, Lanzafame P, Perathoner S, Centi G. New sustainable model of biorefineries: biofactories and challenges of integrating bio-and solar refineries. ChemSusChem 2015;8:2854–66.

[13] Perathoner S, Centi G. CO₂ recycling: a key strategy to introduce green energy in the chemical production chain. ChemSusChem 2014;7:1274–82. https://doi.org/10. 1002/cssc.201300926.

[14] del Anzola Rojas M del P, Zaiat M, Gonzalez ER, De Wever H, Pant D. Effect of the electric supply interruption on a microbial electrosynthesis system converting inorganic carbon into acetate. Bioresource Technol 2018;266:203–10. https://doi. org/10.1016/J.BIORTECH.2018.06.074.

[15] Mohanakrishna G, Vanbroekhoven K, Pant D. Imperative role of applied potential and inorganic carbon source on acetate production through microbial electrosynthesis. J CO2 Utiliz 2016;15:57–64. https://doi.org/10.1016/j.jcou.2016.03. 003.

[16] Bajracharya S, ter Heijne A, Dominguez Benetton X, Vanbroekhoven K, Buisman CJN, Strik DPBTB, et al. Carbon dioxide reduction by mixed and pure cultures in microbial electrosynthesis using an assembly of graphite felt and stainless steel as a cathode. Bioresource Technol 2015;195:14–24. https://doi.org/10.1016/j.biortech. 2015.05.081.

[17] Bajracharya S, Yuliasni R, Vanbroekhoven K, Buisman CJN, Strik DPBTB, Pant D. Long-term operation of microbial electrosynthesis cell reducing CO₂ to multi-carbon chemicals with a mixed culture avoiding methanogenesis. Bioelectrochemistry 2017;113:26–34. https://doi.org/10.1016/j.bioelechem.2016. 09.001.

[18] Callaway TR, Dowd SE, Wolcott RD, Sun Y, McReynolds JL, Edrington TS, et al. Evaluation of the bacterial diversity in cecal contents of laying hens fed various molting diets by using bacterial tag-encoded FLX amplicon pyrosequencing. Poult Sci 2009;88:298–302. https://doi.org/10.3382/ps.2008-00222.

[19] Reeder J, Knight R. Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. Nat Methods 2010;7:668–9. https://doi.org/10. 1038/nmeth0910-668b.

[20] Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 2009;75:7537–41. https://doi.org/10.1128/AEM.01541-09.

[21] Malvankar NS, Lovley DR. Microbial nanowires for bioenergy applications. Curr Opin Biotechnol 2014;27:88–95. https://doi.org/10.1016/J.COPBIO.2013.12.003.

[22] Tan Y, Adhikari RY, Malvankar NS, Pi S, Ward JE, Woodard TL, et al. Synthetic biological protein nanowires with high conductivity. Small 2016;12:4481–5.

[23] Croese E, Pereira MA, Euverink GJW, Stams AJM, Geelhoed JS. Analysis of the microbial community of the biocathode of a hydrogen-producing microbial electrolysis cell. Appl Microbiol Biotechnol 2011;92:1083–93. https://doi.org/10. 1007/s00253-011-3583-x.

[24] Semenec L, Franks AE. Delving through electrogenic biofilms: from anodes to cathodes to microbes. AIMS Bioeng 2015;2:222–48. https://doi.org/10.3934/ bioeng.2015.3.222.

[25] Schwartz E, Friedrich B. The H2-metabolizing prokaryotes; 2006.

[26] Rabaey K, Boon N, Höfte M, Verstraete W. Microbial phenazine production enhances electron transfer in biofuel cells. Environ Sci Technol 2005;39:3401–8. https://doi.org/10.1021/es048563o.

[27] Saheb-Alam S, Singh A, Hermansson M, Persson F, Schnürer A, Wilén B-M, et al. Effect of start-up strategies and electrode materials on carbon dioxide reduction on bio-cathodes. AEM.02242-17 Appl Environ Microbiol2017. https://doi.org/10. 1128/AEM.02242-17.

[28] Nevin KP, Hensley SA, Franks AE, Summers ZM, Ou J, Woodard TL, et al. Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. Appl Environ Microbiol 2011;77:2882–6. https://doi.org/10.1128/AEM.02642-10.

[29] Patil SA, Arends JBA, Vanwonterghem I, Van Meerbergen J, Guo K, Tyson GW, et al. Selective enrichment establishes a stable performing community for microbial electrosynthesis of acetate from CO₂. Environ Sci Technol 2015;49:8833–43.

[30] Marshall CW, Ross DE, Handley KM, Weisenhorn PB, Edirisinghe JN, Henry CS, et al. Metabolic reconstruction and modeling microbial electrosynthesis. Sci Rep 2017;7:1–12. https://doi.org/10.1038/s41598-017-08877-z.

[31] ElMekawy A, Hegab HM, Mohanakrishna G, Elbaz AF, Bulut M, Pant D. Technological advances in CO₂ conversion electro-biorefinery: a step toward commercialization. Bioresour Technol 2016;215:357–70. https://doi.org/10.1016/j. biortech.2016.03.023.

[32] Christodoulou X, Okoroafor T, Parry S, Velasquez-Orta SB. The use of carbon dioxide in microbial electrosynthesis: advancements, sustainability and economic feasibility. J CO2 Utiliz 2017;18:390–9. https://doi.org/10.1016/J.JCOU.2017.01. 027.

[33] Tartakovsky B, Mehta P, Santoyo G, Guiot SR. Maximizing hydrogen production in a microbial electrolysis cell by real-time optimization of applied voltage. Int J Hydrogen Energy 2011;36:10557–64. https://doi.org/10.1016/J.IJHYDENE.2011. 05.162.

[34] Andersen SJ, Pikaar I, Freguia S, Lovell BC, Rabaey K, Rozendal RA. Dynamically adaptive control system for bioanodes in serially stacked bioelectrochemical systems. Environ Sci Technol 2013;47:5488–94.

[35] Nam J-Y, Tokash JC, Logan BE. Comparison of microbial electrolysis cells operated with added voltage or by setting the anode potential. Int J Hydrogen Energy 2011;36:10550–6. https://doi.org/10.1016/J.IJHYDENE.2011.05.148.