Elucidating the impact of power interruptions on microbial electromethanogenesis

Guillermo Pelaz[a], Rubén González[b], Antonio Morán [a], Adrián Escapa* [a,b].

[a] Chemical and Environmental Bioprocess Engineering Group, Natural Resources Institute (IRENA), University of León, Av. Portugal, 41, 24071 León, Spain

[b] Department of Electrical Engineering and Automatic Systems, Campus de Vegazana s/n, Universidad de León, E-24071 León, Spain

*Corresponding author. E-mail address: aescg@unileon.es (A. Escapa)

Highlights

- Electromethanogenesis is resilient to power supply interruptions.
- Power interruptions with no H_2 supply had a large impact on methanogens.
- Power interruptions with no H_2 supply induced large variability.
- Power interruptions with H_2 supply limited the metabolism of electrotrophs.
- Shifts in microbial populations do not correlate methane production rates.

Abstract

The need to accommodate power fluctuations intrinsic to high-renewable systems will demand in the future the implementation of large quantities of energy storage capacity. Electromethanogenesis (EM) can potentially absorb the excess of renewable energy and store it as CH_4. However, it is still unknown how power fluctuations impact on the performance of EM systems. In this study, power gaps from 24 to 96 h were applied to two 0.5 L EM reactors to assess the effect of power interruptions on current density, methane production and current conversion efficiency. In addition, the cathodes where operated with and without external H_2 supplementation during the power-off periods to analyse how power outages affect the two main metabolic stages of the EM (i.e.: the hydrogenic and methanogenic steps).

Methane production rates kept around 1000 mL per m^2 of electrode and per day regardless of the duration of the power interruptions and of the supplementation of hydrogen. Interestingly, current density increased in the absence of hydrogen (averaged current density during hydrogen supplementation was 0.36 $A \cdot m^{-2}$, increasing up to 0.58 $A \cdot m^{-2}$ without hydrogen). However current was less efficiently used in the production of methane with no hydrogen supplementation. Nevertheless, when the electrical power was restored after the power interruption experiments, performance parameters were similar to those observed before. These results indicate that EM is resilient to power fluctuations, which reinforces the opportunity of using EM as a technology for renewable energy storage.

Keywords

Electromethanogenesis, power-to-gas, hydrogen, methane, biocathode

1. Introduction

According to the International Energy Agency, the growth of renewable power capacity will account for almost 95% of the increase in global capacity in 2026 [1]. To integrate these shares of variable electrical energy into the power system without compromising the grid stability and power quality, large amounts of energy storage capacity would be required. In this regard, and according to the same source, it is expected that energy storage will expand by 56% in 2026 (year 2021 as a basis).

Currently, there is a wide range of energy storage alternatives that, depending on their energy and power capacities, provide different benefits to the power grid [2]. Among them, power-to-gas (P2G) represents a developing energy storage technology capable of absorbing excess of renewable electricity and store it as hydrogen. Further combining this hydrogen with CO_2 to produce methane (either through chemical or biological methanation) can provide a more beneficial and flexible storage option [3,4], for it can be readily converted back into electricity (by means of a CHP unit), injected in the gas grid (as a carrier of renewable energy) or used as automotive fuel.

Bioelectrochemical methane production (also termed as Microbial electromethanogenesis (EM)) can be seen as a sub-branch of P2G. The distinctive feature of EM is that, thanks to the “catalytic” activity of a certain type of electroactive microorganisms, it allows direct conversion of CO_2 and electricity into methane in an

engineered environment known as biocathode [5–7]. Unlike other CO_2 methanation alternatives, EM can occur without an external source of H_2, which avoids the need for hydrogen compression (an energy intensive process) and the problems associated to hydrogen storage [8]. EM could thus provide in the future an environmentally-friendly, long-term energy storage solution to reduce the levelized cost of intermittent renewables [9]. EM also enjoys a fair degree of operational flexibility as it can find application in other sectors such as biogas upgrading or wastes treatment [10].

However, because of the relatively high capital costs of EM (chiefly explained by the cost of electrodes and membranes), and the low merit figures and efficiencies [11], EM has not yet entered the phase of commercial development. In addition, it is still uncertain how power interruptions, —derived from the intermittent and inconsistent nature of renewable power sources— will impact on the cathodic microbial communities and on the overall EM performance. Previous studies on microbial electrosynthesis systems (a bioelectrochemical technology similar to EM able to convert CO_2 and electricity into multicarbon organic compounds [12]) have revealed that the absence of electricity for periods of up to 64h affected production rates, although electro-autotrophic activity resumed once the power supply was restored [13]. In another study it was shown that after a long period (6 months) of power interruption in an acetate-producing microbial electrosynthesis reactor, acetogenic activity recovered after reconnection [14]. However, few days later, methanogens became dominant and methane was the main product of CO_2 bioelectroreduction. To the best of our knowledge, no studies have yet described the behaviour of an EM system subjected to power disconnections. Therefore, in this study we seek to investigate the influence of power-off periods on the performance and microbial communities of an EM system. We also aim at understanding how power outages impact on the two main metabolic stages of EM (i.e.: the hydrogenic and methanogenic steps), by supplementing externally-generated hydrogen during certain power-off periods.

2. Material and methods

2.1. Reactors

Experiments were conducted in two standard H-type reactors (Adams & Chittenden Scientific, CA, USA) with an internal volume of 500 mL per chamber (Figure 1). Each reactor consisted of an anodic chamber and a cathodic chamber separated by a cation exchange membrane (CMI7000, Membranes International, USA). Two graphite felts (SGL Group, Germany) separated and kept in place by two silicon sheets were used as

the biocathodes (working electrode). The total effective area of the biocathodes was 90 cm^2 (6 x 3 x 0.5 cm each of the two felts). Prior to inoculation, the graphite felts were pre-treated by subsequent immersion in nitric acid 1 M, acetone 1 M and ethanol 1 M during 24 h each to avoid hydrophobicity and impurities [15]. In addition, a 2 x 2 cm platinum mesh electrode (Goodfellow, UK) was fixed under biocathode (separated 2 cm by a silicon sheet). The purpose of this additional electrode (cathode) was to provide hydrogen to the biocathode during the power-interruption periods in the hydrogen-supply experiments (see section 2.3.2.). Two 2 x 2 cm platinum mesh (Goodfellow, UK) were used as anodes that acted as counter-electrodes for the cathodes.

The biocathodes were operated at a poised potential of -1.0 V vs an Ag/AgCl reference electrode (Sigma-Aldrich, USA), using a Biologic VSP multichannel potentiostat and an EC-Lab software. Unless otherwise indicated, all the potentials will be referred to the Ag/AgCl electrode, whose stability was checked prior to every batch cycle.

The platinum mesh cathodes were operated at a set current of 4 mA to generate abiotic H_2 when required. The biocathode and the platinum mesh cathode were connected to two independent channels of the potentiostat (see Figure 1) through titanium wires covered with heat-shrinkable tubes to avoid unwanted electrochemical reactions.

Appropriate connections and sealings were designed for sampling ports and substrate supply as illustrated in Figure 1. Gas was collected using a 1 L gas-bag (Ritter, Germany). Reactors were kept inside a thermal chamber (Fitotron, SANYO) that maintained the temperature at 30 ± 0.5 °C. The catholytes were continuously stirred using a magnetic stirrer at 200 rpm in order to prevent mass transfer limitations on current efficiency [16].

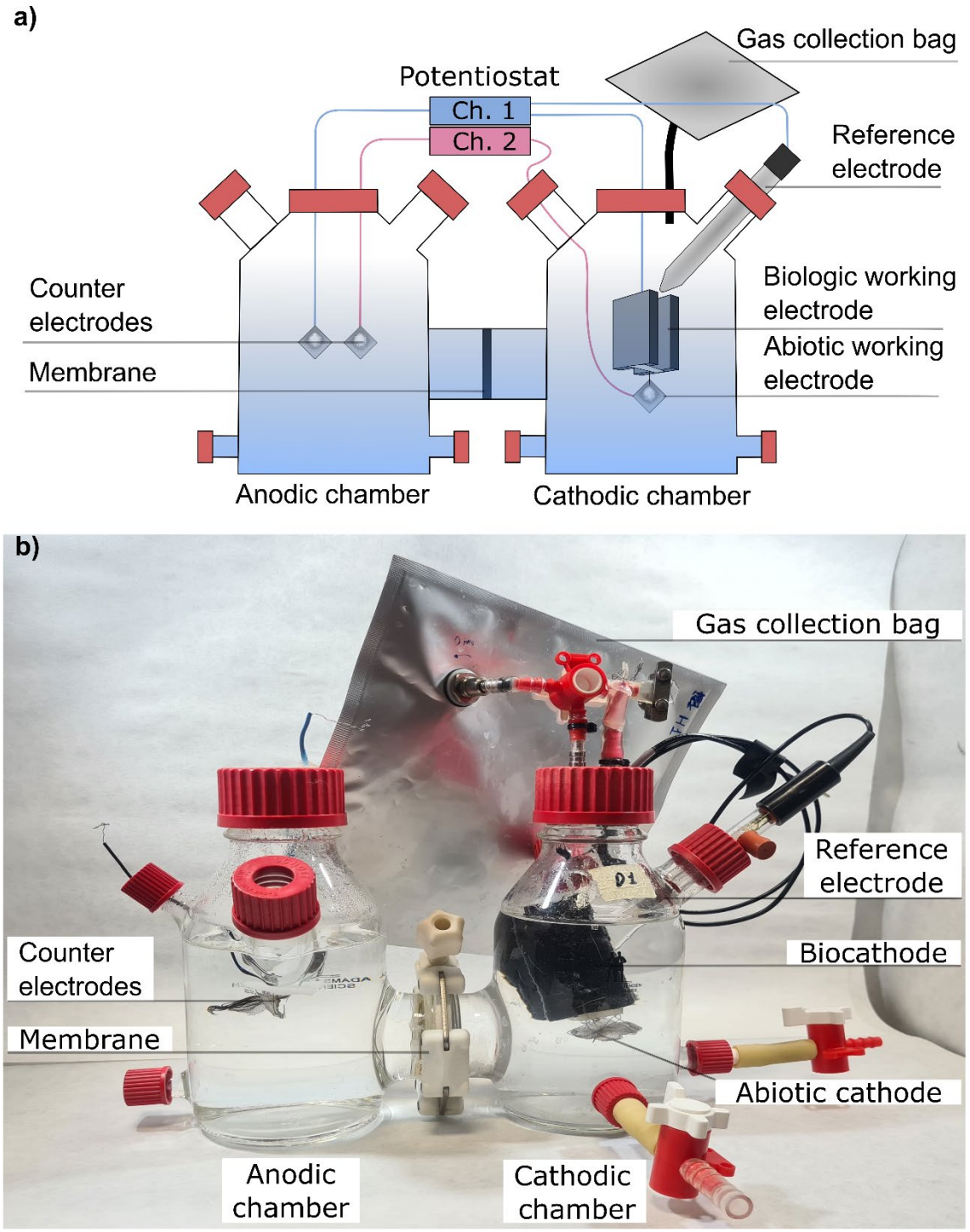

***Figure 1**. a) Schematic diagram of reactor; b) Lab set-up.*

2.2. Electrolytes

Catholyte and anolyte consisted of 20 mM potassium phosphate buffer, macronutrients (280 mg·L^{-1} NH_4Cl, 5.7 mg·L^{-1} $CaCl_2$, 10 mg·L^{-1} $MgSO_4 \cdot 7H_2O$, and 90 mg·L^{-1} $MgCl_2 \cdot 6H_2O$), 1 mL·L^{-1} of a micronutrients solution, and 1 mL·L^{-1} of a vitamin solution

as described in [17]. Electrolytes were replaced at the end of each cycle (96 h each). 0.4 L of CO_2 (99.9% purity) were supplied to the cathodic chamber with the help of the gas bag at the beginning of each cycle.

2.3. Operation

The biocathodes were inoculated using anaerobic sludge obtained from the anaerobic digester of a local wastewater treatment plant in 1:5 ratio of sludge to medium [18]. In addition, 0.4 L of CO_2 (99.9% purity) were supplied as the only carbon source and the biocathodes were polarised at -1.0V. After 16 consecutive cycles current density profiles recorded in both reactors tended to be repeatable between cycles. At that point the platinum mesh cathode was placed in the cathode chamber as explained before and the experimental phases began.

The experimentation was divided into 11 tests (numbered from 0 to 10 in Table 1). Each test condition was repeated in triplicate and they were randomly scheduled (allowing a rest period of 72 h between test in which the biocathodes were polarised at −1.0V) to avoid any habituation effect. The 11 tests were grouped into 3 set of tests with different aims and different operating conditions, as described below:

2.3.1. Power interruption with hydrogen supplementation tests **(PI+HS)**

In the course of the PI+HS tests (tests 1 to 4 in Table 1) the biocathodes were subjected to power supply interruptions of 24 h, 48 h, 72 h and 96 h, that were programmed at the beginning of each cycle. After that, the biocathodes were polarised at −1V for the remainder of the cycle (the total duration of each cycle was 96 h). During the power interruption interval (i.e.: the beginning of the cycle), hydrogen was supplied to the biocathode by means of the platinum mesh cathode, that was operated at a constant current of 4 mA. This value was selected to be similar to the mean current produced by the biocathode when polarised at −1.0 V. With this, we sought that methanogens receive an almost similar amount of reducing equivalents during the entire tests regardless of the duration of the power gaps. In addition, and to prevent the formation of any biofilm on the platinum mesh cathodes, they were immersed in a nitric acid 1 M solution before the beginning of each test.

2.3.2. Power interruption tests **(PI)**

In the course of the PI tests (tests 6 to 9) the biocathodes were subject to the same schedule of power interruptions, although no hydrogen was supplied during the power

interruption intervals (i.e.: the abiotic cathode remained inactive). Following the power gap, the biocathodes were polarised at −1V in the same way as in the PI+HS tests.

2.3.3. Normal operating conditions **(NOC)**

During tests 0, 5 and 10 the biocathodes were operated in normal conditions, which means that they were polarised at −1.0 V vs Ag/AgCl during the 96 h duration of the cycles, while the abiotic platinum cathodes remained inactive. These tests provided a baseline against which compare the performance of the reactors during the PI and PI+HS tests.

Table 1. *Summary of tests. All tests had a duration of 96 h. "Power interruption" indicates the number of hours in which the biocathodes remained disconnected from the power supply. "Power supply" indicates the number of hours along which the biocathodes were polarised.* ***NOC:*** *normal operating conditions.* ***PI+HS****: power interruption with hydrogen supplementation.* ***PI****: power interruption without hydrogen supplementation.*

Operating conditions	Tests number	Power interruption (h)	Power supply (h)
NOC	0	0	96
PI+HS	1	24	72
	2	48	48
	3	72	24
	4	96	0
NOC	5	0	96
PI	6	24	72
	7	48	48
	8	72	24
	9	96	0
NOC	10	0	96

2.4. Analytical techniques

Liquid samples were analysed for total organic carbon (TOC) and total inorganic carbon (IC). Dissolved oxygen (Hach, HQ40d two-channel digital multimeter), redox (pH Meter, pH 91; Wissenschaftlich Technische Werkstätten, WTW), pH (pH Meter BASIC 20+, Crison) and ammonium (781 pH/Ion Meter, Metrohm) were measured following standard methodologies [15].

At the end of each batch cycle, the gas bag was disconnected from the reactor and the amount of gas in the bag (V_g) was measured with the aid of a gastight syringe (100 mL, Hamilton SampleLock syringe). Gas composition (hydrogen (H_2), carbon dioxide (CO_2), oxygen (O_2), nitrogen (N_2) and methane (CH_4)), were determined by a gas chromatograph (Varian CP3800 GC) equipped with a thermal conductivity detector (TCD) [15]. The volume of hydrogen and methane produced in each cycle was calculated from V_g and the gas mole fraction in the gas bag, and was corrected to the standard temperature and pressure conditions.

2.5. Electrochemical techniques

The electrochemical performance of the biocathodes was characterised by means of cyclic voltammetry (CV) tests using a Biologic VSP potentiostat. CV tests were performed at the end of each cycle at a scan rate of 1 $mV \cdot s^{-1}$, a voltage range between −1.0 V and 0.0 V at a temperature of 30 ºC.

2.6. Molecular biology techniques

At the end of test 0, 4 and 9 (Table 1), small cathode samples were cut from biocathodes (around 300 mg of each electrode), and bulk samples (300 mL) were collected and concentrated by centrifugation. These samples were used to characterise the microbial communities.

Microbial communities were analysed by high throughput sequencing of massive 16S rRNA gene libraries. Total Bacteria and Archaea were analysed. Genomic DNA was extracted with a DNeasy PowerSoil Kit (Qiagen) according to manufacturer's instructions. All PCR reactions were carried out in a Mastercycler (Eppendorf, Hamburg, Germany), and PCR samples were checked for size of the product on a 1% agarose gel and quantified by NanoDrop 1000 (Thermo Scientific). The entire DNA extract was used for high-throughput sequencing of 16S rRNA gene-based massive libraries with 16S rRNA gene-based primers for Bacteria 27Fmod -519R and for Archaea 349F-806R. The Novogene Company (Cambridge, UK) carried Illumina sequencing out using a HiSeq 2500 PE250 platform.

The obtained DNA reads were compiled in FASTq files for further bioinformatics processing carried out using QIIME software version 1.7.0 [19]. Sequence analyses were performed by Uparse software (v7.0.1001) using all the effective tags. Sequences with ≥97% similarity were assigned to the same OTUs. Representative sequence for each OTU was screened for further annotation. For each representative sequence,

Mothur software was performed against the SSUrRNA database of SILVA Database [20] for species annotation at each taxonomic rank (Threshold:0.8~1).

The quantitative analysis of all samples was carried out by means of quantitative-PCR (qPCR) using PowerUp SYBR Green Master Mix (Applied Biosystems) in a StepOnePlus Real-Time PCR System (Applied Biosystems) as described previously [18]. The qPCR amplification was performed for the 16S-rRNA gene in order to quantify the entire eubacterial community and for the mcrA gene to quantify the total methanogen community. The primer sets 314F qPCR (5′-CCTACGGGAGGCAGCAG-3) and 518R qPCR (5′-ATTACCGCGGCTGCTGG-3′) at an annealing temperature of 60 °C for 30 s was used for Bacteria and Arc 349F (5′-GYGCASCAGKCGMGAAW-3′) and Arc 806R (5′-GGACTACVSGGGTATCTAAT-3′) for Archaea quantification.

3. Results and discussion

After inoculation, the cathodic biofilms of both reactors (denominated as D1 and D2) were allowed a maturation period of 16 cycles (96 h duration per cycle). Throughout cycles 13 to 16 current density and methane production tended to stabilise (results not shown), so it was assumed that biofilms were mature enough to start the experimental phase.

3.1. PI+HS test

Figure 2 shows that during the hydrogen supplementation tests (PI+HS tests), averaged current density kept fairly stable (roughly 0.38 A m^{-2} in D1 and 0.33 A m^{-2} in D2) with no clear dependence on the duration of the power interruptions —excluding, of course, test 4 where the power supply was disconnected for the entire cycle—. Methane production rates (i.e. the amount of methane produced per unit of time and per unit of surface area of the cathodes) followed a similar trend, and kept around 1.22 $L \cdot m^{-2} \cdot d^{-1}$ in D1 and 1.09 $L \cdot m^{-2} \cdot d^{-1}$ in D2, regardless of the duration of the power interruptions. This result indicates that the methanogens proliferating on the cathodic biofilm can effectively use the externally added hydrogen during the power gaps, and that their metabolism is not dependent on the biocathode potential, thus confirming the role of hydrogen as a mediator in EM methane formation [6,21]. The contribution of planktonic communities to methane production can be neglected as the catholyte (i.e.: growth medium) was completely renewed at the beginning of every test.

At the end of the PI+HS set of tests normal operating conditions (NOC tests) were established again on both reactors (test 5). Current density values were similar to those measured during the power interruption tests, which indicates that the electrotrophic step is not affected by the power interruption. Methane production rates also resumed previous values, highlighting thus the resilience of the methanogens to power interruptions.

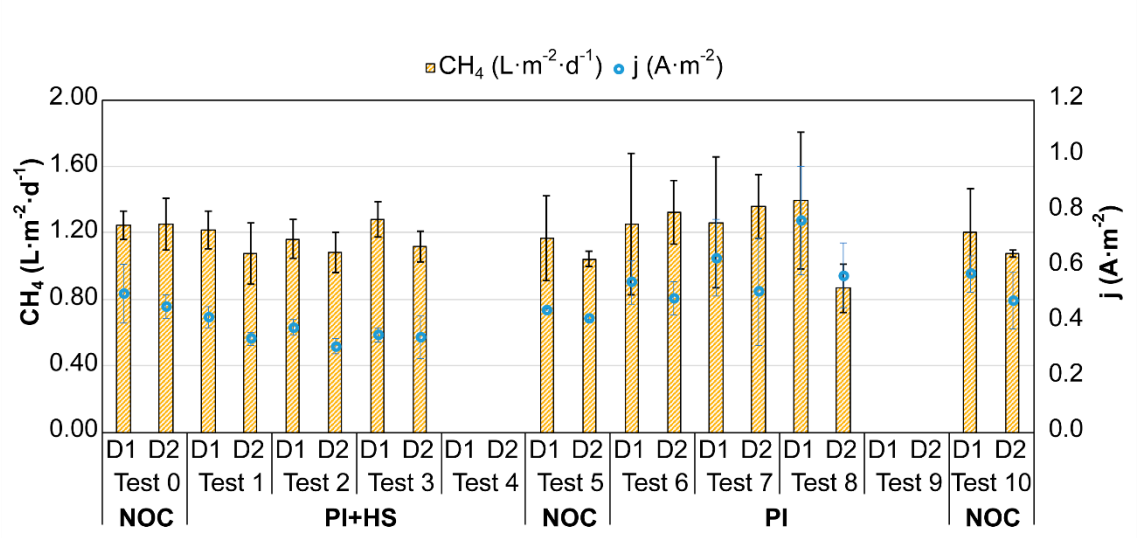

Figure 2. *Methane production rates (in L·per m² of electrode and per day) for reactors D1 and D2 across the different tests.*

3.2. PI test

Interestingly, when reactors where subjected to the same program of power interruptions ─although with no hydrogen supplementation (PI tests in Table 1)─, current density values (see Figure 2) were significantly larger than those observed during the PI+HS tests for both D1 and D2 (p-values of 0.0031 and 0.0027 for D1 and D2 respectively). Taking a closer look at the current density profiles (Figure 3), important differences between the PI+HS and the PI tests can be observed. Typically, when a biocathode is re-connected after a power interruption, current increases sharply during the first few minutes, and then it tends to stabilise [13]. In our experiments, this phenomenon was only visible in the PI tests, and it became more pronounced the longer the duration of the power interruptions (Figure 3). Contrastingly, this trend was totally reversed during the PI+HS tests, where the size of the initial peak decreased with the duration of the power gap.

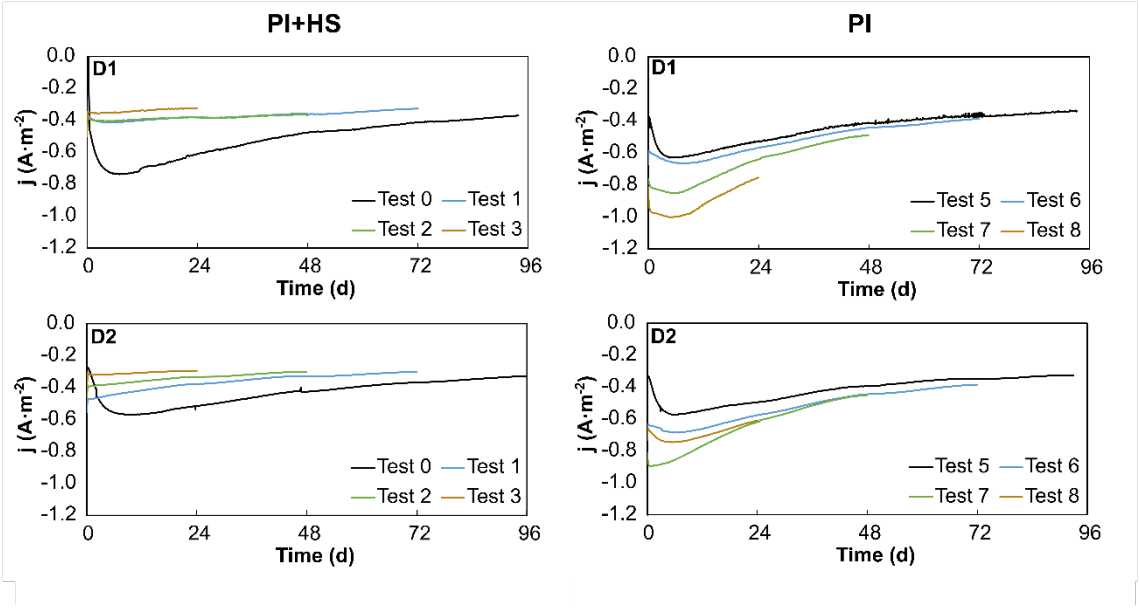

Figure 3. *Typical current density profiles recorded for the different tests (for clarity reasons, only one current profile out of the three replicates was selected for each tests). To facilitate the comparation between tests, all the profiles are represented starting at time 0. However, as explained in section 2.3, the biocathodes were polarised right after the power interruption; so, for instance test 3 in PI+HS and 8 in PI would really begin at the hour 72.*

This finding can most probably be linked to the presence/absence of externally added hydrogen on the cathode chamber. Indeed, if we assume that during the power gaps hydrogen partial pressure regulates the intracellular redox state of the electroactive hydrogenic bacteria –as it happens for instance with fermentative hydrogen producing bacteria [22,23]– then, in the absence of hydrogen (PI tests), the oxidised form of the redox cofactor inside the cells would tend to accumulate. As a result, once the biocathode is polarised again after the power gap, larger currents will be demanded by the electroactive bacteria to rebalance their redox state. In addition, and following the same logic, when hydrogen is externally added (PI+HS tests), the reduced form of the redox cofactor would predominate, and consequently lower current densities would be demanded by bacteria after reconnection.

This hypothesis would be partially supported by the research published in [24], where the addition of NAD+ (the oxidised form of a common redox cofactor) to a hydrogen-producing biocathode increased both current density and hydrogen production. However, when the reduced form (NADH) was supplemented, no impact was observed on current nor on hydrogen production.

Regarding methane, no significant differences where observed between the production rates with and without hydrogen supplementation (p-values of 0.4394 and 0.2729 for D1 and D2 respectively), which indicates that the methanogenic activity is resilient to power interruptions of up to 72 h even in the absence of hydrogen. However, it is important to note that both, current and methane production rate were less reproducible without hydrogen supplementation as evidenced by the larger error bars (see Figure 2). In addition, current was used less efficiently in the production of methane during the PI tests. The columbic efficiency (CE), computed as the ratio between the electrons that end-up in methane to the total of coulombs entering the cathode chamber, was significantly lower when there was no external hydrogen supplementation (p-values of 0.0007 and 0.0256 for D1 and D2 respectively). Indeed, whereas CE kept around 90% during the PI+HS tests (Figure 4), it seemed to decline with the duration of the power-off periods along the PI tests (from around 90 % with no power interruption to 40-60% for the 72 h of power interruption). The lack of any electron donor on the cathode chamber (either in the form of electrons or externally added hydrogen) for long periods of time might be inducing a metabolic shift —or even a change in the populations—, that would explain the large variability in current and methane production and also the decline in CE. The slight decrease of the pH, together with the presence of significant amounts of TOC in the catholyte at the end of the PI tests (Figure 5), would support any of the two possibilities. It is also important to note that the qPCR analysis, as it will be shown in the next section, revealed a notable decline in both bacteria and archaea after the PI experiments.

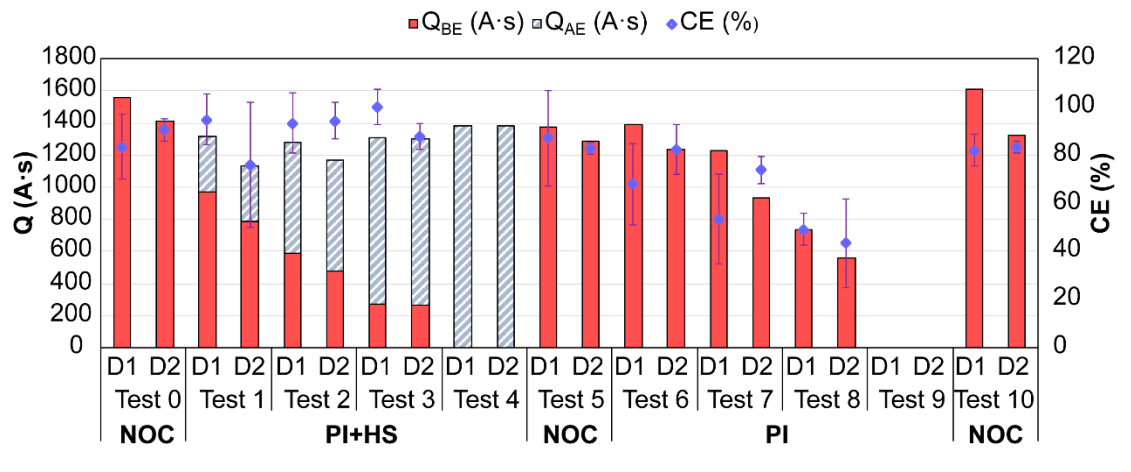

Figure 4. *Columns represent the total electrical charge consumed by the biocathode (Q_B) and the abiotic platinum electrode (Q_P). The blue squares represent the coulombic efficiency.*

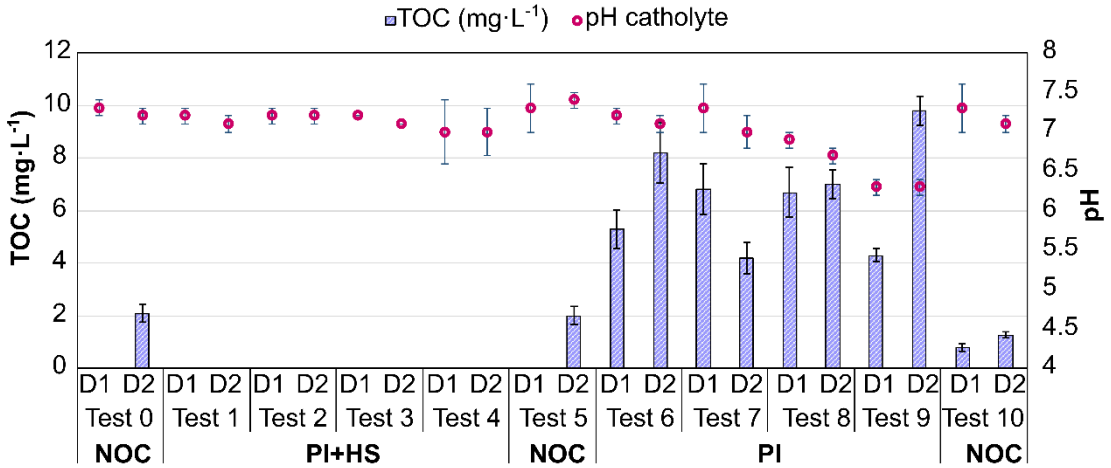

Figure 5. *Total organic carbon (TOC) and pH in the catholytes.*

Finally, and despite the frequent interruptions of the power supply during the PI tests, the reactors recovered their previous performance when normal operating conditions were imposed again (test 10). No significant differences were observed between the values of the current density, methane production rate and coulombic efficiency measured in Tests 5 and 10 (i.e.: beginning and end of the PI tests respectively).

3.3. Cyclic voltammetry tests

Cyclic voltammetry tests (CV) were conducted at the end of each test (see Figure 6). The voltammograms recorded during the PI+HS experiments showed how the catalytic wave associated to hydrogen evolution (i.e.: potentials bellow −0.9V [25–27]) contracted as the length of the power interruption increased. Contrastingly, when there was no externally added hydrogen (PI tests), the length of the tails increased with the length of the interruption period. This result is coherent with the significantly higher current densities measured during the PI tests, and supports the hypothesis that the presence of externally added hydrogen limits the activity of the eletrothrophic hydrogens.

When the biocathodes were disconnect for the entire cycle (96 h) in the presence of hydrogen supplementation (test 4 in PI+HS), the resulting voltammogram underwent a large transformation compared to those recorded under shorter power gaps. The most prominent feature was perhaps the appearance of a large reductive wave starting at −0.5V that might be linked to acetate production [28]. This peak also appeared —though less apparent— at large disconnection periods (72-96 h) in the absence of

hydrogen supplementation (PI experiments). Another peculiarity of the 96 h-disconnect voltammograms was the total disappearance of the oxidation peak that occurred between -0.8V to -0.6V that has been linked to H_2 oxidation [28]. All these evidences suggest a shift in the metabolism of the hydrogen producing bacteria induced by long inactivity as argued in the previous section. Nevertheless, this shift was temporary and reversible, because when biocathodes were polarised again at −1V for the entire cycle (96 h), the voltammograms adopted a shape similar to those recorded during the power interruption experiments.

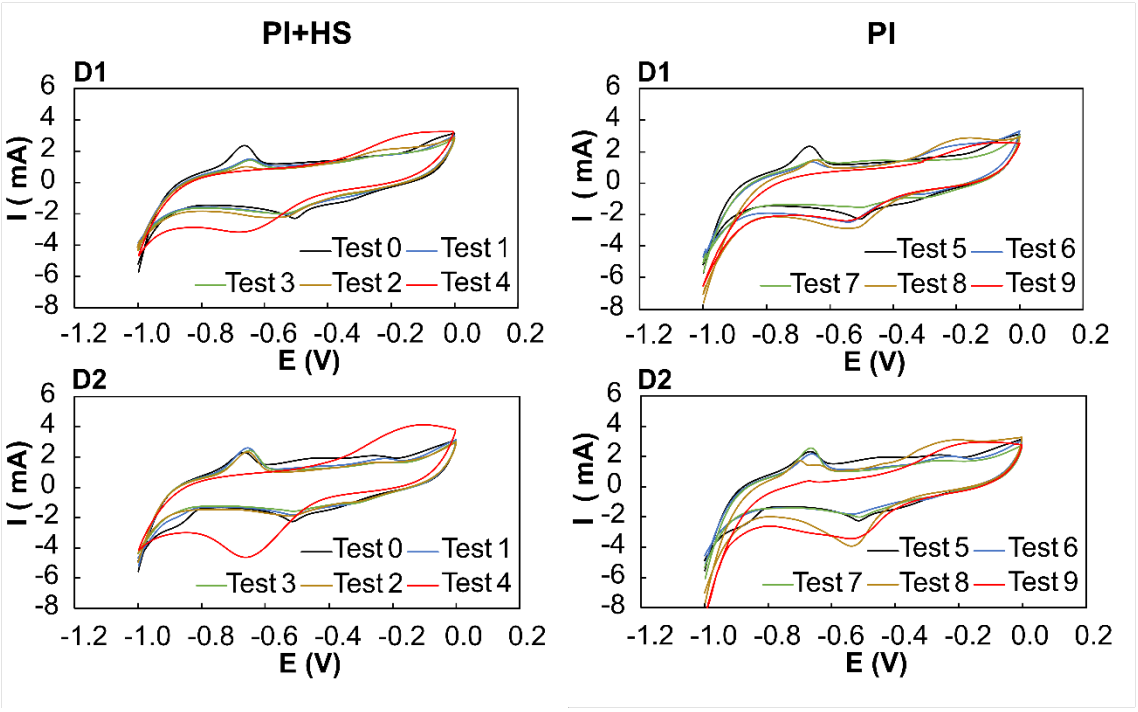

Figure 6. Cyclic voltammograms for D1 and D2 during the PI+HS and PI set of tests. For clarity reasons, only one voltamogram for each power interruption period and set of experiments is shown.

3.4. Microbiology

Before commencing the power interruption tests, the biofilms population was dominated by bacteria (around 70% bacteria vs. 30% archaea) (Figure 7), although the single most abundant genus in both reactors was the archaea *Methanobacterium* (29 and 28% for D1 and D2 respectively) (Figure 8). *Methanobacterium* species live on the reduction of CO_2 with H_2 to produce CH_4, which is coherent with the hypothesis that H_2 is the main intermediary in the electron transfer between the electrodes and CO_2 [6,7,29,30]. Organisms catalysing hydrogen production such as *Desulfovibrio* (10% and 15% for D1 and D2) [28,31,32] or *Petrimonas* (2% and 2% for D1 and D2) [33] would confirm the syntrophic relationship between the electron uptake of the biocathode and

the final methane production. Another relevant organism found was *Lentimicrobium*, whose species are able to produce acetate, propionate and hydrogen, and are commonly found in syntrophy with hydrogenotrophic methanogens [34].

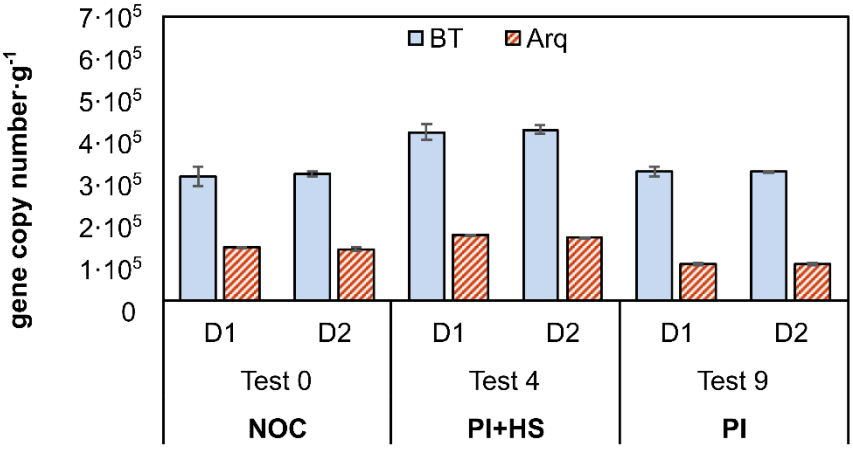

Figure 7. qPCR analyses for reactors D1 and D2 at the end of tests 0, 4 and 9 in terms of Bacteria (BT) or Archaea (Arc)

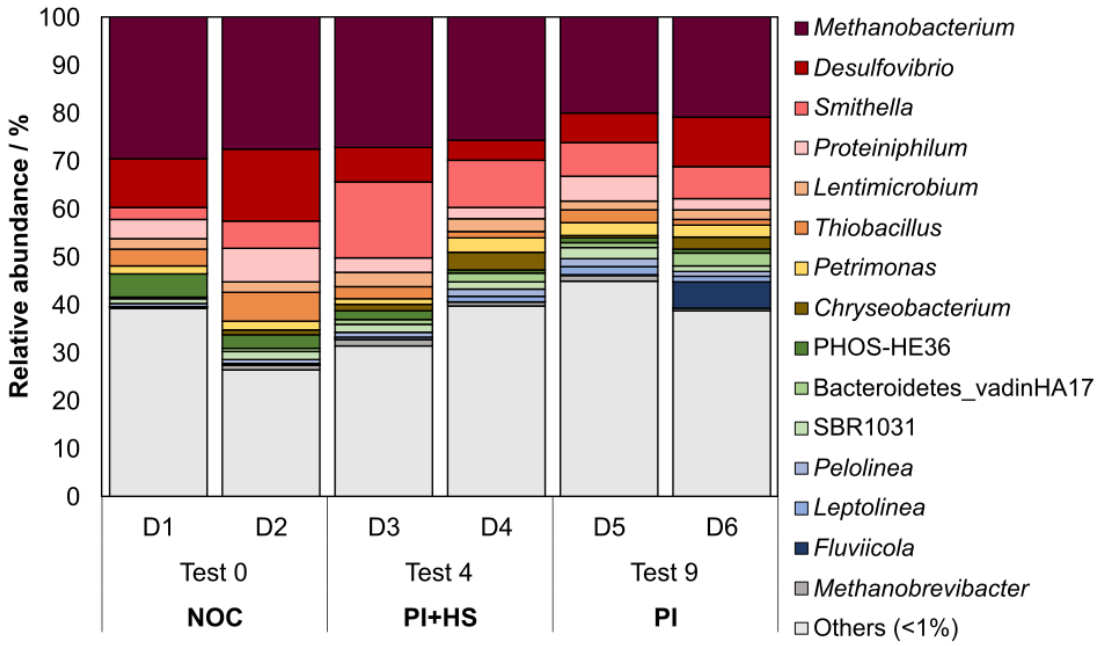

Figure 8. Relative abundance for Reactors D1 and D2 during test 0 (initial conditions), test 4 (last 96 h outage with H2 supplementation), test 9 (last 96 h outage without H_2 supplementation).

Biofilm samples taken at the end of the PI+HS set of tests (test 4) showed a marked growth in both bacterial (around 40%) and archaeal populations (around 27%) (Figure 7). The presence of externally added hydrogen would obviously account for the archaeal increase, as most of the archaeal genus found in our reactors are hydrogenotrophic. However, the bacterial growth is more difficult to clarify. *Desulfovibrio*, which was the most important bacterial genus before the PI+HS tests underwent a sharp decline in its relative abundance, from 10% to 4% in D1 and from 15% to 7% in D2. This genus includes electroactive hydrogen-producing species, and its decline can be justified by the presence of externally added hydrogen, being also coherent with the lower current density measured during the PI+HS tests as discussed in sections 2.2 and 2.3. Therefore, if hydrogen affected negatively some bacteria, it must have also created a niche opportunity for other bacteria if we are to account for the overall bacterial growth. Interestingly, the *Smithella* species ─which are propionate-oxidizing syntrophic bacteria─ experienced a notable growth (from 3% to 10% in D1 and from 6% to 16% in D2). Therefore, the cathodic biofilm must have supported the development of other bacteria that produce the metabolites required for their proliferation. The large catalytic wave detected at −0.5 V in CVs at the 96 h disconnection tests (Figure 6) would support this conjecture. The absence of any organic carbon in the catholytes during PI+HS test (Figure 5), might be revealing a fast-local consumption of this metabolites in the biofilms.

The PI set of tests resulted in a large decrease of archaea (around 40% decline at the end of test 9) that in in our reactors are represented by the genus *Methanobacterium.* It seems then that the hydorgenotrophic methanogens, which are at the top of the methanogenic syntrophic community, are very sensitive to a prolonged lack of any reducing agent (either hydrogen or electrons) in the cathode chamber. Bacterial communities also suffered a significant decline (around 20%), although the main hydrogenic electrotrophic group (*Desulfovibrio*) showed an increase in relative abundance (6% for D1 and 10% for D2). This is again in accordance with the larger current density observed during the PI tests, and might be related to the absence of externally added hydrogen, which favoured its metabolism as discussed before. It is important to note that only during the PI tests TOC was consistently detected in the catholytes (Figure 5), so it seems that the loss of activity of hydrogenotrophic methanogens may have induced a metabolic shift that contributed to the formation of other products such as VFAs.

Conclusions

This study investigates the effect of electrical power interruptions on the performance of a microbial electromethanogenic system. Results indicate that power gaps of up to 96 h do not have a significant impact on methane production rates regardless of the supplementation of hydrogen. However, when these power gaps occurred in the absence of externally added hydrogen, current underwent a significant increase, although it was less efficiently used in the production of methane. In addition, both current density and methane production rate became less reproducible (larger variations between replicates) in the absence of hydrogen, which can be attributed to a disruption in the metabolic routes or a shift in the microbial populations caused by a prolonged lack of electron donors. Overall, this study shows that EM can stand relatively large periods of power interruption without a significant impact on performance.

Acknowledgments

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