Communication

Long-term open circuit microbial electrosynthesis system promotes methanogenesis

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Abstract

Microbial electrosynthesis (MES) can potentially provide a mean for storing renewable energy surpluses as chemical energy. However, the fluctuating nature of these energy sources may represent a threat to MES, as the microbial communities that develop on the biocathode rely on the continuous existence of a polarized electrode. This work assesses how MES performance, product generation and microbial community evolution are affected by a long-period (6 weeks) power off (open circuit). Acetogenic and H_2-producing bacteria activity recovered after reconnection. However, few days later syntrophic acetate oxidation bacteria and H_2-consuming methanogens became dominant, producing CH_4 as the main product, via electromethanogenesis and the syntrophic interaction between eubacterial and archaeal communities which consume both the acetic acid and the hydrogen present in the cathode environment. Thus, the system proved to be resilient to a long-term power interruption in terms of electroactivity. At the same time, these results demonstrated that the system could be extensively affected in both end product generation and microbial communities.

Keywords: Biocathode; Electromethanogenesis; Microbial electrosynthesis; Microbial community dynamics; Methanogens; Acetogens

Renewable energy production is beating records in past few years around the world. However, the unpredictable nature and the variability of renewable power represent the key hurdles for a widespread use of these technologies. Efficient storage systems that allow to exploit the electricity surpluses can be a part of the solution to this challenge [1].

Microbial electrosynthesis (MES) is a novel technology capable of converting a CO_2 stream and electricity into easily storable and transportable fuels and chemicals using electroactive microorganisms as biocatalyst [2]. First studies were able to produce mainly acetate, although the spectrum of products has been enlarged over the years enabling the production of longer chain fatty acids, alcohols and fuels like methane [3]. In MES, electrotrophic microorganisms are capable of accepting electrons from a solid cathode and use inorganic carbon as the sole carbon source for their metabolism and growth [4–7]. This technology shows several advantages for CO_2 fixation and energy surplus exploitation [8] as it is independent from land use, requires reduced nutrients and water consumption compared to other biomass to biofuel approaches, and can be installed next to CO_2 or renewable energy sources with minor instrumentation [9]. Another interesting feature of MES is that they can be easily coupled to other bio-based systems to exploit synergies or to overcome limitations. On example of the later is the integration of MES with anaerobic digestion (AD), an approach that helps to improve the quality of the biogas produced in AD by promoting the conversion of CO_2 into CH_4 [10,11]. In addition, it is widely admitted that MES can only develop its full potential as a sustainable environmental technology when powered by renewable energy [2,8]. This integration between MES and renewable energy systems may bring additional advantages to the latter, as MES can provide a mean to store surpluses of electricity during peak production [12]. However, the unpredictable interruptions and fluctuations, typical of renewable power, can represent a potential threat to the stability of the microbial communities that thrive off the electrons that arrive at the cathode. The impact of short power interruptions have been examined in previous studies [13,14] showing that MES can be resilient to power interruptions in the range of hours, recovering its previous stable performance in 7–16 h after power gaps. Longer periods of power disconnection, that can be potentially originated from unexpected generator failure (e.g.: when operating in island mode) or from maintenance operations can also have an impact on MES performance.

The aim of the present work is to evaluate the impact of longer (6 weeks) power disconnections on the performance and microbial communities of a MES that has been producing acetate in a stable and efficient manner for a period over a year.

Previous operating history. The experimental cell was inoculated with the enriched supernatant of a long-term working acetogenic MES [15] around one year before the present study was carried out. That inoculum was analyzed for archaeal and eubacterial composition, confirming that homoacetogenic and H_2-producing microorganisms were dominant at that point and archaea were not present (See section "*Role of Microbial communities involved in the process"*). This inoculated cell was acclimated for 3 months with bicarbonate as carbon source in order to allow for the development of a robust biofilm. During this period the cell reached an average acetic acid production of 236 mg L^{-1} d^{-1} with a peak value of 550 mg L^{-1} d^{-1}. Maximum titers over 1 g L^{-1} and up to 78% bicarbonate conversion into acetic acid, together with the previously mentioned production rates led to the conclusion that the biofilm was stable and ready to undergo a defined experimental plan.

At this point, the cell was used in a 6 month experiment in which short power gaps (4 to 64 h) were subsequently applied to assess how unexpected electrical disconnections affect MES performance [13]. The cell was resilient to these short power interruptions always restoring bioelectrochemical acetic acid production, although its production rate decreased by 77% after the longer gap. The average production rate during this period was 135 mg L^{-1} d^{-1}, and no methane was found as product.

After this experimental period, the cell was left in open circuit for 6 weeks, not adding any substrate in the medium, or gases in the headspace, and reconnected in the frame of the present study to evaluate the impact of this long power interruption on MES performance and the microbial communities present in the cathode.

Biocathode evolution and performance. After replacing the catholyte by a fresh culture medium, the cell was reconnected by poising the WE (cathode) at −1 V vs. Ag/AgCl and flushed with pure nitrogen. Electrical current was produced almost immediately, which contrasts with the slow start-up of new biocathodes [16]. Fig. 1 shows substrate (IC) and products (acetic acid, methane and hydrogen) evolution during the experimental time.

At the moment of first sampling (day 2), and despite the sharp decrease in the substrate (inorganic carbon), no products were found in the gas headspace or the culture medium. This, together with the

electrical charge consumption measured (863 C), and the small amount of CO_2 in the headspace (less than 8%) during this period, suggests that microorganisms were using the available inorganic carbon source and energy to proliferate and adjust their metabolic pathways to the new conditions after the long power interruption. Six days after reconnection, hydrogen and acetic acid began to appear although in low quantities: acetate reached only 49 mg L^{-1} d^{-1} and despite the percentage of hydrogen in the headspace was important (62%), negligible net gas production rate was measured.

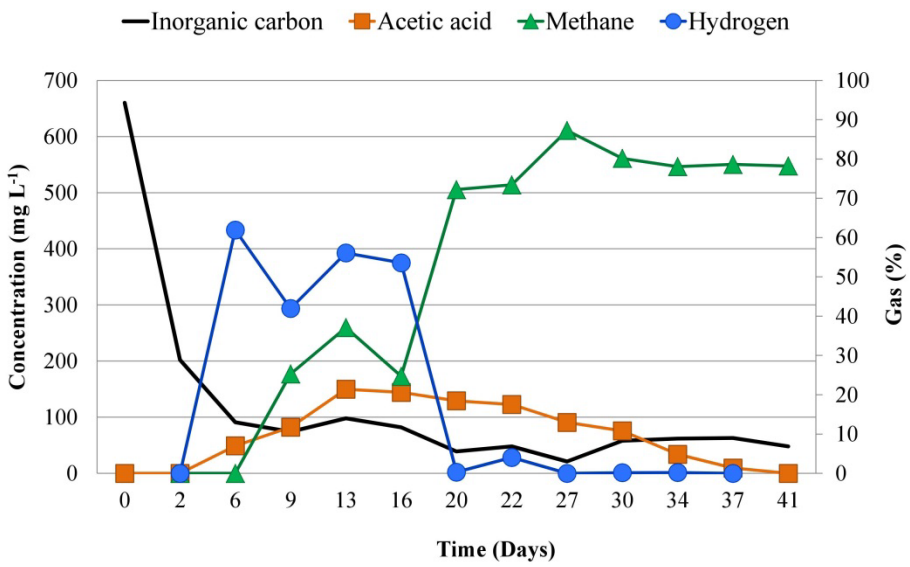

Fig. 1. Substrate and acetic acid concentration in the liquid medium (black and orange) and gaseous products proportion in the outlet gas (blue and green). Day 0 corresponds to power supply reconnection.

From day 6 to 16 the product profile diversified, adding methane to acetate and hydrogen. Acetate production grew slowly during this period and then decreased gradually until no net production was found by the end of the experiment. Importantly, total net gas production became measurable, growing up to 75 mL L^{-1} d^{-1}. Hydrogen concentration in the off gas was steady in the range between 40% and 60%, and methanogenic activity grew drastically boosting methane proportion from 0% to 37%. Between days 16 and 20, methanogenesis clearly overtook acetogenic activity and after day 20 methanogenesis was absolutely dominant, with most of the product formation corresponding to this gas and only small quantities to hydrogen and acetate. Methane percentage in the off-gas is consistently maintained around 80% reaching a peak of 87% corresponding to rates from 30 to 55 mL L^{-1} d^{-1} of pure methane. Similar values can be found in other MES systems [10] intended for methane production which suggests that

methanogenic activity completely takes over the acetogenic. Rates, after the methanogenesis becomes dominant, are shown in Fig. S2 (Supporting information) together with methane cathodic efficiency.

Role of microbial communities involved in the process. SEM images were taken to confirm microbial attachment on the electrode surface. Clean graphite electrode can be seen in Fig. S3(A, B), while images corresponding to inoculated electrode are shown in Fig. S3(C–F). Biofilm coverage was not regular and it was scattered in clumps upon the electrode, showing thick biofilm formation in some regions together with areas in which the graphite surface is not covered.

The microbial community analysis at family level (Fig. 2) shows differences among inoculum, biofilm and supernatant at the end of the experiment. Although main families are common, the relative abundance shows great difference in all the samples. As mentioned above, the inoculum was dominated mainly by homoacetogenic and H_2-producing bacteria. In contrast, the biofilm was enriched in a greater diversity of families. *Porphyromonadaceae* (a VFA producing family that shows an important increase in the biofilm) together with *Clostridiaceae* (already present in high proportion in the inoculum), could probably be the main family responsible for the acetic acid production. *Desulfovibrionaceae* also increases in the biofilm sample and is widely described as electroactive in biocathodes, where they are able to catalyze hydrogen production [16,17]. Another H_2-producing family found in all the samples in relevant proportion is *Rhodocyclaceae* also described in biocathodes [16]. *Veillonellaceae* family, which is one of the most important electroactive bacteria in bioelectrosynthesis [18] was also present in the biofilm and absent in the supernatant.

Around 90% of the present bacteria are represented by only 13 genera as shown in Fig. 3 for both biofilm and supernatant samples. The biofilm is composed mainly of acetogens, such as *Desulfovibrio, Clostridium* or *Sporomusa*, together with hydrogen producers such as *Symbiobacterium* [19] and *Azonexus*. In contrast, the supernatant is mainly composed of hydrogen producers and other fermentative bacteria although *Clostridium* in also present.

Archaea community analysis is also relevant to understand the behavior of MES systems in which methane is being produced. As shown in Table S1, the archaeal community is represented by almost only one family with one genus.

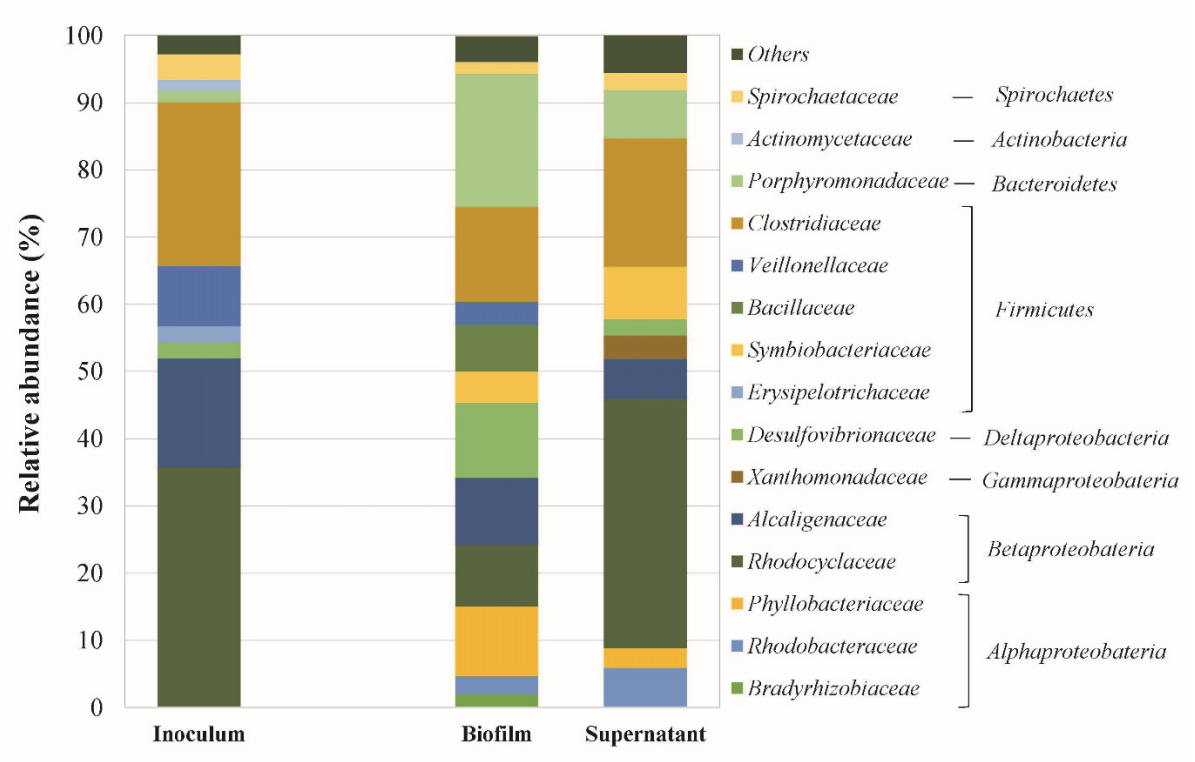

Fig. 2. Inoculum, biofilm and supernatant eubacterial composition at family level.

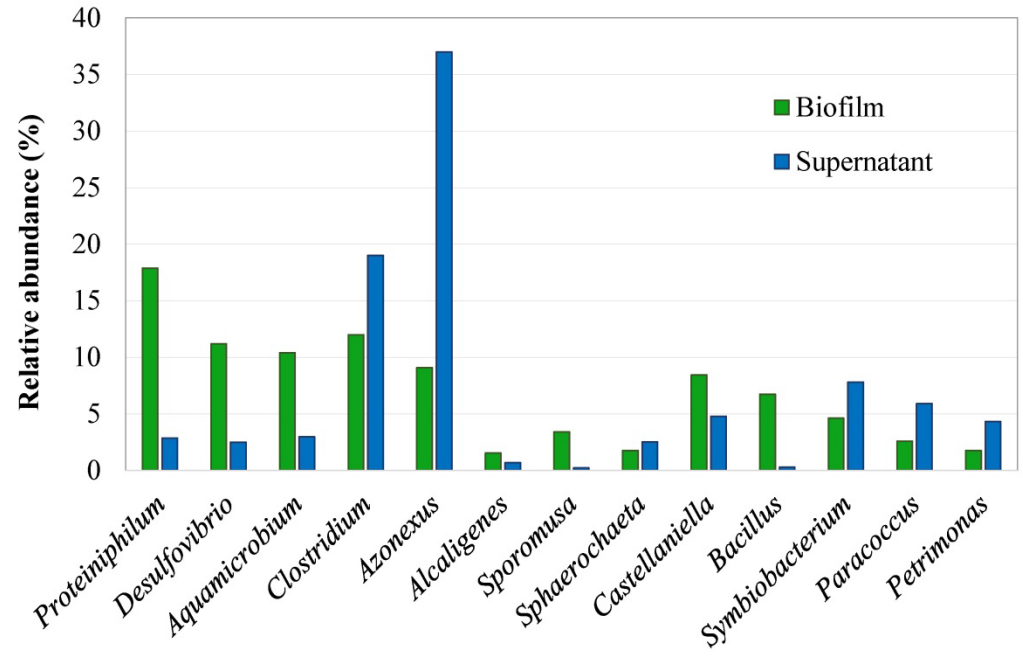

Fig. 3. Biofilm and supernatant microbial community at genera level.

Methanobacteriaceae is clearly dominant both in the biofilm and the supernatant, accounting for >99.4%, being represented by the genus *Methanothermobacter*. This is a hydrogenotrophic archaea that produces CH_4 from CO_2 and H_2, which could explain the methane production and hydrogen depletion

observed during the experiment via hydrogen mediated electromethanogenesis [20]. The rest of the archaeal families found in the MES are mainly acetoclastic like *Methanosaetaceae* (See Table S1). The initial inoculum was enriched using a methanogenesis inhibitor and accordingly no archaea were found on it. This fact, together with the absence of methane during the previous cell history in which the inhibitor was not added (before power interruption), led us to hypothesize that open-circuit conditions might have favored the growth of residual archaeal communities up to a dominant position during the unpowered period. Moreover, the strong presence of produced hydrogen during the first days after reconnection could also favor the proliferation of *Methanobacteriaceae.* Here it is important to remember that hydrogenotrophic methanogenesis is energetically more favorable than homoacetogenesis in the presence of hydrogen gas, and thus hydrogenotrophic methanogens might be outcompeting homoacetogens [21]. Despite the operational conditions (temperature, pH, conductivity, etc) were the same before power interruption and after reconnection [13] and very similar to those reported for other electromethanogenic systems [22–24], the power gap promoted the development of a new environment in which acetogens and methanogens could coexist. Still, it must be acknowledged that other possibilities such as external contamination, slight changes in pH or high biological hydrogen gas presence could also explain this shift. Overall, the presence of hydrogen and acetic acid producers in the microbial community of the biofilm and supernatant lead us to believe that the acetic acid was being produced following two (widely described) pathways simultaneously: (i) direct bioelectrosynthesis [23] and (ii) hydrogen mediated bioelectrosynthesis [4]. During the first 2 weeks after reconnection, the appearance of hydrogen and acetic acid can be mainly attributed to the presence of microorganisms such as *Symbiobacterium, Azonexus*, *Desulfovibrio* and *Sporomusa*. However, after those 2 weeks, a quick hydrogen and acetic acid depletion was observed accompanied by simultaneous rise in methane production, suggesting an increment in the electromethanogenic process. It is important to note that the fact that methane was almost the unique product found from day 20 onwards does not necessarily mean that acetic acid and hydrogen were not being produced any more, on the contrary these compounds are most probably acting as reaction intermediates in the methanogenic process. In this sense, up to 10 different electromethanogenic routes have been described to be able to individually or simultaneously occur in mixed culture biocathodes [22]. Thus, it might be possible that Syntrophic Acetate Oxidizers (SAO) such as *Clostridium* [25] could be consuming acetic acid and producing hydrogen, which in turn would explain the lack of acetic acid

observed in the medium [21,26]. Although hydrogen production from acetate is thermodynamically unfavorable, SAO bacteria are in a syntrophic relationship with the H_2-consuming methanogens, making the whole process thermodynamically favorable [26,27]. All this suggests that hydrogen and acetic acid can be acting as intermediates for methane production.

This study shows how an acetogenic MES is prone to undergo a radical shift in the product formation (in our case from acetic acid to methane) after a long-term power interruption (6 weeks). However, this does not necessarily mean that acetic acid production has stopped. In fact, our results suggest that acetic acid is still being produced, although it has become a metabolic intermediate for methane formation, as a result of an extensive change in the cathodic microbial community. With an eye on future prospects and practical application of MES, this represents an important operational challenge and thus future research should be oriented towards identifying the mechanisms that would trigger this shift in the microbial community, and towards envisaging strategies to prevent them.

In summary, this study demonstrated that a MES system fed with inorganic carbon is resilient to a long-term interruption of power supply, quickly recovering its electroactivity after reconnection. However, a prolonged electrical disconnection extensively affects MES microbial communities and end product, promoting electromethanogenesis.

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References

[1] A.G. Olabi, Energy 136 (2017) 1–6.

[2] K.P. Nevin, T.L. Woodard, A.E. Franks, Z.M. Summers, D.R. Lovley, MBio 1 (2010) e00103-10.

[3] S. Bajracharya, S. Srikanth, G. Mohanakrishna, R. Zacharia, D.P. Strik, D. Pant, Journal of Power Sources 356 (2017) 256–273.

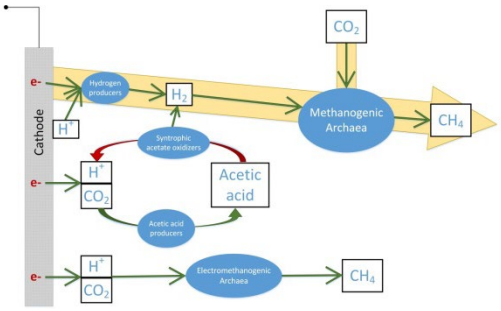
[4] K. Rabaey, R.A. Rozendal, Nature Reviews Microbiology 8 (2010) 706–716.

[5] S.A. Patil, J.B.A. Arends, I. Vanwonterghem, J. Van Meerbergen, K. Guo, G.W. Tyson, K. Rabaey, Environmental Science & Technology 49 (2015) 8833–8843.

[6] G. Kumar, R.G. Saratale, A. Kadier, P. Sivagurunathan, G. Zhen, S.-H. Kim, G.D. Saratale,

Chemosphere 177 (2017) 84–92.
[7] G.D. Saratale, R.G. Saratale, M.K. Shahid, G. Zhen, G. Kumar, H.-S. Shin, Y.-G. Choi, S.-H. Kim, Chemosphere 178 (2017) 534–547.
[8] S. Abate, P. Lanzafame, S. Perathoner, G. Centi, ChemSusChem 8 (2015) 2854–2866.
[9] J. Desloover, J.B.A. Arends, T. Hennebel, K. Rabaey, Biochemical Society Transactions 40 (2012) 1233 LP-1238.
[10] R. Mateos, A. Escapa, K. Vanbroekhoven, S.A. Patil, A. Moran, D. Pant, Microbial Electrochemical Technology (2019) 777–796.
[11] N. Aryal, T. Kvist, F. Ammam, D. Pant, L.D.M. Ottosen, Bioresource Technology 264 (2018) 359–369.
[12] A. Escapa, R. Mateos, E.J. Martínez, J. Blanes, Renewable and Sustainable Energy Reviews 55 (2016) 942–956.
[13] M. del P. Anzola Rojas, R. Mateos, A. Sotres, M. Zaiat, E.R. Gonzalez, A. Escapa, H. De Wever, D. Pant, Energy Conversion and Management 177 (2018) 272–279.
[14] M. del P. Anzola Rojas, M. Zaiat, E.R. Gonzalez, H. De Wever, D. Pant, Bioresource Technology 266 (2018) 203–210.
[15] S. Bajracharya, R. Yuliasni, K. Vanbroekhoven, C.J.N. Buisman, D.P.B.T.B. Strik, D. Pant, Bioelectrochemistry 113 (2017) 26–34.
[16] R. Mateos, A. Sotres, R.M. Alonso, A. Escapa, A. Morán, Bioelectrochemistry 121 (2018) 27–37.
[17] H.D. May, P.J. Evans, E. V LaBelle, Current Opinion in Biotechnology 42 (2016) 225–233.
[18] P.-L. Tremblay, T. Zhang, Frontiers in Microbiology 6 (2015) 201.
[19] A. Kadier, M.S. Kalil, K. Chandrasekhar, G. Mohanakrishna, G.D. Saratale, R.G. Saratale, G. Kumar, A. Pugazhendhi, P. Sivagurunathan, Bioelectrochemistry 119 (2018) 211–219.
[20] M. Hara, Y. Onaka, H. Kobayashi, Q. Fu, H. Kawaguchi, J. Vilcaez, K. Sato, Energy Procedia 37 (2013) 7021–7028.
[21] I. Díaz, C. Pérez, N. Alfaro, F. Fdz-Polanco, Bioresource Technology 185 (2015) 246–253.
[22] R. Blasco-Gómez, P. Batlle-Vilanova, M. Villano, M. Balaguer, J. Colprim, S. Puig, R. Blasco-Gómez, P. Batlle-Vilanova, M. Villano, M.D. Balaguer, J. Colprim, S. Puig, International Journal of Molecular Sciences 18 (2017) 874.
[23] S. Cheng, D. Xing, D.F. Call, B.E. Logan, Environmental Science & Technology 43 (2009) 3953–3958.
[24] M. Villano, F. Aulenta, C. Ciucci, T. Ferri, A. Giuliano, M. Majone, Bioresource Technology 101 (2010) 3085–3090.
[25] M. Cai, D. Wilkins, J. Chen, S.-K. Ng, H. Lu, Y. Jia, P.K.H. Lee, Frontiers in Microbiology 7 (2016) 778.
[26] S. Hattori, Microbes and Environments 23 (2008) 118–127.
[27] J. Dolfing, Applied and Environmental Microbiology 80 (2014) 1539–41.

Graphical abstract



Methanogenesis overtakes acetic acid production after an acetogenic MES reactor is disconnected for a long period of time. The hydrogenotrophic methanogenic pathway (highlighted in yellow) is found to predominate over other methanogenic pathways.