



Conditions for high resistance to starvation periods in bioelectrochemical systems

Yolanda Ruiz, Edgar Ribot-Llobet, Juan Antonio Baeza *, Albert Guisasola

GENOCOV, Departament d'Enginyeria Química, Escola d'Enginyeria, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain

ARTICLE INFO

Article history:

Received 2 February 2015

Received in revised form 20 June 2015

Accepted 21 June 2015

Available online 28 June 2015

Keywords:

Microbial fuel cell

Microbial electrolysis cell

Starvation

Substrate accumulation

Exoelectrogenic bacteria

ABSTRACT

The present work aims at understanding the performance of bioelectrochemical systems when subjected to different starvation periods, which is very relevant in view of their industrial application or use as biosensor. The results show that both microbial fuel cells (MFC) and microbial electrolysis cells (MEC) could resist starvation periods up to 10–11 days without any significant decrease in their performance when endogenous consumption was enabled by closing the circuit in MFC or applying an external voltage in MEC. By contrast, starvation periods longer than 5 days in both MFC and MEC when the flow of electrons from the anode to the cathode was not permitted thereby avoiding endogenous consumption, led to a reversible decrease in the cells performance. A longer starvation period of 21-days under open-circuit caused an irreversible performance loss of the MFC.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Bioelectrochemical systems (BES) comprise the biotechnological applications resulting from the ability of some microorganisms, known as exoelectrogens or anode respiring bacteria (ARB), to transfer electrons outside the cell. Extensive research has been conducted in the last decade on BES aiming at current generation or production of value added products, such as hydrogen [1]. In short, a BES consists of an anode, a cathode and an electrical circuit. Oxidation occurs in the anode, where exoelectrogenic bacteria convert organic matter into CO₂, protons and electrons. Microorganisms transfer these generated electrons to an anode and from there, electrons flow through an electrical circuit to the cathode, where a reduction reaction occurs. If oxygen is reduced to water, the process is thermodynamically favourable and then, electricity is generated (this device is known as microbial fuel cell, MFC). In contrast, if the generated protons are to be reduced to hydrogen, a certain potential has to be added to drive the system (in a device known as microbial electrolysis cell, MEC).

BES have been mainly applied for producing electricity from waste-waters. However, the enormous research conducted in the last years on this field has resulted in a plethora of alternative applications: from microbial electrosynthesis to bioremediation or biosensors development. With respect to the latter, different biosensors, in which anodic exoelectrogenic bacteria act as the biological sensing element, have

been proposed so far for the measurement of biological oxygen demand [2–4], microbial activity [5], toxicity [6–9], dissolved oxygen [10] and single molecules such as glucose [11], lactate [12], volatile fatty acids [13,14] and arabinose [15].

However, when dealing with biological processes, one has to be aware of the need of maintaining bacterial activity. In this frame, starvation periods (i.e. periods in absence of substrate) may occur often in real systems due to technical plant stops. Under this scenario, it is very important to know i) how detrimental will this period be to the biomass activity and ii) which are the best conditions for biomass maintenance in case starvation is unavoidable. Regarding the particular case of biosensors, starvation periods will occur as part of their usual operation, during which the maintenance of an active ARB-enriched biomass must be ensured.

For this reason, it is essential to understand the behaviour of BES under different periods of starvation as well as to estimate how detrimental will these periods be to the biomass and, finally, which are the best conditions for the biomass in case starvation is unavoidable. To the best of our knowledge, there is little information about starvation in both MFC and MEC. In Oh and Logan [16] the relationship between starvation and voltage reversal in two MFC stacked together was studied and it was concluded that starvation periods were detrimental for the MFC performance. However, this detrimental effect could have been caused not only by starvation but also by the voltage reversal experienced by the cell. In contrast, Kaur et al. [17] evaluated starvation as strategy to avoid electron losses derived from methanogenesis and the results suggested that 12 days of starvation were not overly harmful for ARB in MFC. Nevertheless, only closed loop starvation conditions were tested and the causes of such high resistance to starvation were not

* Corresponding author.

E-mail addresses: yolanda.ruiz@uab.cat (Y. Ruiz), edgarribot@gmail.com

(E. Ribot-Llobet), Juanantonio.baeza@uab.cat (J.A. Baeza), Albert.guisasola@uab.cat (A. Guisasola).

addressed. Gao et al. [18] studied the syntrophy between biopolymer-accumulating bacteria and ARB both in MFC and MEC, which allowed current generation without the addition of an external substrate for several days. Moreover, the system recovered the initial current generation when external substrate was added. Experiments were conducted by operating both MFC and MEC in two chamber configuration and with a set anode potential at -0.4 vs Ag/AgCl, but no experiments were performed in open loop for MFC or without applied voltage in MEC. Therefore, considering the few works in the literature where BES starvation was studied and the lack of a systematic evaluation, the aim of this study is to shed light on the effect of starvation processes in MFC and MEC under a wider set of operating conditions. Open-circuit and closed-circuit conditions for MFC and the application or not of an electrical voltage for MEC were studied in order to know which condition is better to maintain ARB activity and where is the limit of starvation time for each case.

2. Materials and methods

2.1. Reactors set-up

Two different type of cells were used, MFC for electricity generation and MEC for hydrogen production. MFC consisted of a 30 mL cylindrical cell with a lateral 3 cm diameter aperture where the cathode was assembled (7.07 cm^2). The cathode was carbon cloth coated with carbon powder and platinum suspension on the inner side (0.5 mg/cm^2 , ETEK C1-10 10% Pt on Vulcan XC-72, ElectroChem Inc.), whereas the outer side was coated with a polytetrafluoroethylene (PTFE) solution, which permitted oxygen diffusion into the cell while preventing water leakage [19,20]. The anode was a carbon fibre brush (PANEX®33 160 K, ZOLTEK) [21] of 20 mm diameter $\times$ 25 mm length wound into a titanium core. It was thermally treated at 450°C for 30 min prior to its utilization to enhance biomass adhesion [22]. The two electrodes, spaced 2 cm apart, were connected through an external resistance ($1000\ \Omega$ or $100\ \Omega$). Current intensity was determined from the monitoring of the voltage drop across this resistance. A reference electrode (Ag/AgCl NaCl 3 M, model RE-1B, BAS Inc.) was placed inside the cell. The MEC was similar to the MFC but the cathode was not exposed to air and the cell was provided with a glass cylinder that enabled gas collection. The gas produced was further collected in a 0.1 L gas sample bag with a twist type valve (Cali-5-Bond, Ritter), connected to the glass cylinder. Both electrodes were connected to a power source (HQ Power, PS-23,023) applying a voltage of 0.8 V. Current intensity was determined from the monitoring of the voltage drop across a $12\text{-}\Omega$ resistor connected in series to the circuit. A low external resistance was selected in MEC to minimize the voltage losses across the resistor, so that the voltage applied by the power supply was approximately the voltage between the anode and the cathode.

Voltage evolution for both MFC and MEC was monitored by means of a 16-bit data acquisition card (Advantech PCI-1716) connected to a personal computer with a software developed in LabWindows CVI 2013 for data acquisition.

2.2. MFC and MEC operation

The cells operated with acetate as substrate (1.5 g/L) in batch mode. The medium contained per litre: Na_2HPO_4 (12.04 g) and KH_2PO_4 (2.06 g), so that the final phosphate buffer saline (PBS) concentration was 100 mM , NH_4Cl (0.2 g), FeCl_2 (4 mg), Na_2S (6 mg) and mineral media (5 mL). The mineral media stock solution contained (g/L): EDTA (1), $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ (0.164), $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ (0.228), H_3BO_3 (0.02), $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ (0.04), Na_2SeO_3 (0.002), $\text{Na}_2\text{WO}_4\cdot 2\text{H}_2\text{O}$ (0.02), $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ (0.04), MgCl_2 (2.32), $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ (1.18), ZnCl_2 (0.1), $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ (0.02) and $\text{AlK}(\text{SO}_4)_2$ (0.02). A 50 mM 2-bromoethanesulfonate concentration was used to selectively inhibit methanogenic activity [23]. Anodes were inoculated from the effluent

of an existing MFC. Both MFCs and MECs were working with substrate for 15 days prior to starvation experiments.

Starvation tests were conducted in two MFCs and two MECs. The effects of different starvation periods in MFCs were studied by maintaining the cell in closed-circuit (MFC_{CC}) and in open-circuit (MFC_{OC}) during these periods. The external resistance was $1000\ \Omega$ and $100\ \Omega$ for MFC_{CC} and MFC_{OC} , respectively. In the case of MFC_{OC} , the external resistance was only connected under feast conditions (i.e. substrate presence). Starvation tests in MECs were studied while maintaining the applied voltage (MEC_{AV}) and without it (MEC_{WV}).

The cell was filled with medium without acetate before each starvation period to ensure fully starvation conditions. The medium was replaced with fresh medium with substrate for one batch cycle after each starvation period. Cycles after starvation periods were monitored to evaluate the effects of each starvation period on the cell performance. The cell was filled again with medium without acetate once current density started to decrease, provided that the performance was similar previous to any starvation period and a recovery time with substrate was not required. MECs were sparged with nitrogen for 10 min after replacing the medium to guarantee anaerobic conditions. All experiments were conducted at room temperature ($T = 25^\circ\text{C}$).

2.3. Chemical analyses

Acetate concentration was analysed with gas chromatography (Agilent Technologies, 7820-A) using a flame ionization detector (FID) and helium as carrier gas. Gas composition was analysed with the same gas chromatograph using a thermal conductivity detector (TCD) [24]. Hydrogen production was calculated as proposed in Ambler and Logan [25].

2.4. Electrochemical analyses

Power and polarization curves were obtained with a multi-resistance board which allowed changing the external resistance between $470,000$ and $25\ \Omega$. The cell was left in open circuit (OC) for 1 h previous to any measurement. A 10 min period was used for voltage stabilization at each resistance. The voltage drop across each resistance was measured by means of a multimeter. Medium was renewed previous to polarization curves recording.

2.5. System performance indexes

Coulombic efficiency (CE) (i.e. the fraction of electrons recovered as current intensity versus that theoretically generated from substrate oxidation) was evaluated as described in equation 1.

$$\text{CE} = \frac{\int_{t_0}^{t_f} I \, dt}{F \cdot b \cdot \Delta S \cdot V_R} \quad (1)$$

where t_0 and t_f are the initial and final times of a batch experiment (s), F is the Faraday's constant ($96,485\text{ C/mol e}^-$), b is the stoichiometric number of electrons produced per mol of substrate ($8\text{ mol e}^-/\text{mol acetate}$), ΔS is the substrate consumption (mol/L) and V_R the liquid volume (L).

The cathodic gas recovery (r_{CAT}), the ratio of coulombs recovered as hydrogen compared to those recovered as current intensity, was calculated as in equation 2.

$$r_{\text{CAT}} = \frac{V_{\text{H}_2} \cdot 2 \cdot F \cdot V_m^{-1}}{\int_{t_0}^{t_f} I \, dt} \quad (2)$$

where V_{H_2} is the volume of produced hydrogen and V_m is the molar gas volume (24.03 L/mol) at 20°C .

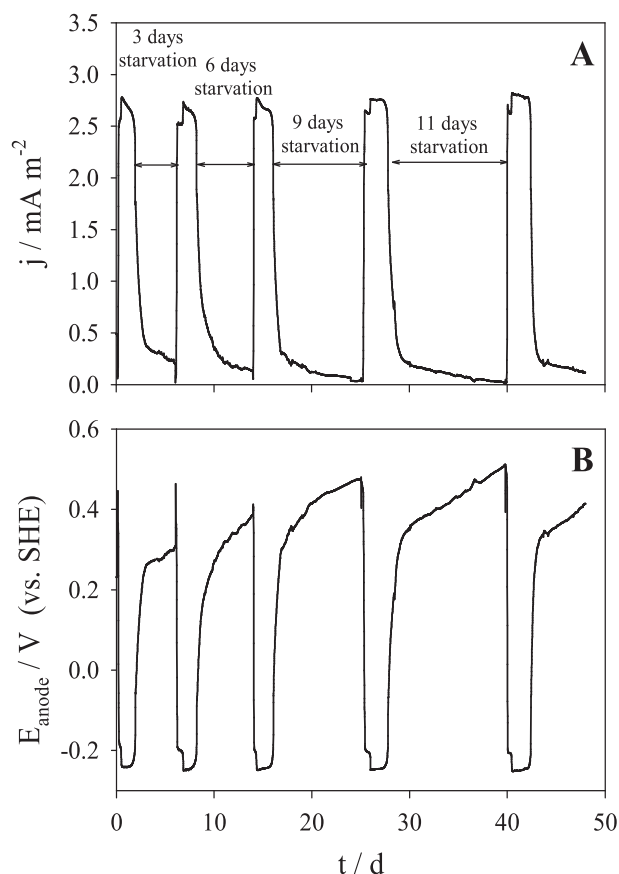


Fig. 1. Starvation experiments in MFC under closed-circuit conditions (MFC_{cc}). (A) Current density and (B) anode potential.

The energy recovery of the cell, i.e. the amount of energy contained in the produced hydrogen with respect to the energy inputs of the system, was calculated in relation to the electrical input (r_E) (equation 3) and the electrical input and the energy content of the substrate (r_{E+S}) (equation 4) as in Selembo et al. [26].

$$r_E = \frac{n_{H_2} \cdot \Delta H_{H_2}}{\int_{t_0}^{t_F} (I \cdot E_{ap} - I^2 \cdot R_{ext}) dt} \quad (3)$$

where n_{H_2} are the moles of produced hydrogen, ΔH_{H_2} is the heat of combustion of hydrogen (-285.83 kJ/mol), E_{ap} is the applied voltage and R_{ext} is the external resistance used for monitoring.

$$r_{E+S} = \frac{n_{H_2} \cdot \Delta H_{H_2}}{n_S \cdot \Delta H_S + \int_{t_0}^{t_F} (I \cdot E_{ap} - I^2 \cdot R_{ext}) dt} \quad (4)$$

where n_S are the moles of consumed substrate and ΔH_S is the heat of combustion of the substrate (-870.28 kJ/mol for acetate).

3. Results and discussion

3.1. Starvation in MFC systems

The effect of starvation periods was tested in MFCs under different conditions: (i) closed-circuit (MFC_{cc}) and (ii) open-circuit (MFC_{oc}) both in the short and in the long term.

Fig. 1A summarizes the experimental current density profiles of MFC_{cc} subjected to different short term starvation periods. The starvation periods ranged from 3 to 11 days. Current density profiles did not show any significant variation with respect to the initial batch cycle, where a current density of 2.8 mA/m² was reached. This indicated that short term starvation periods (up to 11 days) under closed-circuit mode did not have any detrimental effect on the MFC operation. Moreover, during the starvation periods, the current density remained positive indicating that electrons were flowing from the anode to the cathode regardless of the lack of substrate in the medium.

Table 1 compares the experimental coulombic efficiency and maximum power of the different batch cycles. Both coulombic efficiency and power remained practically constant in all cycles in agreement with the observed current density profiles. Coulombic efficiency ranged between 23 and 27%, whereas maximum power density was in a narrow range between 1.3 and 1.4 mW/m² in all batch cycles.

Under feast conditions, the anode potential (Fig. 1B) reached a constant value in all the cycles (-210 mV vs Standard Hydrogen Electrode, SHE). Under starvation conditions (i.e. substrate absence), the anode potential showed a sharp increase to $+260$ mV vs SHE and, then, a slower increase. The anode potential profiles remained similar after each starvation period.

Fig. 2 displays the experimental current density profiles of MFC_{oc} obtained after short term starvation periods. MFC_{oc} was subjected to 2, 5 and 7 days of starvation. The selected starvation periods for MFC_{oc} were different than for MFC_{cc} since the resistance to starvation under open circuit was expected to be lower. Therefore, starting with a period of 2 days seemed more convenient. Moreover, starvation periods of both 9 and 11 days were not tested because, as discussed below, after 7 days without substrate, a performance decrease was already observed. The current density profile of those cycles after 2 and 5 days of starvation was very similar to that of the initial batch cycle, in which a current density of 8.3 mA/m² was achieved. However, the maximum current density after 7 days of starvation in open-circuit mode decreased to 6.1 mA/m², which means a decrease of more than 25%. Hence, the activity was affected within one week of starvation. Nevertheless, subsequent cycles with substrate allowed a fast recovery of the initial cell activity (results not shown). This decrease in performance was not caused by oxygen diffusion to the anode. A natural bio-film on the cathode (which was visible with the naked eye) ensured

Table 1
Experimental coulombic efficiency (CE) and maximum power density of MFC after different starvation periods. Results for closed-circuit (MFC_{cc}) and open-circuit conditions (MFC_{oc}) are presented.

MFC _{cc}				MFC _{oc}			
Cycle	Starvation length (days)	CE (%)	Power density (mW/m ²)	Cycle	Starvation length (days)	CE (%)	Power density (mW/m ²)
0	-	23.6	1.4	0	-	38.9	1.2
1	3	24.0	1.3	1	2	32.5	1.1
2	6	23.0	1.4	2	5	35.0	1.1
3	9	26.7	1.4	3	7	21.6	0.7
4	11	24.8	1.4	-	-	-	-

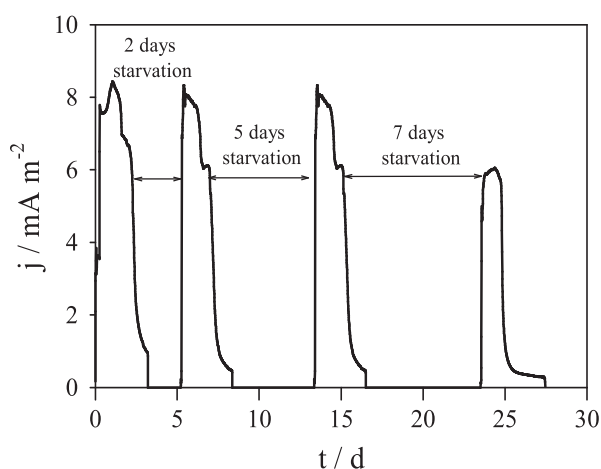


Fig. 2. Current density in MFC starvation experiments under open-circuit conditions (MFC_{oc}).

fully anaerobic conditions in the anode even when substrate was not available [27,28].

Table 1 presents the coulombic efficiency and power of MFC_{oc} after the short term starvation periods. As observed, the initial coulombic efficiency was 38.9% and remained fairly constant after 2 (32.5%) and 5 (35.0%) days. The same occurred to the maximum power density, which remained practically constant (1.2–1.1 mW/m²). Coulombic efficiency decreased to 21.6% and power density to 0.67 mW/m² after 7 days of starvation, as expected according to the current density profiles. The initial coulombic efficiency and power values were again obtained after some cycles with substrate.

As previously stated, short term starvation did not show any irreversible negative effect on the MFC operation. MFC_{oc} performance only decreased after 7 days of starvation and could be easily recovered. Hence, it was decided to increase the starvation period to 21 days for both cases (closed and open-circuit). The power and polarization curves obtained before and after the long-term starvation experiment are shown in Figs. 3A and 3B, respectively. The initial power curves reached a maximum power density of around 3.5 and 3.2 mW/m² for MFC_{cc} and MFC_{oc}, respectively. The initial polarization curves, meanwhile, showed similar open-circuit potentials (OCP) and maximum current density values. The maximum power density of MFC_{cc} after a 21-days starvation period severely decreased to a value of 1.7 mW/m² and the current density at this maximum power density was 3 times lower (3.3 mA/m²) than the initial value (9.4 mA/m²). The OCP value remained the same, although the maximum current density decreased to 6.1 mA/m².

Figs. 3C and 3D show the experimental current density and anode potential profiles of MFC_{cc} during the long-term starvation experiment. After a 21-days starvation period, MFC_{cc} reached a lower current density (1.8 mA/m²) with respect to the initial batch cycle (2.7 mA/m²). Hence, 21 days of starvation under closed-circuit conditions were clearly negative for the cell operation. Nevertheless, as shown in Fig. 3C, MFC_{cc} could recover its initial activity with an additional batch cycle with substrate.

Regarding open-circuit conditions (MFC_{oc}), the maximum power density after 21 days in starvation was far lower ($8 \cdot 10^{-3}$ mW/m²) than its initial value (3.2 mW/m²). The current density at this point fell from 8.9 mA/m² to 0.1 mA/m². The polarization curve showed that both the OCP and the maximum current density drastically decreased to 170 mV and 0.1 mA/m², respectively. Several batch cycles with substrate were later performed but the cell could not recover its activity, suggesting an irreversible damage after a 21-days starvation period under open-circuit conditions.

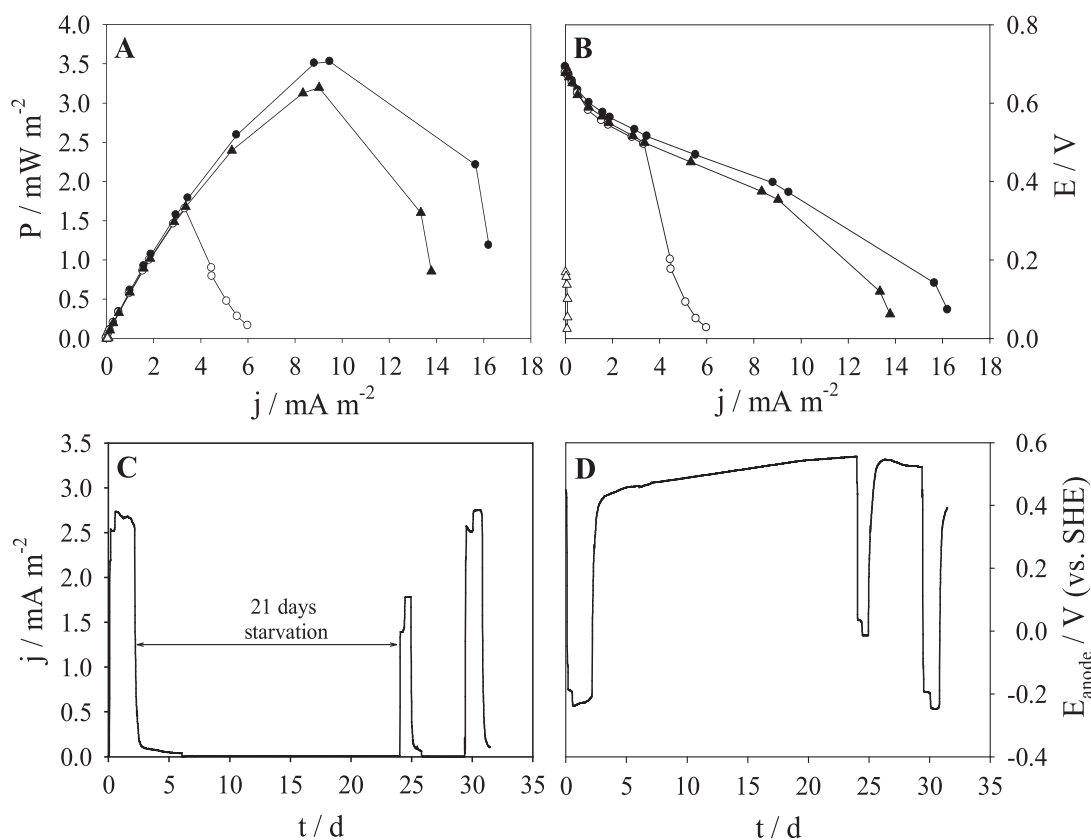


Fig. 3. MFC performance after a 21-days starvation period. (A) Power density and (B) polarization curves comparing the initial MFC performance (filled symbols) with the performance after the 21-days starvation period (open symbols) under closed (MFC_{cc}, ○) and open-circuit conditions (MFC_{oc}, △). (C) Experimental current density and (D) anode potential of MFC under closed-circuit conditions (MFC_{cc}).

The results show that short starvation periods (up to 11 days) are not detrimental to MFC performance under closed circuit conditions. Longer starvation periods (21 days) under closed circuit conditions negatively affected MFC performance, although it could be easily recovered after a short period with substrate. MFC are usually operated under batch mode and, as such, subjected under alternating feast and starvation conditions. Biomass can easily adapt to this scenario by using storage polymers as carbon and energy source under famine conditions. This observation has been already detailed in conventional activated sludge systems [29]. Hence, under alternating feast and starvation conditions, the population selected is able to survive under substrate limitation by accumulating substrate as a polymer inside of the bacterial cell under high substrate concentrations, and consuming it under starvation conditions. According to the literature, this could be accomplished either by only ARB [30] or by a syntrophic consortium [18]. This endogenous behaviour can explain the observed current density under the starvation periods under closed-circuit conditions. The results in closed-circuit suggest that the performance decrease observed in Oh and Logan [16] under starvation were probably due to voltage reversal rather than the starvation itself. On the other hand, our results are in agreement with those in Gao et al. [18] and Kaur et al. [17], where acetate-fed MFC were subjected to 7 and 12 days of starvation, respectively. In the former case, the current density recovered its initial value, whereas in the second case, the performance of the cell was only slightly affected by 12 days without substrate. Moreover, Chang et al. [4] showed that an MFC usually fed with glucose and glutamic acid recovered its initial current intensity after 12 days of starvation. However, a recovery time was required in this case.

On the other hand, when the cell was maintained under open-circuit conditions, the utilization of substrate accumulation was not feasible

because there was no electron sink. This is probably the reason why, after only 7 days of starvation in open-circuit conditions, MFC lost activity and after a 21-days starvation period the anode was irreversible damaged. Hence, this is the first report showing that the optimal way to maintain an MFC under starvation conditions is under closed-circuit conditions.

3.2. Starvation in MEC systems

The effect of starvation periods was also tested in MECs aiming at hydrogen production following two different strategies: (i) maintaining an applied voltage of 0.8 V (MEC_{AV}) and (ii) without any applied voltage (MEC_{WV}) during the starvation periods. The experimental current density and anode potential profiles during starvation tests in MEC_{AV} are shown in Fig. 4. The cell performance remained practically constant even after a starvation period of 10 days, since the maximum current density was very similar after 1, 3, 5 and 10 days of starvation (ranging between 28 and 34 mA/m^2). Moreover, the current density remained positive even during the starvation periods as already seen for MFC_{CC} . The anode potential had a value between -0.20 and -0.28 V vs SHE during conventional operation, which indicated a good performance of the anode. However, during the starvation periods it took positive values ranging between 0.02 and 0.18 V vs SHE. Anode potential profiles remained almost constant as well (Fig. 4B).

The common MEC performance indexes obtained in all the tested cycles are displayed in Fig. 5. The coulombic efficiency was practically the same in all batch cycles, being the highest value 88% after 1 day and the lowest value 77% after 5 days of starvation. Hydrogen was almost fully recovered, since r_{CAT} was practically 100% in all cases. No significant differences were found in energy efficiencies, since both r_E and r_{S+E} were in narrow ranges between 164 and 177%, and 64 and 70%, respectively. The differences in hydrogen production were also minimal, since the lowest and the highest hydrogen production were 1.2 and 1.3 $m^3/m^3 \cdot d$, suggesting again that starvation periods up to 10 days with an applied voltage of 0.8 V did not affect the MEC performance.

The results obtained with the starvation tests without any applied voltage (MEC_{WV}) are shown in Fig. 6. The current density profile of MEC_{WV} during the short-term starvation periods shows a lower performance after 10 days of starvation while 1, 3 and 5 days of starvation did not seem to have a significant effect on the cell performance. The maximum current density only ranged between 34 and 39 mA/m^2 for the initial and the 3 subsequent cycles (after 1, 3 and 5 days of starvation), and as a result, substrate was consumed in only 1 day for the three cycles. Current density after 10 days of starvation, meanwhile, decreased to 19 mA/m^2 and the duration of the cycle increased from 1 to 3 days.

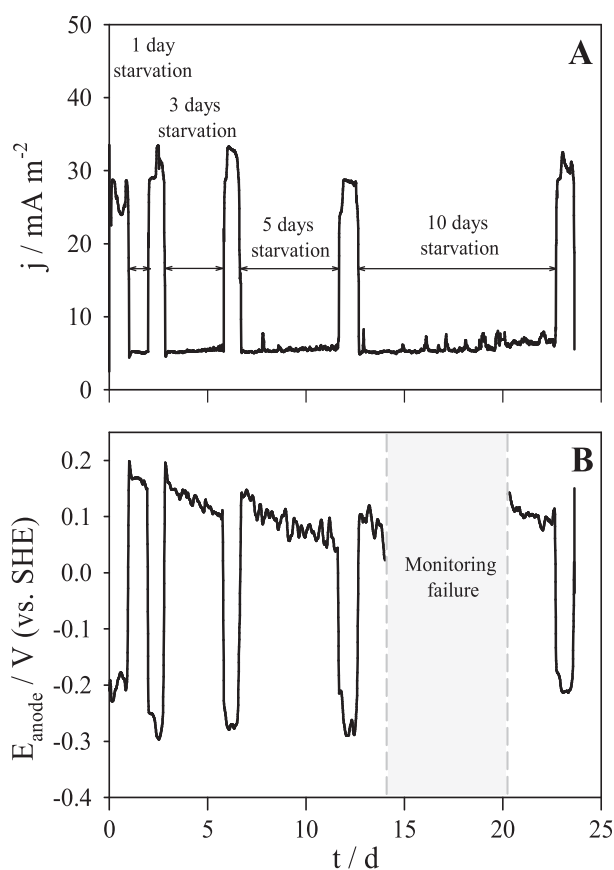


Fig. 4. Evaluating starvation in MEC with an applied voltage of 0.8 V (MEC_{AV}). (A) Current density and (B) anode potential. Note that from days 14 to 20 the anode potential could not be monitored.

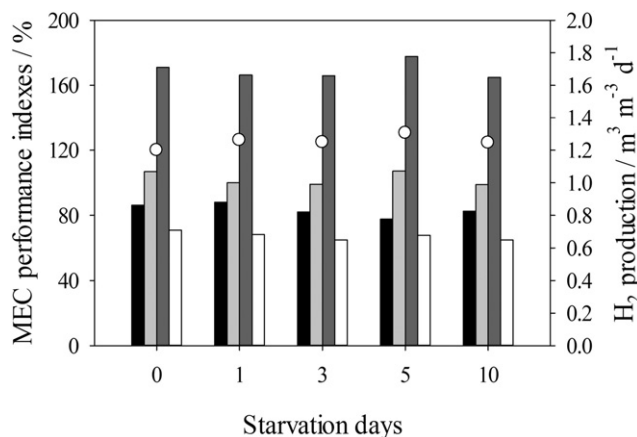


Fig. 5. Cycle performance after different starvation periods in MEC at an applied voltage of 0.8 V (MEC_{AV}). Coulombic efficiency (CE, black bar), cathodic gas recovery (r_{CAT} , light grey bar), energy efficiency (r_E , dark grey bar and r_{S+E} , white bar) and hydrogen production (circles).

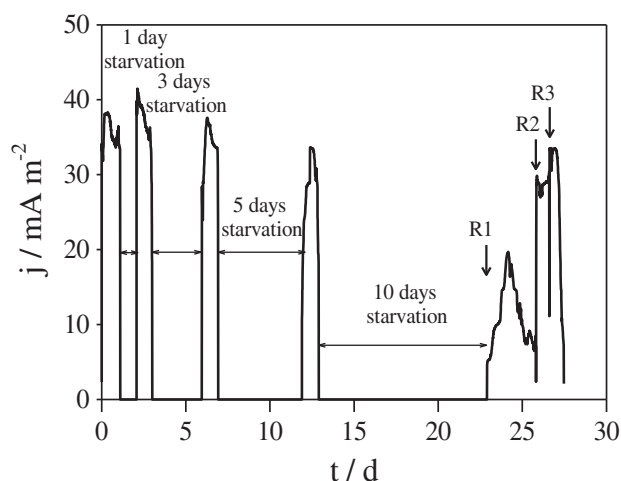


Fig. 6. Evaluating starvation in MEC without any applied voltage (MEC_{WV}).

Two additional batch cycles (R2 and R3) were monitored after the 10 days starvation cycle (R1) and the current density practically recovered its initial value.

The efficiencies and hydrogen production of MEC_{WV} are shown in Fig. 7. The coulombic efficiency decreased as the starvation periods increased, being initially 89% and 40% after 10 days of starvation. The r_{CAT} remained close to 100% after 1, 3 and 5 days of starvation, but significantly decreased to 63% after 10 days of starvation. Following a similar trend, r_E and r_{S+E} remained between 160 and 172%, and 52 and 64% even after 5 days of starvation, but after 10 days without substrate they were 65% and 22%, respectively.

As previously stated, two additional batch cycles were monitored after the 10-days starvation period. The previous performance indexes were also calculated and coulombic efficiency increased to 65 and 102%, r_{CAT} to 77 and 91%, r_E to 127 and 150% and r_{E+S} to 44 and 68%, for R2 and R3 respectively, thus, practically recovering the values previous to any starvation period.

Our results compare for the first time the effect of starvation in MEC with and without applied voltage. The results show that starvation periods up to 10 days did not have any significant effect on MEC performance as long as the applied voltage was maintained. In contrast, when no voltage was applied, 10 days without substrate led to a decrease of the MEC performance, although this negative effect was reversible. MEC outcomes had some analogy to those in MFC, since an applied voltage during the starvation periods would have allowed the utilization of

accumulated substrate. In fact, Fig. 4 shows a positive current density during starvation as already seen in Fig. 2 for MFC_{CC} . These results agree with those obtained in Gao et al. [18] where an MEC operating with a fixed anode potential of -0.4 vs $Ag/AgCl$ (-0.19 vs SHE) recovered its initial current intensity after 4 days without substrate.

MEC without any applied voltage during starvation periods lost part of its activity after 10 days, as accumulated substrate could not be used because the MEC overall process is thermodynamically unfavourable and electrons did not flow spontaneously from the anode to the cathode. Hence, for starvation periods longer than 5 days the best alternative is maintaining an applied voltage.

4. Conclusions

MFC under closed-circuit conditions during starvation periods displayed a high resistance to starvation, probably due to the consumption of the accumulated substrate as polymer inside the bacterial cell. In this sense, bacterial activity in MFC was not affected by a period up to 11 days without substrate. Longer starvation periods (21 days) only showed a reversible decrease in its performance. In the same manner, a starvation period of 10 days did not affect the performance of an MEC when the applied voltage was maintained.

Nevertheless, when the accumulated substrate could not be consumed by exoelectrogenic bacteria during starvation periods, i.e. when the circuit was kept open in MFC or no voltage was applied in MEC, the performance after starvation periods drastically decreased. Starvation periods longer than 5 days produced a negative effect on the cells performance, although it was recovered after one or two cycles with substrate. However, a 21-days open-circuit starvation period in MFC caused an irreversible deleterious effect on the biological anode. Thus, for long starvation periods, the utilization of the accumulated substrate has to be enabled, either by closing the circuit in MFC or applying a voltage in MEC.

Acknowledgments

The authors would like to acknowledge Laura Alemany for her assistance in the lab work developed. The authors are members of the GENOCOV group (Grup de Recerca Consolidat de la Generalitat de Catalunya, 2014 SGR 1225). Yolanda Ruiz thanks the Spanish Government for the FPU PhD grant (AP2010-1960) that allowed her to conduct her research.

References

- [1] H. Liu, S. Grot, B.E. Logan, Electrochemically assisted microbial production of hydrogen from acetate, *Environ. Sci. Technol.* 39 (2005) 4317–4320.
- [2] M. Di Lorenzo, T.P. Curtis, I.M. Head, K. Scott, A single-chamber microbial fuel cell as a biosensor for wastewaters, *Water Res.* 43 (2009) 3145–3154.
- [3] Y.F. Zhang, I. Angelidaki, Submersible microbial fuel cell sensor for monitoring microbial activity and BOD in groundwater: focusing on impact of anodic biofilm on sensor applicability, *Biotechnol. Bioeng.* 108 (2011) 2339–2347.
- [4] I.S. Chang, J.K. Jang, G.C. Gil, M. Kim, H.J. Kim, B.W. Cho, B.H. Kim, Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor, *Biosens. Bioelectron.* 19 (2004) 607–613.
- [5] Z.D. Liu, J. Liu, S.P. Zhang, X.H. Xing, Z.G. Su, Microbial fuel cell based biosensor for in situ monitoring of anaerobic digestion process, *Bioresour. Technol.* 102 (2011) 10221–10229.
- [6] M. Kim, M.S. Hyun, G.M. Gadd, H.J. Kim, A novel biomonitoring system using microbial fuel cells, *J. Environ. Monitor* 9 (2007) 1323–1328.
- [7] S. Patil, F. Harnisch, U. Schroder, Toxicity response of electroactive microbial biofilms—a decisive feature for potential biosensor and power source applications, *ChemPhysChem* 11 (2010) 2834–2837.
- [8] N.E. Stein, H.M.V. Hamelers, G. van Straten, K.J. Keesman, On-line detection of toxic components using a microbial fuel cell-based biosensor, *J. Process. Contr.* 22 (2012) 1755–1761.
- [9] N.E. Stein, H.V.M. Hamelers, G. van Straten, K.J. Keesman, Effect of toxic components on microbial fuel cell-polarization curves and estimation of the type of toxic inhibition, *Biosensors* 2 (2012) 255–268.
- [10] Y.F. Zhang, I. Angelidaki, A simple and rapid method for monitoring dissolved oxygen in water with a submersible microbial fuel cell (SBMFC), *Biosens. Bioelectron.* 38 (2012) 189–194.

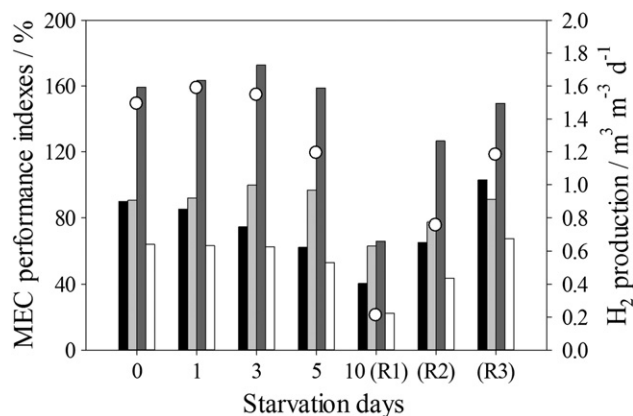
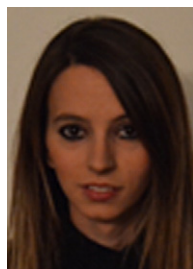


Fig. 7. Cycle performance after different starvation periods in MEC without any applied voltage (MEC_{WV}). Coulombic efficiency (CE, black bar), cathodic gas recovery (r_{CAT} , light grey bar), energy efficiency (r_E , dark grey bar and r_{S+E} , white bar) and hydrogen production (circles).

- [11] A. Kumlanghan, J. Liu, P. Thavarungkul, P. Kanatharana, B. Mattiasson, Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter, *Biosens. Bioelectron.* 22 (2007) 2939–2944.
- [12] J.M. Tront, J.D. Fortner, M. Plotze, J.B. Hughes, A.M. Puzrin, Microbial fuel cell technology for measurement of microbial respiration of lactate as an example of bioremediation amendment, *Biotechnol. Lett.* 30 (2008) 1385–1390.
- [13] A. Kaur, J.R. Kim, I. Michie, R.M. Dinsdale, A.J. Guwy, G.C. Premier, S.E.R. Ctr, Microbial fuel cell type biosensor for specific volatile fatty acids using acclimated bacterial communities, *Biosens. Bioelectron.* 47 (2013) 50–55.
- [14] J.M. Tront, J.D. Fortner, M. Plotze, J.B. Hughes, A.M. Puzrin, Microbial fuel cell biosensor for in situ assessment of microbial activity, *Biosens. Bioelectron.* 24 (2008) 586–590.
- [15] F. Golitsch, C. Bucking, J. Gescher, Proof of principle for an engineered microbial biosensor based on shewanella oneidensis outer membrane protein complexes, *Biosens. Bioelectron.* 47 (2013) 285–291.
- [16] S.E. Oh, B.E. Logan, Voltage reversal during microbial fuel cell stack operation, *J. Power Sources* 167 (2007) 11–17.
- [17] A. Kaur, H.C. Boghani, I. Michie, R.M. Dinsdale, A.J. Guwy, G.C. Premier, Inhibition of methane production in microbial fuel cells: operating strategies which select electrogens over methanogens, *Bioresour. Technol.* 173 (2014) 75–81.
- [18] Y. Gao, J. An, H. Ryu, H.-S. Lee, Microbial fuel cells as discontinuous portable power sources: syntrophic interactions with anode-respiring bacteria, *ChemSusChem* 7 (2014) 1026–1029.
- [19] S. Cheng, H. Liu, B.E. Logan, Increased performance of single-chamber microbial fuel cells using an improved cathode structure, *Electrochem. Commun.* 8 (2006) 489–494.
- [20] S. Cheng, H. Liu, B.E. Logan, Power densities using different cathode catalysts (Pt and CoTMPPE) and polymer binders (nafion and PTFE) in single chamber microbial fuel cells, *Environ. Sci. Technol.* 40 (2006) 364–369.
- [21] B. Logan, S. Cheng, V. Watson, G. Estadt, Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells, *Environ. Sci. Technol.* 41 (2007) 3341–3346.
- [22] X. Wang, S.A. Cheng, Y.J. Feng, M.D. Merrill, T. Saito, B.E. Logan, Use of carbon mesh anodes and the effect of different pretreatment methods on power production in microbial fuel cells, *Environ. Sci. Technol.* 43 (2009) 6870–6874.
- [23] P. Parameswaran, C.I. Torres, H.S. Lee, B.E. Rittmann, R. Krajmalnik-Brown, Hydrogen consumption in microbial electrochemical systems (MXCs): the role of homo-acetogenic bacteria, *Bioresour. Technol.* 102 (2011) 263–271.
- [24] Y. Ruiz, J.A. Baeza, A. Guisasola, Enhanced Performance of Bioelectrochemical Hydrogen Production using a pH Control Strategy, *Chemsuschem* 8 (2015) 389–397.
- [25] J.R. Ambler, B.E. Logan, Evaluation of stainless steel cathodes and a bicarbonate buffer for hydrogen production in microbial electrolysis cells using a new method for measuring gas production, *Int. J. Hydrogen Energ.* 36 (2011) 160–166.
- [26] P.A. Selembo, M.D. Merrill, B.E. Logan, The use of stainless steel and nickel alloys as low-cost cathodes in microbial electrolysis cells, *J. Power Sources* 190 (2009) 271–278.
- [27] J. Ahmed, S. Kim, Effect of cathodic biofilm on the performance of air-cathode single chamber microbial fuel cells, *B. Korean Chem. Soc* 32 (2011) 3726–3729.
- [28] N. Montpart, Hydrogen Production from Wastewater in Single Chamber Microbial Electrolysis Cells: Studies Towards its Scaling-up, *Universitat Autònoma de Barcelona*, Department of Chemical Engineering, 2014.
- [29] G. Sin, A. Guisasola, D.J.W. De Pauw, J.A. Baeza, J. Carrera, P.A. Vanrolleghem, A new approach for modelling simultaneous storage and growth processes for activated sludge systems under aerobic conditions, *Biotechnol. Bioeng.* 92 (2005) 600–613.
- [30] S. Freguía, K. Rabaey, Z.G. Yuan, J. Keller, Electron and carbon balances in microbial fuel cells reveal temporary bacterial storage behavior during electricity generation, *Environ. Sci. Technol.* 41 (2007) 2915–2921.



Yolanda Ruiz is a chemical engineer and PhD (2015) from the Department of Chemical Engineering at Universitat Autònoma de Barcelona. She is a member of the GENOCOV research group and she is conducting research on reducing the applied voltage requirements for hydrogen production in microbial electrolysis cells in view of the scaling-up of this technology.



Edgar Ribot-Llobet is a chemical engineer and a PhD student in the Department of Chemical Engineering at Universitat Autònoma de Barcelona. He is a member of the GENOCOV research group, and his research is focused on understanding the fundamentals of bioelectrochemical systems for wastewater valorisation. He is currently combining his thesis writing with a position in the pharmaceutical sector.



Dr. Juan A. Baeza has been an associate professor in the Department of Chemical Engineering at Universitat Autònoma de Barcelona since December 2004. His research is focused on Environmental Engineering: nutrient removal from wastewater (nitrification – denitrification, enhanced biological phosphorus removal), bioelectrochemical systems (MFC, MEC), modelling and process control. He leads research on nutrient removal from urban wastewater in the “GENOCOV group on biological treatment of liquid and gas effluents” of the UAB. <http://orcid.org/0000-0003-1290-1669>.



Dr. Albert Guisasola is a chemical engineer and Ph.D. in environmental engineering. He is associate professor in the Department of Chemical Engineering at Universitat Autònoma de Barcelona. His main research interest, within the GENOCOV research group, is not only to upgrade current wastewater treatment processes, but also to convert wastewater into a resource of energy and nutrients. Dr. Guisasola is studying these advanced biological and bioelectrochemical systems for wastewater treatment from an engineering approach. <http://orcid.org/0000-0002-3012-7964>.