Revealing the proliferation of hydrogen scavengers in a single-chamber microbial electrolysis cell using electron balances

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Abstract

The bioelectrochemical generation of hydrogen in microbial electrolysis cells (MECs) is a promising technology with many bottlenecks to be solved. Among them, the proliferation of hydrogen scavengers drastically reduces the cell efficiency leading to unrealistic coulombic efficiencies (CE) and cathodic gas recoveries (rCAT). This work provides a novel theoretical approach to understand, through electron equivalent balances, the fate of hydrogen in these systems. It was validated with a long term operated single-chamber membrane-less MEC. In the short term, H₂-recycling (i.e. hydrogen being derived to the anode) resulted in rCAT of only 4% and in CE up to 463%. The 80.5% of the current intensity came from H₂-recycling and only the 19.5% from substrate oxidation. In the long term, methane was produced from hydrogen, thus decreasing rCAT to 0 (rCAT = 94.5% when considering methane production). CE was 74.5% suggesting that H₂-recycling only took place when methanogenic activity was marginal.

1. Introduction

Among all the possible renewable energy sources, H₂ gas is one of the most attracting alternatives for the scientific community. It is a clean and renewable energy carrier, without an impact on the greenhouse gas emission at the point of use and a high combustion heat (120 kJ/g) when compared to other possible biofuels (CH₄, 50 kJ/g or ethanol, 26.8 kJ/g) [1]. Moreover, H₂ can be very efficiently converted into electricity by means of chemical fuel cells when compared to biogas [2]. Nowadays, most H₂ is produced by steam reforming of fossil fuels, a non-sustainable technology. For this reason, research is focused on the development of technologies for sustainable H₂ production. Among the different alternatives, the bioelectrochemical generation of H₂ in microbial electrolysis cells (MECs) is a novel technology introduced in Liu et al. [3] with very promising lab results and theoretical higher yields.

MECs take advantage of the capability of the anode respiring bacteria (ARB) of using insoluble electron acceptors in their respiration process and thus, transferring the electrons to a solid anode under anaerobic conditions. Hence, ARB oxidize organic matter and transfer the electrons to the anode,
which flow through an external circuit to the cathode. The cathode is also kept under anaerobic conditions and thus, the protons generated in the anode are reduced to form H\(_2\). The global process is not thermodynamically spontaneous and a certain voltage has to be applied to drive the reactions [3]. In any case, the energy contained in the produced H\(_2\) has to be higher than the energy added by the power source in order to make MECs a feasible system.

The use of membranes in MECs to separate the anodic chamber from the cathodic chamber is nowadays a controversial issue. On the one hand, membranes theoretically prevent the diffusion of H\(_2\) from the cathode to the anode and avoid potential problems related to H\(_2\) scavengers and impurities in H\(_2\). On the other hand, membranes are expensive and cause potential losses associated to pH gradients across them [4]. Thus, higher voltages need to be applied for the reactions to take place resulting in a severe decrease of energetic efficiency.

Electron flow derived to methanogenesis is one of the major hurdles of bioelectrochemical systems. CH\(_4\) production from organic carbon sources results in a significant decrease of the system efficiency, measured as Coulombic Efficiency (i.e. ratio of electrons contained in the initial substrate that are converted into current). Avoiding methanogenesis in MECs is not a straightforward issue since these microorganisms are strongly favoured in conventional MEC anodic environments (i.e. anaerobiosis with abundance of electron donors and biofilm formation) and this is why the contamination of H\(_2\) with CH\(_4\) has been widely reported (e.g. [5]). Moreover, when working with fermentable substrates, the H\(_2\) generated in fermentation can be used for methanogenesis as electron donor, which can account for important electron losses at the anodic compartment [6]. This hydrogenotrophic methanogenesis becomes even more important when operating single-chamber systems (i.e. membrane-less), since the H\(_2\) electrochemically formed in the cathode can also be used as electron donor. Nowadays, CH\(_4\) formation is mostly prevented using a chemical inhibitor of methanogenesis (being 2-bromoethanesulfonate, BES, the most common). BES utilisation is practical with short-term lab-scale experiments but it is not economically feasible at a real scale. Other approaches for methanogenesis suppression such as low hydraulic retention times [7], intermittent exposure to air [5], low temperature and pH shocks [8,9] have not been totally successful yet even at lab-scale conditions.

The presence of different H\(_2\) scavengers other than methanogens has also been observed. On the one hand, the effect of homoacetogenic bacteria (e.g. strictly anaerobic bacteria that produce acetate with H\(_2\) as electron donor and inorganic carbon) in two-chamber MECs with fermentable substrates was reported to have a positive effect, since they allow the electron recovery from the produced H\(_2\) in fermentation [6]. However, in single-chamber MECs, homoacetogens can have a detrimental effect since they can transform back to acetate the H\(_2\) produced in the cathode. This H\(_2\)-acetate loop can result in an increase of the cycles duration and thus, more input energy requirements and lower H\(_2\) recoveries [10]. Nevertheless, the low H\(_2\) recoveries in single-chamber MECs due to H\(_2\)-recycling are not only as a result of the homoacetogenic activity, but the use of H\(_2\) as electron donor by ARB has also been reported [11]. In this sense, Lee and Rittmann [7] studied the contribution of H\(_2\)-recycling in a continuous single-chamber MEC by minimizing the methanogenic activity, obtaining that from the 62 to the 76% of the total current intensity was as a result of H\(_2\)-recycling. However, methanogenic activity was not completely suppressed and therefore, the contribution of H\(_2\)-recycling could have been even higher.

A whole understanding of the competition between the different H\(_2\) scavengers in single-chamber MEC systems has not been reported yet, although it was found that methanogenesis inhibition could favour homoacetogenic growth [6]. Lee and Rittmann [7] observed that H\(_2\)-recycling and CH\(_4\) production occurred in the system simultaneously. Parameswaran et al. [12] found that homoacetogens could survive in a cell working at low HRT (with high BES concentration) indicating that homoacetogens could compete with hydrogenotrophic methanogens in real systems.

This work is the first study where the long term operation of a single-chamber membrane-less MEC with continuous dosage of BES is experimentally assessed. Long and fully monitored cycles and electron equivalent balances are used to understand the existing H\(_2\) losses due to the competition between homoacetogens, ARB and hydrogenotrophic methanogens for H\(_2\).

2. Materials and methods

2.1. Reactor description and operation

A single-chamber membrane-less MEC of 1300 mL was used (Fig. 1). A carbon fiber brush (PANEX®33 160 K, ZOLTEK) previously inoculated in a microbial fuel cell was used as anode. The cathode was made with carbon cloth coated with carbon powder and platinum suspension on the side facing the anode[14,15]. Both electrodes were arranged concentrically with the cathode in the outer perimeter, so that all ends of the anode were at the same distance from the cathode. An Ag/AgCl reference electrode (+210 mV vs SHE) was used to monitor the electrode potentials. The reactor operated in batch mode and with constant agitation. A constant voltage of 1.2 V was provided by a power supply (TTI QL35STP). The H\(_2\) produced was collected in a 0.5 L gas sample bag with a twist type valve (Calif-5-Bond, Ritter).

Intensity was calculated from the monitoring of the voltage across an external resistance of 12 Ω by using a 16-bit data acquisition card (Advantech PCI-1716) connected to a personal computer with software developed in LabWindows CVI 2010 for data acquisition.

The medium was a 100 mM phosphate buffer with acetate as carbon source prepared as in Parameswaran et al. [10] with the addition of the methanogenic inhibitor BES [16]. The acetate concentration in the medium was 235 mg/L (4 mM) and BES concentration was 50 mM except as indicated, where it was increased to 90 and 120 mM.

2.2. Chemical analyses

Acetate was analysed by gas chromatography (Agilent Technologies, 7820-A) using a flame ionization detector (FID) with
helium as carrier gas. H2 and CH4 were analysed with the same gas chromatograph using a thermal conductivity detector (TCD) with argon as carrier gas to ensure a good response in H2 peak.

2.3. Batch experiments

Batch experiments were carried out to assess the cell performance over time. Culture medium was renewed prior to each cycle monitoring. Acetate concentration, gas production/composition and current intensity were measured along the cycles. Obtaining experimental profiles in time and not only start/end measurements was essential for a better understanding of the system.

Gas production was calculated as in Ambler and Logan [17]. The same gas composition was assumed in both the headspace and the gas sampling bag and therefore, the final volume of each gas (H2 and CH4) was calculated from the total volume (headspace + gas sample bag) and the gas composition of the last analysis of the cycle (equation (1)).

\[
V_{GF,F} = V_{GF,I} \times x_{GF,F}
\]  

where \( V_{GF,F} \) is the final volume of gas and \( V_{GF,I} \) and \( x_{GF,F} \) are the final volume and final composition of a certain gas, respectively.

The moles of H2 corresponding to that volume were calculated assuming a constant pressure of 1 atm in the reactor-bag system and room temperature.

2.4. Presence of homoacetogens

The presence of homoacetogenic bacteria was tested through an experiment similar to that in Parameswaran et al. [10]. Culture medium was replaced and no acetate, but sodium bicarbonate (3 g/L) was added. The MEC was operated with an applied voltage of 1.2 V. H2, stored in a gas sampling bag of 1 L, was intermittently sparged from the bottom of the reactor and collected in another gas sampling bag located at the top of the cell. Once the bag at the top was full, the position of the bags was reversed in order to continue sparging H2 from the bottom of the cell. This operation was repeated nine times between hours 0 and 8 and nine times more between hours 22 and 30 of the experiment.

2.5. Calculations

Coulombic Efficiency (CE) was calculated as in equation (2).

\[
CE = \frac{\text{Coulombs recovered as current intensity}}{\text{Coulombs in substrate}} = \frac{\int_{t_0}^{t_f} I dt}{F b_{Ac} V_L \Delta c M}
\]  

where \( t_0 \) and \( t_f \) are the initial and final times of an experiment, \( \Delta c \) is the acetate concentration change between \( t_0 \) and \( t_f \) (g acetate/L), \( M \) is the molecular weight of acetate (59 g/mol), \( b_{Ac} \) is the number of e\(^-\) transferred per mole of acetate (8 mol e\(^-\)/mol acetate), \( F \) is the Faraday constant (96485 C/mol e\(^-\)), \( I \) is the current intensity and \( V_L \) is the volume of liquid in the reactor.

Cathodic gas recovery (\( r_{CAT} \)) was calculated as in equation (3).

\[
r_{CAT} = \frac{\text{Coulombs in H}_2}{\text{Coulombs recovered as current intensity}} = \frac{V_{H2,F}}{V_m} \frac{2 F V_m^{-1}}{\int_{t_0}^{t_f} I dt}
\]  

where \( V_{H2,F} \) is the final volume of H2 and \( V_m \) is the molar gas volume (24.03 L/mol) at 20 °C.
3. Results and discussion

3.1. CE and $r_{\text{CAT}}$ as MEC performance indicators

The performance of a MEC is commonly assessed through the calculation of the coulombic efficiency (CE) and the cathodic gas recovery ($r_{\text{CAT}}$). CE compares the coulombs recovered as current intensity with the coulombs that could theoretically generated from the substrate oxidation by ARB, while $r_{\text{CAT}}$ compares the coulombs consumed in H$_2$ production with the coulombs arriving to the cathode as current intensity. However, under certain scenarios, these efficiencies may be misleading and some considerations need to be taken into account when analysing the results.

H$_2$ is a suitable electron donor and, as such, its presence may induce the growth of hydrogenotrophic bacteria. H$_2$ is either electrochemically produced at the cathode or appears as a subproduct from the fermentation of organic products. Then, the proliferation of H$_2$ scavengers in MEC systems is frequent, particularly when operating under single-chamber configuration. The most common scenarios in acetate-fed single-chamber MECs are: i) neither methanogenesis nor H$_2$-recycling, ii) only H$_2$-recycling, iii) only methanogenesis and iv) both H$_2$-recycling and methanogenesis taking place.

In view of simplification, it has been assumed that CH$_4$ formation comes only from hydrogenotrophic methanogens and thus, acetate is not a carbon source for methanogenesis. This suppression of aceticlastic methanogenesis in single-chamber acetate-fed systems has already been reported and it is justified by the ARB having higher acetate affinity than methanogens [18]. Anyway, the absence of aceticlastic methanogens in our systems was ensured by monitoring acetate concentration in a batch experiment during 70 h without applying any voltage (Fig. S1, supplementary data). Acetate concentration remained practically constant indicating that acetate consumption related to non-ARB microorganisms was negligible. The absence of aceticlastic methanogens was also corroborated through advanced microbiological analyses showing that only 2% of the Archaea present in the anode were aceticlastic [19]. It should be noted that if a fermentable substrate different than acetate was used, H$_2$ from fermentation should be also considered and the system would become much more complex.

The utilisation of CE and $r_{\text{CAT}}$ to evaluate the MEC performance is not valid when H$_2$-recycling is occurring. Moreover, $r_{\text{CAT}}$ cannot be used when hydrogenotrophic methanogenesis is taking place. In these cases, an extended approach should be used. Nevertheless, obtaining unrealistic CE and $r_{\text{CAT}}$ results would be a good indicator of some H$_2$ being lost: CE higher than 100% suggests H$_2$-recycling, whereas very low $r_{\text{CAT}}$ denotes H$_2$ losses probably as a consequence of methanogenesis or H$_2$-recycling.

3.2. Including H$_2$-recycling (with or without hydrogenotrophic methanogenesis)

When H$_2$-recycling is taking place the estimated CE values are excessively high (even higher than 100%). Then, the MEC performance becomes much more complex to evaluate and a different approach is needed. In this case, we have used electron equivalent balances (i.e. balances in terms of coulombs) for a better description of the cell performance. As it can be observed in Fig. 2, electron equivalent balances are stated for both anodic and cathodic processes, which are linked by the coulombs recovered as current intensity and the coulombs recycled as H$_2$ by ARB and homoacetogens.

Regarding anodic processes, the coulombs recovered as current intensity may come from three different sources: i) the oxidation of the external acetate initially added, ii) the oxidation of the acetate resulting from homoacetogenesis and iii) the oxidation of part of the H$_2$ produced in the cathode. Moreover, it should be considered that a fraction of this

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**Fig. 2** – Reaction pathways and parameters of electron equivalent balances in an acetate-fed single-chamber MEC.
The stoichiometry of the possible reactions acetate/H$_2$ is not addressed to current intensity but to the growth of the biomass. The balance in the anodic side can be written as in equation (4).

$$C_{\text{CI}} = C_{\text{Ac}} + C_{\text{H}} + C_{\text{H}_2,\text{r}} - C_{\text{ARB}} - C_{\text{Ac}}$$  \hspace{1cm} (4)

where $C_{\text{CI}}$ are the coulombs recovered as current intensity, $C_{\text{Ac}}$ are the coulombs obtained from the oxidation of the external acetate, $C_{\text{H}}$ are the coulombs obtained from the oxidation of the acetate produced by homoacetogens, $C_{\text{H}_2,\text{r}}$ are the coulombs obtained from the oxidation of the $H_2$ produced in the cathode by ARB while $C_{\text{ARB}}$ and $C_{\text{Ac}}$ are the acetate and $H_2$ fractions addressed to biomass growth in terms of coulombs.

In the case of cathodic processes, the coulombs recovered as current intensity are all used for $H_2$ production which, in turn, has four theoretical different endings: i) being captured in the gas bag, the most desirable, ii) being consumed by methanogens, iii) being consumed by homoacetogens, iv) being consumed by ARB. Equation (5) represents the previous equations system.

$$C_{\text{CI}} = C_{\text{H}_2} + C_{\text{CH}_4} + C_{\text{H}} + C_{\text{H}_2,\text{r}}$$  \hspace{1cm} (5)

where $C_{\text{H}_2}$ are the coulombs consumed in the production of the measured $H_2$ and $C_{\text{CH}_4}$, $C_{\text{H}}$, $C_{\text{H}_2,\text{r}}$, are the coulombs consumed in the production of $H_2$ subsequently consumed for the production of $CH_4$, acetate and current intensity.

Although $H_2$ losses due to leakage ($C_{\text{H}_2,\text{L}}$) are not considered in equation (5), practical knowledge suggests that, in some cases, they might be required to completely solve the equations system. $C_{\text{H}_2,\text{L}}$ can be taken into account in terms of coulombs by modifying equation (5) as follows:

$$C_{\text{CI}} = C_{\text{H}_2} + C_{\text{CH}_4} + C_{\text{H}} + C_{\text{H}_2,\text{r}} + C_{\text{H}_2,\text{L}}$$  \hspace{1cm} (6)

Thus, the fate of the electrons would be completely described with equations (4) and (5) or (6). However, each of the parameters in these equations needs to be estimated/measured.

### 3.2.1. Contribution of the growth processes

The fraction of acetate addressed to ARB growth in terms of coulombs, $C_{\text{Ac}}$, can be estimated from equation (7).

$$C_{\text{Ac}}^{\text{ARB}} = Y_{\text{Ac}}^{\text{ARN}} \cdot (C_{\text{Ac}} + C_{\text{H}}) \cdot \frac{100 - CE_{\text{A1}}}{100} \cdot (C_{\text{Ac}} + C_{\text{H}})$$  \hspace{1cm} (7)

where $Y_{\text{Ac}}^{\text{ARN}}$ is the biomass/substrate yield of ARB when consuming acetate and $CE_{\text{A1}}$ is the real coulombic efficiency of the cell, i.e. the CE of the cell when $H_2$-recycling does not occur and thus, current intensity is entirely produced from the oxidation of the externally added acetate. Thus, equation (7) calculates the product between the fraction of acetate consumed but not recovered as current intensity and the coulombs obtained from acetate oxidation either from the externally added or produced by homoacetogens. Note that using either $Y_{\text{Ac}}^{\text{ARN}}$ or $CE_{\text{A1}}$ in the calculation of $C_{\text{Ac}}^{\text{ARB}}$ implicitly assumes that acetate is only consumed by ARB. Sleutels et al. [20] used CE to assess the competition between ARB and methanogens with acetate as substrate by considering the electrode and methane as the main electron sinks. As previously stated, the presence of acetoclastic methanogens in our system was negligible and therefore, it could be assumed that the acetate not recovered as current intensity was uniquely addressed to ARB growth.

The $CE_{\text{A1}}$ could be either theoretically estimated or experimentally assessed. For the latter, two additional experiments besides the abovementioned standard monitoring are required. On the one hand, acetate evolution and current intensity are measured in a cell with constant $N_2$ sparging to evaluate the ARB activity without $H_2$-recycling (experiment A1). The obtained results could be misleading if acetate stripping is simultaneously occurring and this is why the extent of this stripping is evaluated in a second experiment where acetate is monitored with constant $N_2$ sparging and no applied voltage (experiment A2). The experimental estimation of $CE_{\text{A1}}$ should be more reliable if it is calculated specifically for each system.

Part of the $H_2$ consumed by homoacetogens (Table 1) is also addressed to biomass growth and can be calculated as follows:

$$C_{\text{H}_2}^{\text{HOMO}} = C_{\text{H}} - C_{\text{H}}'$$  \hspace{1cm} (8)

where $C_{\text{H}_2}^{\text{HOMO}}$ are the coulombs equivalent to the $H_2$ addressed to homoacetogens growth.

Similarly, part of $H_2$ oxidized by ARB is also consumed for growth and not recovered as current intensity ($C_{\text{H}_2}^{\text{ARB}}$). Both $C_{\text{H}_2}^{\text{HOMO}}$ and $C_{\text{H}_2}^{\text{ARB}}$ can be also calculated from the biomass/substrate yield as shown in equations (9) and (10).

$$C_{\text{H}_2}^{\text{HOMO}} = Y_{\text{H}_2}^{\text{HOMO}} \cdot C_{\text{H}}$$  \hspace{1cm} (9)

$$C_{\text{H}_2}^{\text{ARB}} = Y_{\text{H}_2}^{\text{ARB}} \cdot C_{\text{H}_2,\text{r}}$$  \hspace{1cm} (10)

where $Y_{\text{H}_2}^{\text{HOMO}}$ and $Y_{\text{H}_2}^{\text{ARB}}$ are the biomass/substrate yields of homoacetogens and ARB when consuming $H_2$.

$C_{\text{Ac}}$, $C_{\text{H}_2}$, $C_{\text{CH}_4}$ and $C_{\text{CI}}$ can be calculated from off-line/on-line measurements. The following paragraphs detail how to do so.

### 3.2.2. Coulombs obtained from the oxidation of the externally added acetate, $C_{\text{Ac}}$

The moles of electrons obtained from acetate oxidation are calculated from the amount of the external acetate consumed (Table 1) and converted to coulombs using the Faraday constant (equation (11)). The reactor volume remained practically constant during all the experiment (less than the 2% of the total liquid volume was extracted for sampling).

$$C_{\text{Ac}} = \Delta c \cdot M^{-1} \cdot V_L \cdot b_{\text{Ac}} \cdot F$$  \hspace{1cm} (11)

<table>
<thead>
<tr>
<th>Table 1 – Stoichiometry of the possible reactions occurring in a MEC.</th>
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<tbody>
<tr>
<td>Reaction/microorganisms</td>
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<td>-------------------------</td>
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<tr>
<td>Acetate oxidation/ARB</td>
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<tr>
<td>CH$_4$ formation/hydrogenotrophic methanogens</td>
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<tr>
<td>Acetate formation/homoacetogens</td>
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<tr>
<td>H$_2$ oxidation/ARB</td>
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<tr>
<td>H$_2$ formation/chemical reaction</td>
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3.2.3. Coulombs consumed in the production of the measured H₂, \( C_{\text{H₂}} \)

\( C_{\text{H₂}} \) is estimated by calculating the moles of electrons consumed during the production of \( H₂ \) (Table 1) and converting them to coulombs (equation (12)).

\[
C_{\text{H₂}} = n_{\text{H₂,f}} b_{\text{H₂,F}} F
\]

where \( n_{\text{H₂,f}} \) are the moles of \( H₂ \) captured and \( b_{\text{H₂,F}} \) is the number of \( e^- \) transferred per mole of \( H₂ \) (2 mol \( e^- \)/mol \( H₂ \)).

3.2.4. Coulombs consumed in the production of \( H₂ \) converted to \( CH₄ \), \( C_{\text{CH₄}} \)

\( C_{\text{CH₄}} \) includes the coulombs consumed in the production of \( H₂ \) converted to \( CH₄ \) without considering biomass growth (\( C_{\text{CH₄,l}} \)) and the \( H₂ \) consumed for hydrogenotrophic methanogenesis growth in terms of coulombs (\( C_{\text{H₂,CH₄}} \)). \( C_{\text{H₂,CH₄}} \) can be calculated with equation (13).

\[
C_{\text{CH₄}} = C_{\text{CH₄,l}}' + C_{\text{H₂,CH₄}} = n_{\text{CH₄,f}} b_{\text{H₂,F}} F
\]

where \( n_{\text{CH₄,f}} \) are the moles of \( H₂ \) consumed to produce \( CH₄ \).

3.2.5. Coulombs recovered as current intensity, \( C_{\text{CI}} \)

\( C_{\text{CI}} \) is calculated by integrating the current intensity from the initial to the final time of the batch experiment.

\[
C_{\text{CI}} = \int_{t_0}^{t_f} Idt
\]

Note that being able to calculate \( C_{\text{AC}}, C_{\text{H₂}}, C_{\text{CH₄}} \) and \( C_{\text{CI}} \) (equations (11)–(13) and (15)) we have a system of six linear equations (equations (4) and (5), and (7)–(10)) and six degrees of freedom (\( C_{\text{Ac}}, C_{\text{H₂}}, C_{\text{H₂,Ac}}, C_{\text{AC}}, C_{\text{CH₄,MO}}, C_{\text{CH₄,AR}} \)). Thus, electron equivalent balances can be solved. All the parameters used to calculate the electron equivalent balances are summarized in Table 2.

Moreover, two interesting performance parameters, the fraction of the current intensity generated due to the oxidation of the externally added acetate (\( f_{\text{CL,Ac}} \)) and due to recycled \( H₂ (f_{\text{CL,H₂}}) \), can be also estimated from the parameters calculated by the electron equivalent balances (equations (16) and (17)).

\[
f_{\text{CL,Ac}} = \frac{1 - Y_{\text{Ac}}^{\text{AB}}} {C_{\text{CI}}} = \left( 1 - \frac{100 \cdot C_{\text{CH₄,l}}}{C_{\text{CI}}} \right) \frac{C_{\text{Ac}}} {C_{\text{CI}}} = \frac{CE_{\text{H₂}}}{CE_{\text{H₂,CH₄}}} \frac{C_{\text{Ac}}} {C_{\text{CI}}}
\]

\[
f_{\text{CL,H₂}} = \frac{1 - Y_{\text{Ac}}^{\text{AB}}} {C_{\text{CI}}} \frac{C_{\text{H₂,l}}'} {C_{\text{CI}}} - \frac{C_{\text{H₂,AB}}}{C_{\text{CI}}} = \frac{CE_{\text{H₂}}}{CE_{\text{H₂,CH₄}}} \frac{C_{\text{H₂,l}}'} {C_{\text{CI}}} - \frac{C_{\text{H₂,AB}}}{C_{\text{CI}}}
\]

3.3. Including hydrogenotrophic methanogenesis when no \( H₂ \)-recycling is occurring

The previously developed electron equivalent balances can be used even when no \( H₂ \)-recycling is occurring but most parameters would be zero. In this sense, the following simplified approach can be more practical. Thus, if hydrogenotrophic methanogens are present in the system, \( r_{\text{CAT}} \) will be underestimated since the amount of \( H₂ \) produced and sequentially diverted to \( CH₄ \) would not be considered. Although \( CE \) would not be affected, the calculation of \( r_{\text{CAT}} \) would need a correction by including the \( H₂ \) theoretically converted into \( CH₄ \). Then, the real volume of \( H₂ \) produced (\( V_{H₂,f}^T \)) would include the measured \( H₂ \) and the \( H₂ \) converted to \( CH₄ \) according to the proper stoichiometry (Table 1). Then, \( V_{H₂,f}^T \) should be used in equation (3) when estimating \( r_{\text{CAT}} \).

\[
V_{H₂,f}^T = V_{H₂,f} + V_{CH₄,f}^T
\]

where \( V_{H₂,f}^T \) is the total volume of \( H₂ \) produced and \( V_{CH₄,f} \) is the measured \( H₂ \) production.

3.4. Experimental study: occurrence of \( H₂ \)-recycling

A 1.3L MEC was operated for 8 months with BES dosage using an ARB-enriched anode. BES concentration was initially set at 50 mM, a value theoretically high enough to suppress methanogenic activity [10]. Under these conditions (i.e. single-chamber membrane-less MEC with BES and under batch operation), methanogenesis could be avoided. However, \( H₂ \)-recycling was favoured and then, efficient \( H₂ \) production was still hindered. Practically from the first days of operation it was observed that the duration of the cycles was not in agreement with the monitored intensity resulting in \( CE \) higher than 100%. Moreover, the highest \( H₂ \) production was detected after adding fresh medium in the cell, whereas \( H₂ \) concentration in the gas sampling bag was decreasing along the cycle, resulting in \( r_{\text{CAT}} \) values close to 0%. Thus, the most plausible option was \( H₂ \)-recycling either by homoacetogens or \( H₂ \)-consumers ARB. Fig. 3 shows an experiment where sodium bicarbonate and \( H₂ \) were added as sole carbon source and sole electron donor, respectively. Acetate concentration was initially zero and it increased over time reaching values of around 70 mg/L. Meanwhile, current density also increased and reached values close to 7 A/m². Thus, homoacetogens were present and consumed \( H₂ \) and \( CO₂ \) to form acetate. Acetate could be subsequently used by ARB to generate current from acetate. However, current intensity due to direct oxidation of \( H₂ \) could not be ruled out.

Electron equivalent balances were calculated to gain insight on the cell performance under \( H₂ \)-recycling conditions and hence a cycle was monitored during approximately 100 h.

Fig. 4 shows the experimental results obtained during the characterisation of the operation with \( H₂ \) recycling. As previously detailed, two additional experiments were required for the calculation of \( C_{\text{Ac}}^{\text{AB}} \). A1) ARB activity was measured in a MEC with continuous \( N₂ \) sparging to avoid \( H₂ \) utilisation by both homoacetogens and ARB and A2) acetate concentration was measured with \( N₂ \) sparging but with no applied voltage to estimate acetate stripping. Fig. 4A compares the cell current density.
with (A1) and without N2 sparging (conventional operation). As it can be observed, the duration of the cycle was completely different (in spite of having the same initial acetate concentration): the cycle was completed after 50 h with N2 sparging whereas under conventional operation, the current density remained at values around 17 A/m³ after 100 h. In A1 H2 was removed from the system by stripping, while under conventional operation, H2 was used by homoacetogenic bacteria to produce acetate or by ARB to generate electricity thus, extending the cycles. Regarding acetate measurements, acetate decreased

![Graph A](image1)

**Fig. 3** – Batch experiment with the addition of sodium bicarbonate and H2 sparging (A) Acetate concentration and (B) Current density over time. Current density is shown from time 5 h due to monitoring problems.

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<th>Parameter</th>
<th>Description</th>
<th>Dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_{Ac}$</td>
<td>Number of e⁻ transferred per mole of acetate (8 mol e⁻/mol Ac) and H₂ (2 mol e⁻/mol H₂)</td>
<td>mol e⁻/mol substrate</td>
</tr>
<tr>
<td>$C_{Ac}$</td>
<td>Coulombs obtained from the oxidation of the initially added acetate</td>
<td>C</td>
</tr>
<tr>
<td>$C_{H2}$</td>
<td>Coulombs consumed in the production of H₂ converted to CH₄</td>
<td>C</td>
</tr>
<tr>
<td>$C_{H2}′$</td>
<td>Coulombs consumed in the production of H₂ converted to CH₄ (without considering hydrogenotrophic methanogens growth)</td>
<td>C</td>
</tr>
<tr>
<td>$C_{i}$</td>
<td>Coulombs recovered as current intensity</td>
<td>C</td>
</tr>
<tr>
<td>$C_{Ac}$</td>
<td>Coulombs consumed in the production of H₂ converted to acetate by homoacetogens</td>
<td>C</td>
</tr>
<tr>
<td>$C_{i}′$</td>
<td>Coulombs obtained from the oxidation of acetate produced by homoacetogens</td>
<td>C</td>
</tr>
<tr>
<td>$C_{H2}$</td>
<td>Coulombs consumed in the production of the measured H₂</td>
<td>C</td>
</tr>
<tr>
<td>$C_{H2,L}$</td>
<td>H₂ losses due to leakage</td>
<td>C</td>
</tr>
<tr>
<td>$C_{H2,j}$</td>
<td>Coulombs obtained from the oxidation of H₂</td>
<td>C</td>
</tr>
<tr>
<td>$C_{BA}$</td>
<td>Acetate consumed for ARB growth in terms of coulombs</td>
<td>C</td>
</tr>
<tr>
<td>$C_{MET}$</td>
<td>H₂ consumed for homoacetogens growth in terms of coulombs</td>
<td>C</td>
</tr>
<tr>
<td>$CE$</td>
<td>Coulombic efficiency</td>
<td>–</td>
</tr>
<tr>
<td>$CE_{A1}$</td>
<td>Coulombic efficiency in experiment A1 (no H₂-recycling)</td>
<td>–</td>
</tr>
<tr>
<td>$\Delta C$</td>
<td>Acetate concentration change over $t_F$ and $t_0$</td>
<td>g Ac/L</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant (96485 C/mol e⁻)</td>
<td>C/mol e⁻</td>
</tr>
<tr>
<td>$j_{CI,Ac}$</td>
<td>Fraction of the current intensity generated due to the oxidation of the external acetate initially added</td>
<td>–</td>
</tr>
<tr>
<td>$j_{CI,H2}$</td>
<td>Fraction of the current intensity generated due to H₂-recycling</td>
<td>–</td>
</tr>
<tr>
<td>$I$</td>
<td>Current intensity</td>
<td>A</td>
</tr>
<tr>
<td>$M$</td>
<td>Molecular weight of acetate (59 g/mol)</td>
<td>g/mol</td>
</tr>
<tr>
<td>$\eta_{H2,F}$</td>
<td>Moles of H₂ at the end of a batch experiment</td>
<td>mol</td>
</tr>
<tr>
<td>$\eta_{H2,C}$</td>
<td>Moles of H₂ converted into CH₄ at the end of a batch experiment</td>
<td>mol</td>
</tr>
<tr>
<td>$I_{CAT}$</td>
<td>Cathodic gas recovery</td>
<td>–</td>
</tr>
<tr>
<td>$t$, $t_0$ and $t_F$</td>
<td>Time/initial and final times of the batch experiments</td>
<td>s</td>
</tr>
<tr>
<td>$V_{i,F}$</td>
<td>Final volume of gas</td>
<td>L</td>
</tr>
<tr>
<td>$V_{H2,F}$</td>
<td>Final volume of H₂</td>
<td>L</td>
</tr>
<tr>
<td>$V_{i,F}$</td>
<td>Final volume of gas i</td>
<td>L</td>
</tr>
<tr>
<td>$V_L$</td>
<td>Volume of liquid in the reactor (1.3 l)</td>
<td>L</td>
</tr>
<tr>
<td>$V_{m}$</td>
<td>Molar gas volume (24.03 L/mol at 20 °C)</td>
<td>L/mol</td>
</tr>
<tr>
<td>$V_{H2,L}$</td>
<td>Volume of the H₂ consumed to produce CH₄</td>
<td>L</td>
</tr>
<tr>
<td>$V_{H2,F}$</td>
<td>Volume of H₂ produced including that consumed to produce CH₄</td>
<td>L</td>
</tr>
<tr>
<td>$X_{i,F}$</td>
<td>Final composition of gas i</td>
<td>–</td>
</tr>
<tr>
<td>$Y_{ARB}$</td>
<td>Biomass/substrate yield for ARB when consuming acetate</td>
<td>mol e⁻ biomass/mol e⁻ substrate</td>
</tr>
<tr>
<td>$Y_{ARB}$</td>
<td>Biomass/substrate yield for ARB when consuming H₂</td>
<td>mol e⁻ biomass/mol e⁻ substrate</td>
</tr>
<tr>
<td>$Y_{HOMO}$</td>
<td>Biomass/substrate yield for homoacetogens when consuming H₂</td>
<td>mol e⁻ biomass/mol e⁻ substrate</td>
</tr>
<tr>
<td>$Y_{MET}$</td>
<td>Biomass/substrate yield for hydrogenotrophic methanogens when consuming H₂</td>
<td>mol e⁻ biomass/mol e⁻ substrate</td>
</tr>
</tbody>
</table>
under conventional operation during the first 20 h of the cycle and remained almost constant during the following 80 h. In contrast, when N₂ was sparged, acetate was consumed in 50 h. The decrease in acetate concentration was not related to stripping: Fig. 4B shows that when the cell was disconnected and sparged with N₂ (A2), acetate concentration did not decrease but slightly increased, probably as a result of water evaporation. Finally, Fig. 4C presents the bag composition and shows that the H₂ volume increased, reached a maximum (100 mL) and then decreased. CH₄ concentration was scarce indicating that H₂ consumption was not addressed to methanogenesis.

On the one hand, the CE under conventional operation was, as expected, much higher than 100% (463%). However, when N₂ was sparged, CE decreased to 90.4% (Table 3), thus only the 9.6% of the acetate was consumed for the growth of the biomass (Yᵦᵢₑ). Therefore, CEₐ₁ (i.e. the real CE excluding the H₂-recycling effect) was 90.4%. On the other hand, fᵣₑ was around 4%. The coulombs generated from acetate oxidation according to the experimental acetate measurements, Cᵦₑ, were 1555 C, whereas the coulombs recovered as current intensity, Cᵦᵢ, were 7203 C and the coulombs consumed in H₂ production, Cᵦᵢₑ, 292 C. For Yᵦᵢₑ and Yᵦᵢₑ it was assumed a value of 0.1 mol e⁻/biomass/mol e⁻/substrate, i.e. a value similar to that estimated for ARB when consuming acetate.

Substituting the values of Cᵦₑ, Cᵦᵢₑ, Cᵦᵢₑ, Cᵦᵢₑ, Yᵦᵢₑ and Yᵦᵢₑ in equations (4) and (5) and (7)–(10) it was obtained that:

\[
\begin{align*}
5648 &= Cᵢ₋ + Cᵦᵢₑ - Cᵦᵢₑ - Cᵦᵢₑ \\
6911 &= Cᵦᵡ + Cᵦᵢₑ \\
Cᵦᵢₑ &= 149.18 + 0.096 Cᵦᵡ \\
Cᵦᵢₑ &= Cᵦᵡ - Cᵦᵡ \\
Cᵦᵢₑ &= 0.10 Cᵦᵡ \\
Cᵦᵢₑ &= 0.10 Cᵦᵡ
\end{align*}
\]

The equation system (eqs (19)–(24)) solution is summarized in Table 3 (conventional operation). The fraction of H₂ recycled by homoacetogens, calculated as Cᵦᵡ/(Cᵦᵡ + Cᵦᵡₑ) was 71%, whereas the fraction of H₂ recycled by the direct oxidation of H₂ by ARB, calculated as Cᵦᵡₑ/(Cᵦᵡ + Cᵦᵡₑ), was 29%. Moreover, coulombic losses due to biomass growth were mainly caused by the consumption of acetate by ARB (Cᵦᵢₑ) and the consumption of H₂ by homoacetogens (Cᵦᵢₑ).

fᵢᵢᵢₑ and fᵢᵢᵢₑ were 19.5% and 80.5% respectively (equations (16) and (17)), showing that the effect of H₂-recycling can be far from negligible (e.g. in our system, 80.5% of the current intensity was generated due to H₂-recycling). Moreover, the recycled H₂ in terms of coulombs (Cᵦᵡ + Cᵦᵡₑ) was in just five days around 1.7 times the amount of coulombs that could be generated if all the acetate externally added had been consumed.

3.5. Experimental study: presence of methanogens

At week 9 of operation, batch experiments suggested growth of methanogens even though there was a BES concentration of 50 mM. It was increased to 90 and subsequently to 120 mM
and, surprisingly, CH4 formation was detected even at those high concentrations. Our results suggest that methanogens grew in the MEC even at higher BES concentrations, either as a result of a too thick biofilm preventing BES to penetrate inside or as a result of a development of BES resistance by methanogens [21].

Fig. 5 shows the evolution of the methanogenic activity during the cell monitoring performed at different weeks of operation. At weeks 9–10, the ratio H2/(H2+CH4) only started to decrease (i.e. CH4 was formed) approximately 70 h after the renewal of the medium. At week 16, H2/(H2+CH4) decreased to 35% in just 45 h. BES concentration was increased to 120 mM at week 19 and although methanogenic activity was reduced, it was far from suppressed. At week 22 of operation, BES concentration was decreased to 50 mM to obtain results comparable to the literature. Under these operational conditions, most of the H2 produced was converted to CH4 at the end of the monitoring, as shown in Fig. 5 for week 34. Thus, it was observed that BES may not be an adequate long term solution for methanogenic inhibition when H2 is widely available (i.e. batch conditions with high retention time).

Fig. 6 shows an example of the monitoring of a cycle (week 34) where methanogenic activity was significant. As it can be observed the cycle lasted approximately 50 h, during which acetate concentration was decreasing (Fig. 6B). Regarding gas production, H2 reached a maximum volume between hours 3 and 4 of monitoring and then it started decreasing. In contrast, CH4 production was increasing during all the cycle.

The CE of the cell was 74.5%, whereas the rCAT if only comparing the coulombs recovered as H2 to those recovered for methanogenic inhibition when H2 is widely available (i.e. batch conditions with high retention time).

Table 3 – Summary of the electron equivalent balances during a cycle with H2-recycling.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conventional operation</th>
<th>With N2 sparging</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>463%</td>
<td>90.4%</td>
</tr>
<tr>
<td>rCAT</td>
<td>4%</td>
<td>–</td>
</tr>
<tr>
<td>CCl</td>
<td>7203 C</td>
<td>2989 C</td>
</tr>
<tr>
<td>CAc</td>
<td>1555 C</td>
<td>3306 C</td>
</tr>
<tr>
<td>CH2</td>
<td>292 C</td>
<td>–</td>
</tr>
<tr>
<td>CH4</td>
<td>0 C</td>
<td>–</td>
</tr>
<tr>
<td>C12</td>
<td>4893 C</td>
<td>0 C</td>
</tr>
<tr>
<td>C10</td>
<td>4403 C</td>
<td>0 C</td>
</tr>
<tr>
<td>C11,12</td>
<td>2018 C</td>
<td>0 C</td>
</tr>
<tr>
<td>C13,14</td>
<td>572 C</td>
<td>317 C</td>
</tr>
<tr>
<td>C13,14MO</td>
<td>489 C</td>
<td>0 C</td>
</tr>
<tr>
<td>C13,14H</td>
<td>202 C</td>
<td>0 C</td>
</tr>
<tr>
<td>fCl,Ac</td>
<td>19.50%</td>
<td>100%</td>
</tr>
<tr>
<td>fCl,CH4</td>
<td>80.50%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Fig. 5 – Methanogenic activity vs time represented as the ratio H2/H2 + CH4 at different weeks of operation. Week 9 (○), week 10 (●), week 16 (△), week 19 (▲) and week 34 (▼) of operation. Concentration of BES: 90 mM (solid), 120 mM (dashed) and 50 mM (dash-dotted).

Fig. 6 – Monitoring of the MEC with the presence of methanogens (A) Current density, (B) Acetate concentration and (C) Gas production: H2 (●) and CH4 (▼). Note the different scales in (C).
as current intensity was 0. A much more realistic $r_{\text{CAT}}$ value of 94.5% was calculated by computing CH4 into the balance, assuming that all CH4 produced came from H2 [22] and transforming volume of CH4 into volume of H2 by considering a $Y_{\text{H2}}^{\text{MET}}$ of 0.1 mol e−/biomass/mol e− substrate (equations (14) and (18)). Acetate-driven methanogenesis could be discarded since it would have resulted in a much lower CE. These results show that when methanogenesis became important, H2-recycling, if still occurring, lost importance since only the 5.5% of the coulombs recovered as current intensity were not subsequently recovered as H2 or CH4.

As previously stated, the electron equivalent balances can also be used to describe the behaviour of the cell under methanogenesis conditions. In the presented case, the calculated CE suggested that H2-recycling was not occurring, thus $r_{\text{CAT}}$ and $r_{\text{H2},z}$ could be neglected. Therefore, the previous system of equations (equations (4) and (5) and (7)–(10)) could be reduced to only three linear equations:

$$C_{\text{Cl}} = C_{\text{Ac}} - C_{\text{ABB}}^{\text{Ac}}$$

(25)

$$C_{\text{Cl}} = C_{\text{H2}} + C_{\text{CH4}}$$

(26)

$$C_{\text{ABB}}^{\text{Ac}} = \frac{100 - \text{CE}}{100} C_{\text{Ac}}$$

(27)

Note that $C_{\text{ABB}}^{\text{Ac}}$ was replaced by CE in equation (27) since CE did not need to be corrected by H2-recycling. According to the measurements/calculations, $C_{\text{Ac}}$ was 3378 C, $C_{\text{Cl}}$ was 2518 C, $C_{\text{H2}}$ was 0 C and $C_{\text{CH4}}$ was 2379 C. Substituting these values into equations (25)–(27) it was obtained:

$$-860 = -C_{\text{ABB}}^{\text{Ac}}$$

(28)

$$2518 = 2379$$

(29)

$$C_{\text{ABB}}^{\text{Ac}} = \frac{100 - \text{CE}}{100} 3378$$

(30)

As it can be observed, to solve the system $C_{\text{H2},-1}$ had to be included in equation (29) as follows:

$$2518 = 2379 + C_{\text{H2},-1}$$

(31)

However, as deduced from equation (31), the value of $C_{\text{H2},-1}$ was very low and can be assumed as experimental error. Table 4 summarizes the results of the CE, $r_{\text{H2}}$, and electron equivalent balances calculations. The use of electron equivalent balances gives similar information to that provided by CE and $r_{\text{CAT}}$, but returns the values of $C_{\text{ABB}}^{\text{Ac}}$ and $C_{\text{H2},-1}$ in terms of coulombs.

The results so far suggest that H2-recycling took place when the methanogenic activity was not important. Moreover, the CE evolution showed that CE was higher than 100% when methanogens were not dominant. CE decrease to values around 75% was proportional to the methanogenic activity increase. Results could also suggest that CE was decreasing as a consequence of acetate consumption by methanogens. However, this was ruled out taking into account results in the literature and our own experimental results.

Thus, if working with single-chamber MECs, the most feasible strategy to avoid H2 scavengers would be preventing H2 to be available for the microorganisms. Some options would be the use of membranes or using reactors with architectures for a fast H2 separation in order to make H2 unavailable for the microorganisms [11]. On the other hand, other possible strategies based on the selective inhibition of methanogens would not be useful in a system with these characteristics, since H2-recycling would not be avoided.

### 4. Conclusions

In membrane-less single-chamber MEC, the presence of H2 scavengers is a significant hurdle in view of its real application. Under these conditions, the classical indexes CE and $r_{\text{CAT}}$ calculated to estimate its performance are no longer valid.

When methanogens are present, $r_{\text{CAT}}$ should be calculated estimating the amount of H2 converted to CH4.

When methanogens are selectively inhibited, H2-recycling (due to homoacetogenic bacteria or due to direct H2 oxidation) is very likely to occur, causing large deviations in the estimated CE and $r_{\text{CAT}}$ values. A different approach based on electron equivalent balances is presented in this work which, through a better understanding of the process occurring in the cell, results in the calculation of two new parameters, $f_{\text{Cl},\text{Ac}}$ and $f_{\text{Cl},\text{H2}}$, which are much more realistic indicators of the real cell performance.

Two experimental studies under different scenarios (proliferation of homoacetogens or methanogens) were presented. The proposed approach based on balances was successfully applied and under H2-recycling conditions the estimation of the MEC performance was much more accurate.

Moreover, electron balances showed that H2-recycling could be an issue as important as CH4 generation, since the H2-acetate loop increases the operating costs and makes infeasible the production of H2 in MECs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijhydene.2013.10.034.

REFERENCES