



Efficiency parameters that effectively correspond with hydrogen production from VFAs in microbial electrolysis cells

Parámetros de eficiencia que corresponden efectivamente a la producción de hidrógeno a partir de AGVs en celdas de electrólisis microbianas

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Abstract

Microbial electrolysis cells (MECs) are hybrid systems that include characteristics of bioreactors and electrochemical cells. Primary parameters, such as substrate removal, current density and hydrogen production, and secondary parameters, such as coulombic efficiency, cathodic efficiency and hydrogen yield, determine the performance of MECs. The present work aimed to assess primary and secondary parameters in an MEC fed volatile fatty acids (VFAs) to determine those that most reliably describe the MEC performance in a model setup for hydrogen production. MECs were operated at 0.6 V and fed acetic, propionic and butyric acid mixtures in successive feeding cycles. The main performance parameters were chemical oxygen demand removal COD ($84.7 \pm 0.5 \%$), current density ($378 \pm 7 \text{ mA m}^{-2}$) and hydrogen production ($267 \text{ mL L}^{-1} \text{ d}^{-1}$), which resulted in repeatable and more reliable efficiency parameters when MECs were fed acetate than when they were fed VFA mixtures. Both the current density and hydrogen production curves showed similar inflection points, thus giving accuracy to the cathodic efficiency determination (162.1% - 169.6%). Hydrogen yield was not a reliable parameter with the three-VFA mixture since hydrogen production and COD removal curves showed no correlation. These findings indicate that MEC assessment should be verified via the correspondence between primary and secondary parameters.

Keywords: cathodic efficiency, coulombic efficiency, hydrogen yield, MEC, reliability.

Resumen

Las celdas de electrólisis microbianas (CEMs) son sistemas híbridos que incluyen características de biorreactores y de celdas electroquímicas. Los parámetros primarios como remoción de sustrato, densidad de corriente y producción de hidrógeno, así como los parámetros secundarios, tal como eficiencia coulombica, eficiencia catódica y rendimiento de hidrógeno, determinan el desempeño de las CEMs. El presente trabajo se evaluaron parámetros primarios y secundarios en una CEM alimentada con ácidos grasos volátiles (AGVs) para determinar aquellos parámetros confiables en la medición del desempeño de CEMs para producción de hidrógeno. Las CEMs operaron a 0.6 V, fueron alimentadas con mezclas de ácido acético, propiónico, butírico en ciclos. Los parámetros remoción de demanda química de oxígeno DQO ($84.7 \pm 0.5 \%$), densidad de corriente ($378 \pm 7 \text{ mA m}^{-2}$) y producción de hidrógeno ($267 \text{ mL L}^{-1} \text{ d}^{-1}$) llevaron a parámetros de eficiencia repetibles y más confiables cuando las CEMs fueron alimentadas con acetato que cuando fueron alimentadas con mezclas de AGVs. La densidad de corriente y la producción de hidrógeno mostraron curvas con puntos de inflexión similares, dando precisión a la determinación de eficiencia catódica (162.1% - 169.6%). El rendimiento de hidrógeno no fue un parámetro confiable con la mezcla de tres AGVs ya que la producción de hidrógeno y las curvas de remoción de DQO no mostraron correlación. Esos hallazgos indican que la evaluación de CEMs debería ser verificada a través de la correspondencia entre parámetros primarios y secundarios.

Palabras clave: eficiencia catódica, eficiencia coulombica, rendimiento de hidrógeno, CEM, confiabilidad.

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1 Introduction

During dark fermentation, the metabolic pathways allow a maximum of one-third of the substrate to be transformed to hydrogen, and the remaining substrate results in fermentation products, such as volatile fatty acids (VFAs) and solvents (Elbeshbishy *et al.*, 2017). Consequently, VFAs represent a potential source for recovering energy in serial bioprocesses. This strategy has been previously explored for the evaluation of dark fermentation effluents and VFA mixtures in bioelectrochemical systems such as microbial fuel cells (MFCs) to produce an electrical current (Freguia *et al.*, 2010) and microbial electrolysis cells (MECs) to produce hydrogen (Lalauette *et al.*, 2009) because it represents a way of valorizing waste and obtaining an additional energy benefit.

Major challenges to coupling fermentation with MECs are the high organic matter content (García-Amador *et al.*, 2019) and the varied composition of the VFA mixtures that result from fermentations and may be fed to MECs. Studies on actual fermentation effluents in MECs show very dissimilar values in hydrogen production. For instance, a rate of $0.0680 \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ d}^{-1}$ was obtained from fermented activated sludge (Lu *et al.*, 2009), while $3.43 \text{ m}^3 \text{ H}_2 \text{ m}^{-3} \text{ d}^{-1}$ was harvested from corn stalk fermentation effluents (Li *et al.*, 2014). Average performance has been reported for fermentation effluents (Chookaew *et al.*, 2014; Lu *et al.*, 2009; Rivera *et al.*, 2015), swine wastewater (Wagner *et al.*, 2009), food waste (Sasaki *et al.*, 2012), and effluents from an anaerobic reactor (Wu *et al.*, 2013).

Research using VFA synthetic mixtures has focused on hydrogen production and the effect of the applied voltage (Escapa *et al.*, 2013; Xu *et al.*, 2013), the influence of the inoculum source (Xu *et al.*, 2013), and the organic load rate (Escapa *et al.*, 2013). The consumption of VFAs has also been investigated in microbial fuel cells during sulfate reduction (Gonzalez-Paz *et al.*, 2020).

The content or removal of individual VFAs when reported shows divergent performance. The VFA removal rate has been reported to be higher for acetic acid than butyric acid and higher than propionic acid (Yang *et al.*, 2015). Other authors observed that the removal percentage was higher for acetic acid than propionic acid and higher than butyric acid, but the removal percentages for acetic and propionic acids were inverted when the medium was diluted (Liu *et*

al., 2012). Additional research showed that the total amount of consumed VFAs followed the order acetic acid followed by propionic acid and finally butyric acid, but the removal rate was faster for acetic acid than butyric acid and faster than propionic acid (Xu *et al.*, 2013). These results suggest, at first sight, that the VFA removal kinetics may differ between MEC systems; thus, this subject needs more investigation before hydrogen production from actual fermentation effluents can be achieved.

MEC performance is assessed by using primary parameters, including organic matter removal, current density, and hydrogen production, for a subsequent calculation of secondary parameters, such as coulombic and cathodic efficiency and hydrogen yield (Ivanov *et al.*, 2013; Logan and Rabaey, 2012; Paz-Mireles *et al.*, 2019; Sharma *et al.*, 2014). The calculations to evaluate MEC performance were compiled since 2012 (Logan and Rabaey, 2012), and minor modifications have been made to the subject since then. A review of the parameters characterizing microbial electrochemical systems focused only on current density (Sharma *et al.*, 2014).

In another study, MEC assessment was performed in terms of energy by calculating the energy consumed for wastewater treatment ($W_{EL} = \text{kWh kg-COD}^{-1}$) to estimate the water treatability rate ($r_w = I W_{EL}^{-1}$) (Ivanov *et al.*, 2013).

Recently, a correspondence between the evolution of the accumulated coulombs and the accumulated hydrogen volume was demonstrated. The startup time for hydrogen production was graphically interpolated to the accumulated coulomb curve. It was noted that the amount of coulombs agreed for different MEC operation conditions; therefore, it was considered a new parameter for MEC characterization (Paz-Mireles *et al.*, 2019).

Calculation of secondary efficiency parameters that involve COD removal presents a drawback because both planktonic and sessile microbial activity are responsible for COD removal, but only the microorganisms attached to the electrode participate in direct electron transfer (Dorazco-Delgado *et al.*, 2021; Valdez-Ojeda *et al.*, 2014), i.e., in electrical current production. For this reason, it is necessary to determine the parameters that reliably describe cell performance.

The coulombic efficiency is habitually calculated and reported as the percentage of the experimental electrical charge to the electrical charge that could be

produced from the total COD consumed (Eq. 1)

$$CE\% = \frac{\int Idt}{(z F \Delta COD V_a) / MW_{O_2}} \quad (1)$$

where I is the current, t is the test time, z is the electron number related to the oxidation of organic matter (8 e- for COD), F is Faraday's constant (96485 C mol⁻¹), ΔCOD is the organic matter removed in grams per liter, V_a is the anolyte volume and MW is the molecular weight of the oxygen.

In equation 1, a correlation of the primary parameters I and mass removed g-COD is expected to effectively determine $CE\% = f(I, COD_r)$.

A primary parameter is the theoretical number of moles of hydrogen that can be obtained, which is calculated assuming that all the experimental electrical charge is directed to the formation of hydrogen gas, as indicated by Eq. 2.

$$n_{H_2, I} = \frac{\int Idt}{2F} \quad (2)$$

where the number 2 corresponds to the number of electrons needed to form one hydrogen molecule, as shown by Reaction 1.



The cathodic efficiency is then calculated as the percentage of the number of moles of hydrogen collected relative to the theoretical number of moles of hydrogen from the electrical charge (Eq. 3).

$$n_{cat\%} = \frac{n_{H_2, exp}}{n_{H_2, I}} \times 100 \quad (3)$$

where $n_{H_2, exp}$ is the number of moles of hydrogen measured experimentally.

Hydrogen is a small molecule that can easily escape from the capture system. In addition, in two-chamber MECs, gas exchange can occur through the separation membrane, so it is important to determine the parameters that allow the cells to be evaluated reliably.

A correlation between the primary parameters, mol of hydrogen and electrical current, is expected for cathodic efficiency determination $\eta_{cat\%} = f(H_2, I)$.

A secondary parameter, the hydrogen yield based on the COD consumed, is given by Eq. 4

$$Y = \frac{n_{H_2, exp}}{\Delta COD} \quad (4)$$

Hydrogen yield is a habitual parameter in hydrogen-producing bioprocesses when conventional

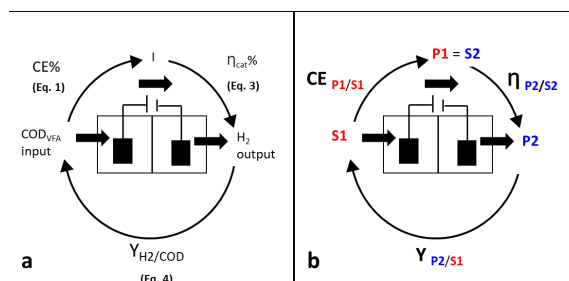


Fig. 1 Schematic representation of a) primary measured parameters (COD, I , H_2) and secondary calculated parameters (CE , η_{cat} , Y); b) Relations of substrates (S) and products (P) used to characterize two-chamber MECs.

reactor tanks are used; therefore, it is also applicable to one-chamber hydrogen-producing MECs. However, if a membrane separates the electrochemical cell into two compartments, the correlation between the primary parameters could not be right forward because of the reactions separated into two reactors (Fig. 1). Therefore, the hydrogen yield in the cathodic chamber as a function of COD mass removal in the anodic chamber should be verified based on the primary parameters.

The validity and reliability of the parameters that determine the performance of MECs has been tacitly accepted but not proven due to the intrinsic variability of biotechnological processes in general, particularly biofilm-based electrochemical processes. For instance, a correlation between current density and volatile solids (suspended biomass) was demonstrated; however, in the same system, no significant difference was found between current and protein from biofilm (attached biomass) (Cercado *et al.*, 2013).

Calculation of secondary parameters without validation of the primary parameters on which they are based could lead to erroneous conclusions both during the investigation and during comparison with other investigations.

Assessment of a two-chamber MEC may be more reliable if the primary parameters of one chamber show a strong correlation for the next calculation of secondary parameters. This work aimed to verify the correspondence of primary parameters in individual anodic and cathodic chambers to determine the accuracy of secondary parameters.

MECs fed VFA mixtures were investigated for COD removal, current density, and hydrogen production as primary parameters. Then, the secondary parameters coulombic efficiency, cathodic efficiency, and hydrogen yield were calculated and

analyzed as a function of the primary parameters. The effect of the correlation between primary parameters and the feed complexity on the reliability of performance parameters was demonstrated.

2 Materials and methods

2.1 Design and operation of the MEC

Two-chamber electrochemical cells with 450 mL of total volume and 300 mL of liquid volume in each chamber were constructed (Fig. 2). The chambers were separated by an anion exchange membrane (3 cm x 3 cm, AMI 7001, Membranes International, Inc., U.S.A.). The anode was constructed from carbon paper (Toray TM, Electrochem, Inc., U.S.A.) with a size of 5 cm x 5 cm. The cathode was prepared from carbon paper with a size of 5 cm x 5 cm with Pt catalyst (0.5 mg cm^{-2}). Titanium wire was utilized for external connections (diameter 0.5 mm, Sigma-Aldrich, Inc., U.S.A.).

Municipal wastewater ($\text{COD } 36.4 \pm 28.6 \text{ g L}^{-1}$) was diluted in a 3:1 ratio with a buffered nutrient solution containing 20 mM sodium acetate at pH 7 (Ruiz *et al.*, 2014). The mixture was used as inoculum in the anodic compartment. Biofilm development was achieved through feeding cycles until the potential was stable in the electrochemical cell operating in MFC mode with an external resistor of 1000Ω . The cathodic compartment was filled with 100 mM phosphate buffer solution (pH 7) supplemented with 50 mM NaCl as the electrolyte support and dissolved oxygen as the electron acceptor.

Before starting the MEC operation, the anodic and cathodic compartments were flushed with nitrogen gas for 10 minutes. A cell voltage of 0.6 V between the anode and the cathode was continuously applied using a power supply (GW Instek, GPS-4303, U.S.A.).

The flowing electrical current was measured through an external resistor and computed via Ohm's law. Voltage and current data were registered using a data acquisition board (NI USB-6008, 8 inputs, 12 bits, 10 Ks/s multifunction I/O) and LabView 7.1 software (National Instruments). The MEC was operated in batch feeding cycles (48 h for each cycle) at room temperature ($20 \pm 2 \text{ }^\circ\text{C}$).

The feeding strategy consisted of testing individual VFAs and two- and three-VFA mixtures, as indicated in Table 1.

Acetic, propionic, and butyric acids in the buffered nutrient solution were fed in batch mode at concentrations of 1500 mg L^{-1} , 250 mg L^{-1} , and 600 mg L^{-1} , respectively. The VFA concentrations were selected to mimic a dark fermentation effluent obtained in our laboratory. The MECs were operated under the same conditions during 6 feeding cycles of 48 h each to obtain repetitions of experimental conditions.

One MEC was operated with acetic acid for 6 feeding cycles (C1-C6), and then, the anolyte was replaced with an acetic-butyric acid mixture, followed by operation for 6 cycles (C7-C12). A second MEC was similarly operated with an acetic-propionic mixture in the first 6 cycles and subsequently with an acetic-propionic-butyric acid mixture for the remaining 6 feeding cycles. The electrodes were retained in the MECs from C1 to C12, while the anolyte was entirely replaced for each feeding cycle.

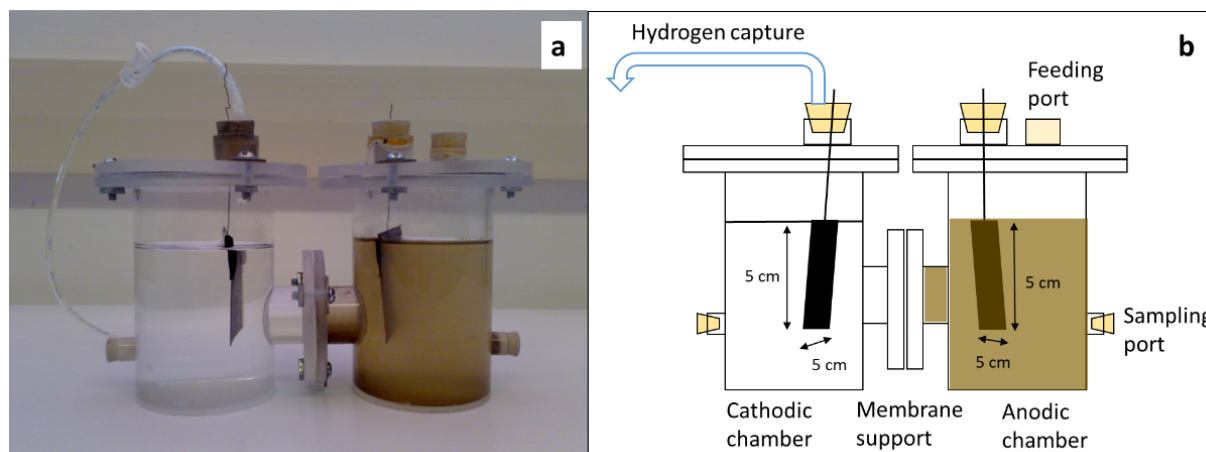


Fig. 2 Two-chamber MEC design. a) Real image; b) Schema of components.

Table 1. VFA mixtures tested in the two-chamber MECs.

MEC	Code	Substrate	Study purpose
1	MEC Ac	Acetic	Control with optimum substrate
1	MEC Ac+Bu	Acetic-Butyric	Effect of the length of the carbon chain
2	MEC Ac-Pro	Acetic-Propionic	Effect of the length of the carbon chain
2	MEC Ac-Pro+Bu	Acetic-Propionic- Butyric	Effect of a complex mixture

2.2 Chemical and electrochemical analyses

The removal of organic matter was measured by the COD using a kit (range 1-1500 mg L⁻¹ Hach, U.S.A.) at the end of each feeding cycle. The removal of VFAs was measured via gas chromatography (GC) in a Varian 3300 GC system equipped with a flame ionization detector (FID) (Ruiz *et al.*, 2014). Samples of 1 mL analyte were taken at the end of the feeding cycles, centrifuged, and filtered through 0.45 μm nitrocellulose filters before analysis. Gas production was monitored by the water displacement method, and the gas composition was determined by GC on an SRI Instruments GC system (SRI 86196, U.S.A.) equipped with a thermal conductivity detector.

3 Results and discussions

3.1 Coulombic efficiency: Correlation of COD (S1) to current density (P1)

The coulombic efficiency was almost constant in MEC-Ac, with an average of 0.8 ± 0.04 %. Then, this parameter fluctuated in MEC-Ac+Bu with a rising trend showing an extreme value of 2.0 % (C7). In the second MEC, the coulombic efficiency was lower than that of the former MEC, but the values obtained were in the same range of magnitude for all feeding cycles (0.19 ± 0.08 %), except for C10 (Fig. 3).

Because a marked difference in the coulombic efficiency was not observed when the substrate was switched in MEC-1 or MEC-2, details of the primary parameters, COD removal and current density, were checked for each cycle (Fig. 4).

A good Pearson's correlation between the removed COD and current density was observed in MEC-Ac ($r = 0.56$); nevertheless, the rest of the data showed no correspondence or a clear trend (Fig. 4a). In MEC-Ac, the highest COD removal was 4.3 g (84.7 ± 0.5 %), which corresponded to the highest current density of 378 ± 7 mA m⁻². After the addition of butyric acid,

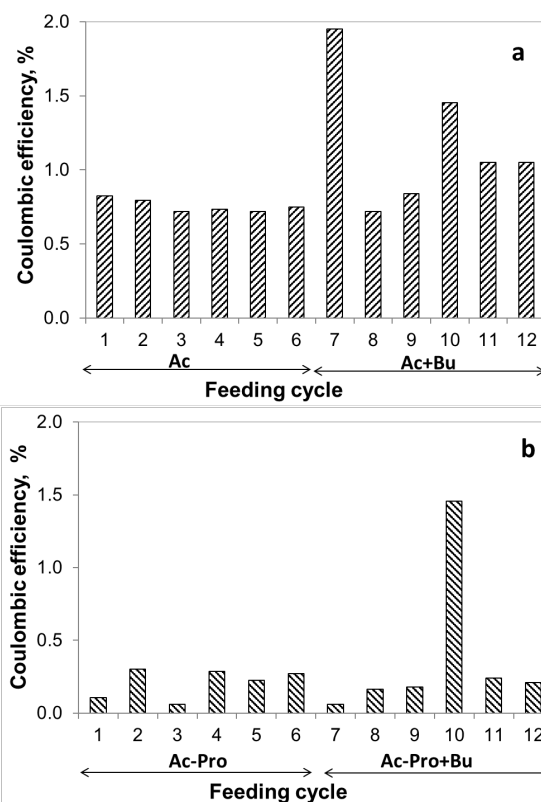


Fig. 3 Coulombic efficiency percentage. a) MEC-1 fed acetic and acetic+butyric mixture; b) MEC-2 fed acetic-propionic and acetic-propionic+butyric mixtures.

COD removal was irregular, but the current density showed a clear diminution of 57 %. The MEC Ac-Pro showed a high dispersion in COD removal, while the corresponding current density was more regular, reaching 26.7 ± 5 mA m⁻². After the addition of butyric acid, the COD remained irregular, but the current density almost doubled, increasing by 84 % (Fig. 4b).

The current observed in the tests was a result of microbial electroactivity associated with their metabolism during nutrient consumption.

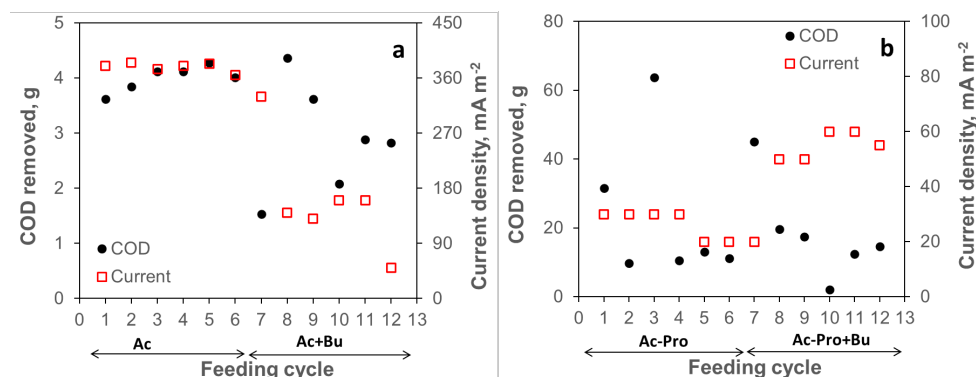


Fig. 4 Chemical oxygen demand removed (COD-mass) associated with current density. a) MEC-1 fed acetic and acetic+butyric mixture; b) MEC-2 fed acetic-propionic and acetic-propionic+ butyric mixtures.

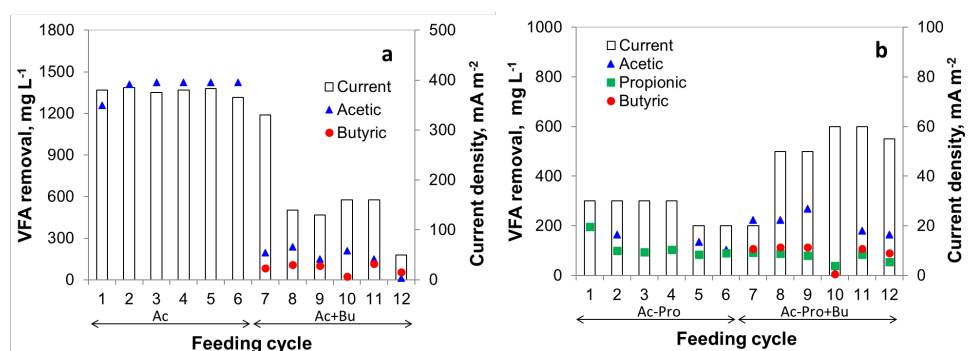


Fig. 5 Volatile fatty acid removal associated with current density. a) MEC-1 fed acetic and acetic+butyric mixture; b) MEC-2 fed acetic-propionic and acetic-propionic+butyric mixtures.

This dual microbial activity was stimulated at a fixed continuous potential applied to the MEC. The total current observed was due to the external stimulus of microorganisms and not to the cell potential developed as a fuel cell (MFC). Although the bioanodes were formed in MFC mode, the developed cell potential was lower (0.23 V) than the potential applied with the power supply (0.6 V); therefore, the spontaneously developed potential was covered and did not contribute to the recorded current density.

The irregular COD removal was attributed to the more complex VFA mixture at higher concentrations than when using only acetate (Table 1). Moreover, low biodegradation of pollutants with high-strength wastewaters has been observed previously in MECs (Rivera *et al.*, 2015), and accumulation of VFAs is a common indicator of inhibition in anaerobic processes (Serrano-Meza *et al.*, 2020).

Conversely, the increase in current density with the more complex substrate Ac-Pro+Bu opposed that expected since a diminution in performance has been

reported when MFCs for electricity production are fed complex substrates (Rosales-Sierra *et al.*, 2017; Sevda *et al.*, 2013). Therefore, it was hypothesized that the electroactive microbial community consumed the actual composition of VFAs, in which acetate predominated due to interconversions of VFAs. This hypothesis was investigated on the basis of VFA removal in each cycle.

Acetic acid removal was the highest among the three acids, reaching 1395 ± 66 mg L⁻¹ in MEC-Ac, but the addition of butyric acid provoked a sort of inhibition removal to 160 ± 80 mg L⁻¹ (Fig. 5a). The acetic acid removal in the presence of propionic acid was 128 ± 29 mg L⁻¹, and unexpectedly, after butyric acid addition, the acetic acid removal increased to 180 ± 90 mg L⁻¹ (Fig. 5b).

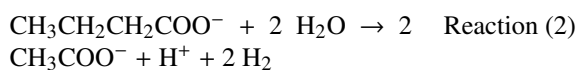
The propionic acid concentration was almost constant in MEC-Ac-Pro and MEC Ac-Pro+Bu, and this observation was consistent with previous reports of propionic acid as a recalcitrant substrate in MECs (Escapa *et al.*, 2013). Moreover, the total removal

of each acid in MEC-A-P+B followed the order acetic followed by butyric and finally propionic, in agreement with previous reports (Teng *et al.*, 2010; Yang *et al.*, 2015).

Regarding the relationship between VFA removal and current production, in MEC-1, the addition of butyric acid decreased acetic acid removal by 8.7 times, and the current density decreased by 2.3 times. Conversely, in MEC-2, the addition of butyric acid increased acetic acid removal by 1.4 times and the current density by 1.8 times.

These calculations verified the direct relation between acetic acid removal and current production, but the change ratio was dissimilar between acetic acid consumption and current production.

This behavior was likely due to interconversions of VFAs; for instance, butyric acid is converted to acetic acid, as given in Reaction 2, thus underestimating acetic acid consumption.



Interconversions of VFAs have been reported previously for the acclimatization of bioanodes, and the adaptation of microbial communities depends on the order in which VFAs are supplied to the anodic compartment. In an MFC, the switch of acetic acid to a two-VFA mixture reduced the performance, but the addition of a third VFA to the MFC operating with a two-VFA mixture improved the performance (Rosales-Sierra *et al.*, 2017). A similar adaptation process was observed in the present study after the addition of butyric acid to the acetic-propionic acid mixture.

All these results indicate that the coulombic efficiency is more reliable for MECs operating with a sole substrate than with a mixture. COD removal in the anodic compartment corresponds to consumption by both suspended and fixed microorganisms and not only for the microorganisms attached to the electrode; thus, any correlation between COD removal and current density can be biased. Moreover, COD removal also refers to a mixture of substrates, and the effect of each substrate on current production may differ or even oppose each other. At least for the above reasons, it is desirable to determine that the primary parameters are reliable for calculating the secondary efficiency parameters.

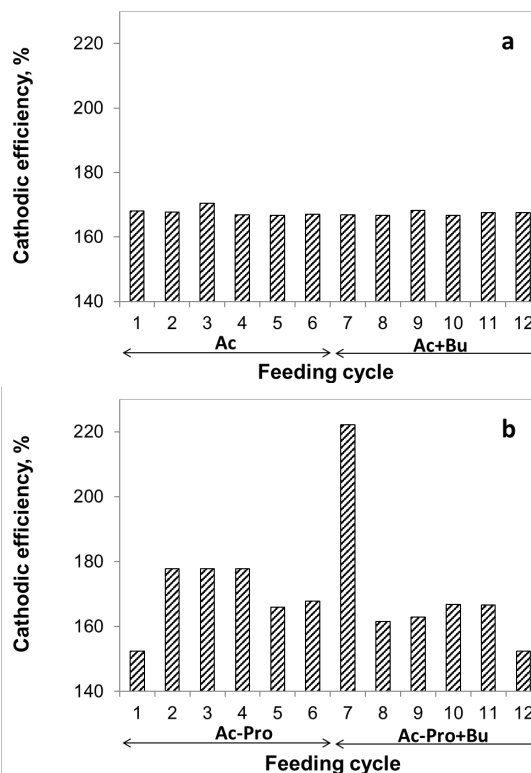


Fig. 6 Cathodic efficiency percentage. a) MEC-1 fed acetic and acetic+butyric mixture; b) MEC-2 fed acetic-propionic and acetic-propionic+butyric mixtures.

3.2 Cathodic efficiency: Correlation of current density (S_2) to hydrogen production (P_2)

The cathodic efficiency was calculated as the ratio of the experimental moles of hydrogen to the theoretical moles of hydrogen based on the current production (Equation 3). The cathodic efficiency percentage was $167.8 \pm 1.4 \%$ in MEC-Ac and very similar in MEC-Ac+Bu ($167.3 \pm 0.6 \%$). The cathodic efficiency in MEC-Ac-Pro was $169.9 \pm 10.1 \%$ and then decreased in MEC-Ac-Pro+Bu up to $162.1 \pm 5.8 \%$ (excluding the extreme data from cycle 7) (Fig. 6).

The cathodic efficiency was similar in MEC-1 and did not allow elucidation of variations due to the feed composition. Therefore, the efficiency percentages were verified through the primary parameters: hydrogen production and current density.

Figures 7a and 7b show that the hydrogen production rate (HPR) ranged from $254 \text{ mL L}^{-1} \text{ d}^{-1}$ to $267 \text{ mL L}^{-1} \text{ d}^{-1}$ for MEC-Ac and MEC-Ac+Bu.

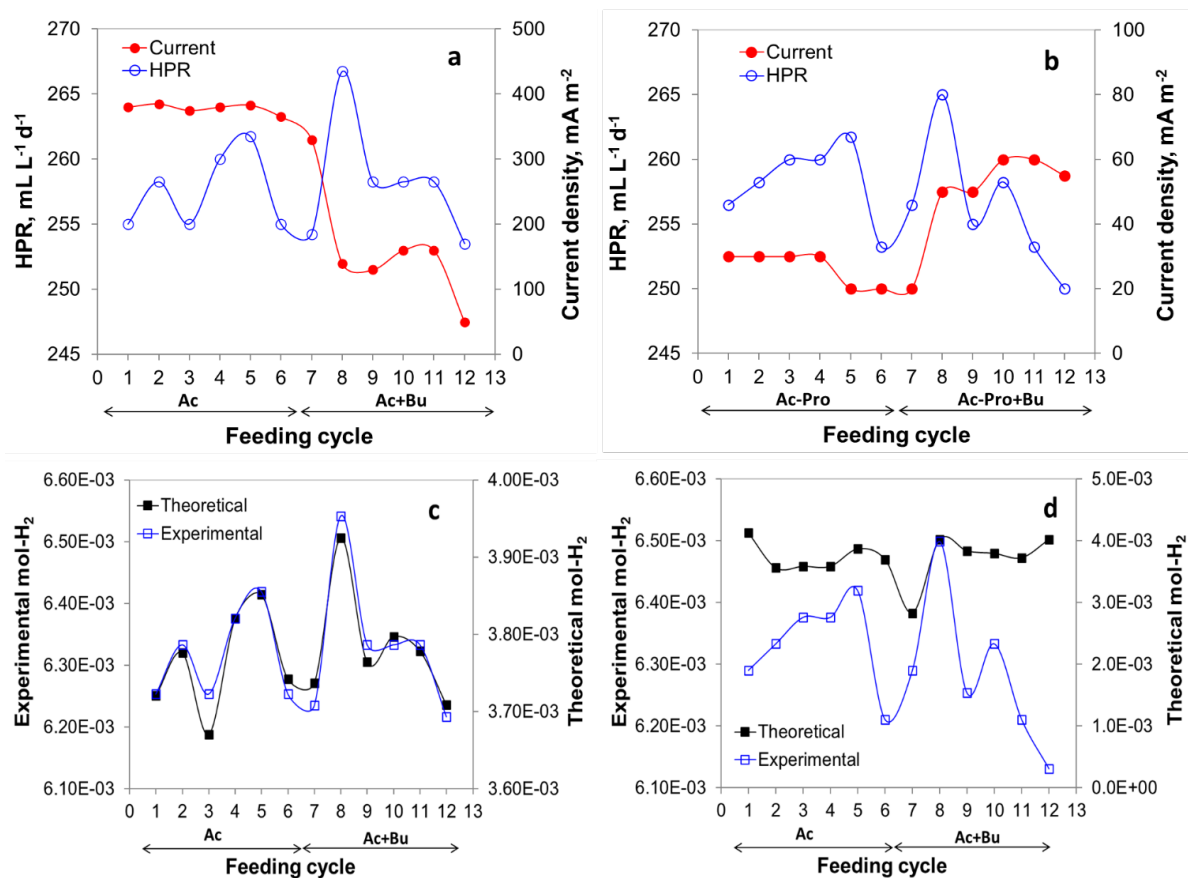


Fig. 7 Curves for hydrogen production rate (HPR), current density and moles of hydrogen as a function of feeding cycles. a) and c) MEC-1 fed acetic and acetic+butyric mixture, b) and d) MEC-2 fed acetic-propionic and acetic-propionic-butyric mixtures.

Two peaks in HPR were observed in cycles C5 (Ac) and C8 (Ac+Bu), corresponding to 157 mL and 160 mL of hydrogen, respectively. HPR for MEC-Ac-Pro and MEC-Ac-Pro+Bu ranged from 250 mL L⁻¹ d⁻¹ to 265 mL L⁻¹ d⁻¹; in MEC-2, hydrogen peaks were also observed in C5 (Ac-Pro) and C8 (Ac-Pro+Bu), with production of 157 mL and 159 mL H₂, respectively.

HPR depends on the electrical charge arriving at the cathode, which is measured as the current density; in consequence, HPR and current density should be closely related. The inflection points for these two parameters showed a good correlation for cycles C1-C6 in MEC-Ac and for C8-C12 in MEC-Ac+Bu. Conversely, only several inflexion points from both curves correlated in MEC-2; in addition, an offset was observed between the curves.

The offset was attributed to the normalization of data to the reactor volume and operation time in the case of HPR calculation and normalization

to electrode area in the case of current density calculation. Therefore, the experimental and theoretical moles of hydrogen were used as equivalent parameters to evaluate the MEC performance.

The curves of moles of hydrogen showed almost identical trends in MEC-1 but continued to diverge at some points for MEC-2 (Fig. 7c and 7d).

Based on the superposition of the experimental and theoretical moles of hydrogen curves, it was concluded that the cathodic efficiency percentage maintained a similar value in MEC-1 even with the switch in the feed composition because electrical charge arrived at the same cathodic system composed of a carbon paper cathode with Pt catalyst in 100 mM phosphate buffer solution. In that sense, the cathodic efficiency enables the comparison of different cathode-catholyte systems rather than the experimental setup in which the same reaction occurs under the same conditions (Reaction 1).

The HPR rate and cathodic efficiency were

reported previously for a similar cathodic system (Zhang and Angeliclaki, 2012). The authors manufactured an MEC with two anodes exposed to wastewater supplemented with acetate and a two-cathode inner chamber containing carbon paper coated with Pt in 10 mM phosphate buffer solution. The HPR in the system ranged from $18 \text{ mL L}^{-1} \text{ d}^{-1}$ to $24 \text{ mL L}^{-1} \text{ d}^{-1}$, and the cathodic efficiency was 93-94 %, which is lower than that obtained in the present work. This difference was attributed to the lower buffer solution concentration (10 mM) compared to that used in the present work (100 mM).

According to the possible comparisons of cathode-catholyte systems, the divergence observed in the curves for MEC-2 could be explained by a malfunctioning of the cathodic compartment. For instance, fouling of the cathode material, pollution of the catholyte, or any hydrogen leakage during gas harvesting. Therefore, the cathodic efficiency calculation for the same conditions in the cathodic compartment makes it possible to verify the installation.

Overall, the findings for cathodic efficiency indicated that current density is a reliable primary parameter to evaluate HPR in two-chamber MECs when they are fed simple substrates. It is opportune then to report cathodic efficiency values along with the primary parameters to verify the reliability of the results and give an extensive overview of the MEC performance.

3.3 Hydrogen yield: Correlation of hydrogen production (P_2) to COD removal (S_1)

Hydrogen yield was calculated as the number of moles of hydrogen collected to the COD consumed in each feeding cycle. The hydrogen yields ($\text{mmol-H}_2 \text{ g}^{-1} \text{ COD}$) were 1.59 ± 0.09 and 2.46 ± 0.94 in MEC-Ac and MEC-Ac+Bu, respectively (Fig. 8a). The yield decreased and was less stable in MEC-Ac-Pro (0.44 ± 0.23) and MEC-Ac-Pro+Bu (0.35 ± 0.12 ; excluding data from C10) (Fig. 8b).

In the previous discussions, a higher reliability for the calculation of performance parameters with acetate as the sole substrate compared to VFA mixtures was demonstrated. This result was verified for hydrogen yield calculation; the inflexion points in the hydrogen production and removed COD curves followed a similar trend in most of the data points for MEC-Ac and MEC-Ac+Bu, indicating a good correlation

between these primary parameters, with Pearson's

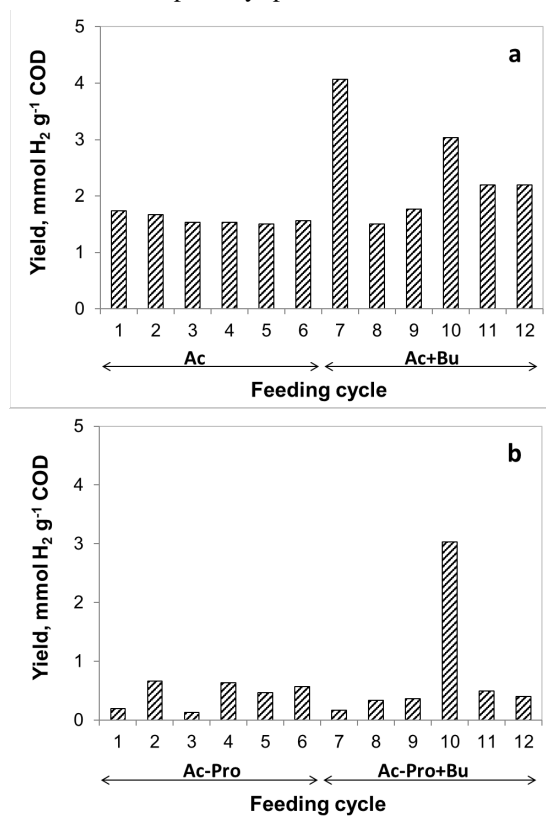


Fig. 8 Hydrogen yield for a) MEC-1 fed acetic and acetic-butyric mixture, b) MEC-2 fed acetic-propionic and acetic-propionic-butyric mixtures.

correlation $r = 0.6$ for MEC-Ac and $r = 0.7$ for MEC-Ac+Bu (Fig. 9a). Conversely, the inflexion points in the hydrogen and COD curves had no correlation in MEC-Ac-Pro and MEC-Ac-Pro+Bu (Fig. 9b).

As stated before, COD removal occurs in the bulk and in the interphase electrode-electrolyte; therefore, part of the COD consumption is not related to electrical current production. Considering this process, the COD mass removal resulted in a coefficient of variation of 30 % for MEC-1 and 80 % for MEC-2, while hydrogen volume had a coefficient of variation of 1.4 % in MEC-1 and 1.6 % in MEC-2, i.e., hydrogen production was a quasi-stable process.

The large difference between the coefficients of variation for COD removal and hydrogen production suggested that although hydrogen was formed from a chain of bioelectrochemical processes that initiated with COD consumption, the relatively stable hydrogen production was due to an additional process.

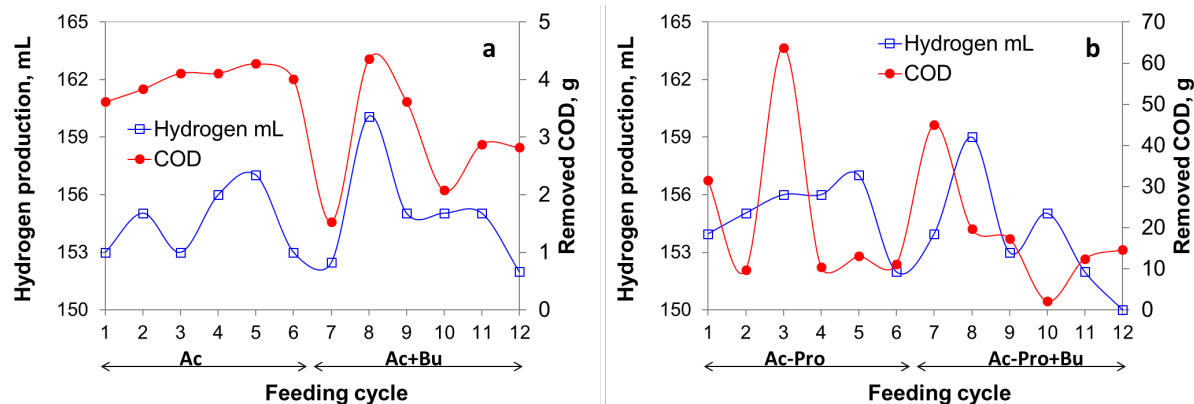


Fig. 9 Hydrogen volume and removed COD curves as a function of feeding cycles. a) MEC-1 fed acetic and acetic+butyric mixture; b) MEC-2 fed acetic-propionic and acetic-propionic+butyric mixtures.

This rationale was supported by the cathodic efficiency values, which were higher than 100%, i.e., more hydrogen gas was experimentally collected than theoretically calculated from the electrical current registered (Fig. 7 c, d).

The source of additional hydrogen could be the anaerobic digestion process occurring in the anodic compartment. Proton exchange membrane (PEM) technology for abiotic fuel cells has a higher technical development than bioelectrochemical technology; a very recent review on the quality control of PEM components reported a series of factors that affect membrane operation (Yuan *et al.*, 2021). The authors signaled that gas crossover is an unavoidable phenomenon in PEMs; moreover, membrane defects, chemical degradation, aging of sealing materials and gas pressure differences in the compartments also contribute to gas crossover leakage.

In the present work, gas pressure differences very likely occurred in the MEC, since biogas (methane, carbon dioxide, and hydrogen) is faster and continuously produced in the anodic compartment than hydrogen gas in the cathodic compartment, as demonstrated previously (Segundo-Aguilar *et al.*, 2021). Therefore, the smallest gas molecules corresponding to hydrogen gas could have crossed the membrane, increasing the net volume harvested and maintaining quasi-stable gas production in the cathodic compartment.

Once a good correlation was determined between the primary parameters (moles of hydrogen and COD consumed) in MEC-1, it was observed that the hydrogen yield tended to increase and stabilize in cycles C7-C-12. This behavior coincided with another secondary parameter, the coulombic efficiency, for

the same cycles; therefore, it could be favorable to increase the number of feeding cycles for further experiments.

Conversely, the lack of a direct correlation between hydrogen produced in the cathodic chamber and COD removed in the anode chamber for complex substrates precludes reliable calculation of yield as a secondary cell efficiency parameter. The possibility of gas cross-leakage across the membrane is latent in all two-chamber cell designs, so it is crucial to first determine the primary parameters that ensure reliability to the estimation of secondary efficiency parameters.

Conclusions

Microbial electrolysis cells are hybrid devices that require evaluation of primary and secondary parameters prior to optimization and technical development toward actual application. In the present work, two-chamber MECs were fed VFA mixtures. The MECs were evaluated for COD and VFA removal, current density production, and hydrogen production rate as primary parameters, as well as coulombic efficiency, cathodic efficiency and hydrogen yield as secondary parameters. The highest COD removal was $84.7 \pm 0.5 \%$, which corresponded to the highest current density of $378 \pm 7 \text{ mA m}^{-2}$ in MEC-Ac. The highest hydrogen production rate was $267 \text{ mL L}^{-1} \text{ d}^{-1}$, which was obtained in MEC-Ac+Bu.

The secondary parameters were more stable and reliable when acetate was fed to MECs instead of VFA mixtures. The coulombic efficiency was influenced by the composition of the mixture and the interconversion

of VFAs in the medium. The cathodic efficiency was a reliable secondary parameter since the electrical current and HPR curves showed a good correlation. In contrast, hydrogen yield was not a reliable parameter for MECs fed two- and three-VFA mixtures.

Based on the analysis of the MEC performance parameters, it is concluded that data on secondary parameters should be accompanied by plots of the corresponding primary parameters to verify its reliability and that the efficiency parameters are more reliable for MECs fed simple substrates.

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