

Methanogenesis in membraneless microbial electrolysis cells

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Abstract Operation of microbial electrolysis cells (MECs) without an ion exchange membrane could help to lower the construction costs while lowering the ohmic cell resistance and improving MEC conversion rates by minimizing the pH gradient between anode and cathode. In this research, we demonstrate that membraneless MECs with plain graphite can be operated for methane production without pH adjustment and that the ohmic cell resistance could be lowered with approximately 50% by removing the cation exchange membrane. As a result, the current production increased from 66 ± 2 to 156 ± 1 A m⁻³ MEC by removing the membrane with an applied voltage of -0.8 V. Methane was the main energetic product despite continuous operation under carbonate-limited and slightly acidified conditions (pH 6.1–6.2). Our results suggest that continuous production of hydrogen in membraneless MECs will be challenging since methane production might not be avoided easily. The electrical energy invested was not always completely recovered under the form of an energy-rich biogas; however, our results indicate that membraneless MECs might be a viable polishing step for the treatment of the effluent of anaerobic digesters as methane was produced under low organic loading conditions and at room temperature.

Keywords Phosphate buffer · Biocatalyzed electrolysis · Overpotentials · Biocatalysts · Ohmic resistance · Microbial fuel cell

Introduction

Microbial electrolysis cells are bio-electrochemical systems (BESs) where a voltage is applied to the cell to drive the (bio)electrochemical reactions (Clauwaert et al. 2008a). In a microbial electrolysis cell (MEC), biocatalyzed oxidation of organic compounds in the anode chamber is typically combined with chemical evolution of hydrogen in the cathode chamber (Liu et al. 2005; Rozendal et al. 2006b). So, the current production is directly proportional to the hydrogen gas produced in anaerobic conditions (Rozendal et al. 2006b). Recently, the possibility of biological hydrogen production in a poised cathode has been described after an anodic acclimatization period with acetate and hydrogen gas (Rozendal et al. 2008).

Separation between the anode and the cathode chambers of a MEC is accomplished with an ion-selective membrane. Ion-selective membranes are used to obtain cathodic hydrogen gas that is as pure as possible. However, ion-selective membranes give rise to a higher ohmic voltage loss in the cell and a pH gradient over the membrane, resulting in a lower current production for a given applied cell voltage (Call and Logan 2008). Removing a part of the membrane helped to minimize the pH gradient between anode and cathode (Clauwaert et al. 2008b). Though the goal of membraneless MECs is to produce hydrogen gas, Call and Logan (2008) reported the production of methane as an undesired side product. Methane production persisted in a membraneless MEC (28 mL volume) despite exposure to the air for 30 to 45 min between batch-feeding cycles

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(Call and Logan 2008). Other strategies for inhibition of methanogenesis in membraneless MECs have been suggested: (1) operation under low pH conditions, (2) washout of methanogens by a short hydraulic retention time, or (3) low carbonate levels (Call and Logan 2008; Rozendal et al. 2008). Since BES favor biofilm-associated biomass, preventing methanogenesis is challenging as biofilms might protect the methanogens from high oxygen concentrations and washout. At the cathode, where local alkalization occurs, creating low pH conditions will be also difficult. Additionally, carbon dioxide, continuously produced as a result of anodic oxidation of organic compounds, may cross over to the cathode, making operation under carbon-limited conditions in a membraneless MEC rather unlikely.

Methane is an energetic compound, and as a product of anaerobic digestion, it is widely used to produce electricity in wastewater treatment facilities (Pham et al. 2006). Though methane contains less energy than hydrogen per unit of mass, a MEC without ion exchange membrane will have lower capital costs. Methane production in a MEC may also be more robust than hydrogen production. Hence, a MEC installed after a conventional anaerobic digester to remove the residual organics present in the effluent of the digester could be a practical polishing step. Indeed, effluents from completely stirred tank digesters treating wastes such as biosolids or manure typically have effluents still containing 0.5 to a few grams of residual volatile fatty acids (van Lier et al. 2001). Interestingly, BES are typically effective at such low substrate concentrations and can also work at ambient temperatures (Pham et al. 2006).

Sulfide is a threat to the long-term performance of a membraneless MEC intended for hydrogen production. Sulfate is often reduced in the anode compartment, and sulfide can end up in the cathode compartment (Rabaey et al. 2006). In the cathode, sulfide can lower hydrogen gas purity as well as the activity of the platinum catalyst often used in MECs (Niessen et al. 2004).

The goal of this research was to determine the effectiveness of a membraneless MEC with high specific surface area granular graphite electrodes (in the order of $6 \times 10^6 \text{ m}^2 \text{ m}^{-3}$; Freguia et al. 2007) for methane production. As has been suggested for microbial fuel cells (MFCs), activation overpotentials may be lowered by increasing the specific surface area of the cathode with granular graphite (Freguia et al. 2007), making chemical catalysts that are fouled by sulfide not necessary. We constructed MECs with and without a cation exchange membrane. The viability of these configurations was evaluated based on the operational performances and polarization behavior of a MEC. The methane production was monitored in the presence and the absence of an applied cell voltage.

Materials and methods

Microbial electrolysis cell construction and operation

The MECs were made of two plexiglass frames ($8 \times 8 \times 2 \text{ cm}^3$ per frame; 0.256 L MEC). The anodic and cathodic frames were filled with graphite granules (type 00514, diameter between 1.5 and 5 mm, Le Carbone, Belgium) and connected to the external circuitry with a graphite rod (5 mm diameter, Morgan, Belgium; Figure S1 of the Electronic Supplementary Material). A cation exchange membrane (CEM; Ultrex CMI7000, Membranes International) was inserted between the anodic and cathodic frames for the tests indicated by an asterisk (*). In the MEC tests completed without a membrane, a nonwoven cloth (Liplisse 3, Libeltex, Belgium) was used to prevent contact between anodic and cathodic graphite granules. During the experiments where sodium acetate (1 g per feeding cycle) was batch fed to the membrane-containing MECs (R1*, R2*, RA*, and RB*), the anode and the cathode had a separate recirculation vessel (2 L) from which the liquid was pumped through the anode or cathode at a rate of 0.35 L h^{-1} (Figure S2 of the Electronic Supplementary Material). During the experiments where sodium acetate or acetic acid were batch fed to the membraneless MECs (RA and RB), a recirculation vessel (0.25 L) was placed in the recirculation loop (0.35 L h^{-1}) so that the cathode effluent was mixed in the recirculation vessel before entering the anode (Figure S3 of the Electronic Supplementary Material). In the other tests, the MEC (RB^c) was operated continuously and fed fresh medium (0.64 L day^{-1} ; hydraulic retention time, 5.3 h) that was flushed for 30 min with nitrogen gas prior to use. A concentrated solution of acetic acid, dissolved in medium used at that time, was continuously added (8.5 mL day^{-1}) to the recirculation loop (0.35 L h^{-1}) to obtain the desired volumetric chemical oxygen demand (COD) loading rate (Bv, $\text{kg COD m}^{-3} \text{ MEC day}^{-1}$; Figure S4 of the Electronic Supplementary Material). The medium always contained $6 \text{ g Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O L}^{-1}$, $3 \text{ g KH}_2\text{PO}_4 \text{ L}^{-1}$, $1 \text{ g NaHCO}_3 \text{ L}^{-1}$, $0.2 \text{ g MgSO}_4 \cdot 7\text{H}_2\text{O L}^{-1}$, $0.1 \text{ g NH}_4\text{Cl L}^{-1}$, $0.0146 \text{ g CaCl}_2 \text{ L}^{-1}$, and trace elements as previously described (Clauwaert et al. 2007b), unless stated differently. All tests were performed at room temperature ($22 \pm 2 \text{ }^\circ\text{C}$).

Electrochemical measurements

The cell voltage of each MEC was applied by a direct current (DC) power supply or by a potentiostat (PAR Bi-Stat Potentiostat, Princeton Applied Research, France). When a DC power supply was used, the current was measured by the voltage difference over a $1\text{-}\Omega$ resistor in

the electrical circuit between the DC power supply and the cathode. The cell voltage, current production, and the cathode potential were recorded every minute with a data-acquisition unit (HP 34970A, Agilent). The hourly averages were then used for further calculations. Polarization curves were obtained with a potentiostat by increasing the applied voltage by -0.050 V increments every hour between -0.100 and -0.800 V. The polarization curves were used to evaluate the electrode potential in function of the current production. The ohmic cell resistance was determined with the current interrupt method based on cell voltage change 0.2 ms after disconnecting the cell during a potentiostatic program. The potential of the cathodic electrode was monitored with a Ag/AgCl reference electrode (assumed to be $+0.197$ V vs Standard Hydrogen Electrode (SHE); model RE-5B, BASi), and the anode potential was calculated from the cathode potential, the cell voltage applied, and the ohmic cell voltage (Clauwaert et al. 2008a). The coulombic efficiency (CE) was determined as previously described (Clauwaert et al. 2007b):

$$CE = \frac{\left(\frac{I}{139.59 \text{ A kg}^{-1} \text{ COD d}}\right)}{B_v} \times 100\% \quad (1)$$

Analytical measurements

Acetate, sulfate, and ammonium concentrations were determined as previously described (Clauwaert et al. 2007a). For the batch-fed tests, a gas bag was connected to the headspace of the recirculation vessel. When operated continuously, a gas trap was used to separate the biogas produced from the effluent. Methane ($\text{CH}_4^{\text{biogas}}$, (vol.) %) and carbon dioxide ($\text{CO}_2^{\text{biogas}}$, (vol.) %) were measured as previously described (Clauwaert et al. 2008b). The specific methane production rate (r_{CH_4} , $\text{L CH}_4 \text{ L}^{-1} \text{ MEC day}^{-1}$) and the theoretical maximum hydrogen production rate ($r_{\text{H}_2}^{\text{Th.MAX}}$, $\text{L H}_2 \text{ L}^{-1} \text{ MEC day}^{-1}$) were calculated from the

biogas production rate (r^{biogas} , $\text{L biogas L}^{-1} \text{ MEC day}^{-1}$) and volumetric fractions of methane and carbon dioxide.

$$r_{\text{CH}_4} = \frac{\text{CH}_4^{\text{biogas}}}{100\%} \times r^{\text{biogas}} \quad (2)$$

$$r_{\text{H}_2}^{\text{Th.MAX}} = r^{\text{biogas}} \times \frac{\left(100\% - \text{CH}_4^{\text{biogas}} - \text{CO}_2^{\text{biogas}}\right)}{100\%} \quad (3)$$

This means that the actual hydrogen production rate could even be lower if traces of nitrogen gas, hydrogen sulfide, etc. would be present in the biogas. The percentage of methane or (theoretical maximum) hydrogen recovered

over acetate dosed ($\eta_{\text{Ac-CH}_4}$ and $\eta_{\text{Ac-H}_2}^{\text{Th.MAX}}$, dimensionless) is calculated as follows:

$$\eta_{\text{Ac-CH}_4} = \frac{\left(\frac{r_{\text{CH}_4} \times 8 \text{ mol e}^{-} \text{ mol}^{-1} \text{ CH}_4 \times 32 \text{ g COD mol}^{-1} \text{ COD}}{24.2 \text{ L mol}^{-1} \times 4 \text{ mol e}^{-} \text{ mol}^{-1} \text{ COD}}\right)}{B_v - E^{\text{Ac}}} \times 100\% \quad (4)$$

$$\eta_{\text{Ac-H}_2}^{\text{Th.MAX}} = \frac{\left(\frac{r_{\text{H}_2}^{\text{Th.MAX}} \times 2 \text{ mol e}^{-} \text{ mol}^{-1} \text{ H}_2 \times 32 \text{ g COD mol}^{-1} \text{ COD}}{24.2 \text{ L mol}^{-1} \times 4 \text{ mol e}^{-} \text{ mol}^{-1} \text{ COD}}\right)}{B_v - E^{\text{Ac}}} \times 100\% \quad (5)$$

with B_v , the volumetric COD loading rate, E^{Ac} , the specific COD flux leaving the microbial electrolysis cell ($\text{kg COD m}^{-3} \text{ MEC day}^{-1}$), and Ac_{COD} the acetate concentration expressed in COD equivalents (g COD L^{-1}).

$$E^{\text{Ac}} = \frac{\left(\text{Ac}_{\text{COD}}^{\text{IN}} - \text{Ac}_{\text{COD}}^{\text{OUT}}\right) \times 0.64 \text{ L day}^{-1}}{\left(256 \times 10^{-6} \text{ m}^3 \text{ MEC}\right) \times \left(1,000 \text{ g kg}^{-1}\right)} \quad (6)$$

In the case of batch-operated tests, r^{biogas} , r_{CH_4} , $r_{\text{H}_2}^{\text{Th.MAX}}$, and E^{Ac} need to be replaced in Eqs. 2 to 5 by V^{biogas} (L), V_{CH_4} (L), $V_{\text{H}_2}^{\text{Th.MAX}}$ (L), and the acetate concentration (g COD L^{-1}) at the end of the batch tests, respectively. The equations to calculate the percentage of the substrate dosed that could be lost as soluble methane and hydrogen gas in the effluent ($F_{\text{CH}_4}^{\text{dis}}$ and $F_{\text{H}_2}^{\text{dis}}$, %) and the specific COD removal rate due to sulfate reduction to sulfide are given in the [Electronic Supplementary Material](#).

Results

Batch-fed microbial electrolysis cells

MECs R1* and R2*, both with a CEM between anode and cathode, were constructed and operated in a batch fed mode (Figure S2 of the Electronic Supplementary Material) with an applied cell voltage of -0.601 ± 0.013 V. The anode chambers were inoculated with 5 mL of anode effluent from a MFC, and the cathode chambers were inoculated with 5 mL nongranular anaerobic sludge obtained from a mesophilic winery digester. After 24 to 48 h of lag phase, the current production remained between 8 and $30 \text{ A m}^{-3} \text{ MEC}$ for both reactors for 28 days (E_{anode} , -0.269 V vs SHE, E_{cathode} , -0.844 ± 0.014 V vs SHE). There was no gas production detected in the cathode.

To verify microbial involvement in the anode and cathode conversion processes, the graphite granules of the anode and the cathode of one of the two MECs were autoclaved. The autoclaved anode was combined with the not autoclaved cathode of (RA*), and the autoclaved cathode was combined with the not autoclaved anode (RB*). Re-inoculation occurred from the recirculation

vessels. It took the reactor with the autoclaved anode 39 h to regain the production of $8 \text{ A m}^{-3} \text{ MEC}$, whereas the current production in the reactor with the autoclaved cathode was not hampered ($12\text{--}20 \text{ A m}^{-3} \text{ MEC}$) during the next 14 days. Polarization curves were obtained before and 3 days after autoclaving the respective graphite electrode granules (Fig. 1), indicating that mainly the anode was affected due to the autoclaving and the disruption by replacing the graphite electrode granules.

Subsequently, the CEMs were removed, and a nonwoven cloth was placed between the anode and the cathode to create the membraneless MEC configuration (Figure S3 of the Electronic Supplementary Material). The reactors (RA and RB) were inoculated with 5 mL of the same nongranular anaerobic sludge as used for R1* and R2*. Removing the CEM lowered the ohmic cell resistance from $2.2 \pm 0.6 \ \Omega$ ($N=14$) to $1.1 \pm 0.3 \ \Omega$ ($N=14$). The MECs were operated for 22 days with an applied cell voltage of $-0.610 \pm 0.006 \text{ V}$. The current production immediately increased to $35 \text{ A m}^{-3} \text{ MEC}$ after removing the CEM and further increased up to $55 \pm 6 \text{ A m}^{-3} \text{ MEC}$ in the following days (E_{anode} , -0.225 V vs SHE , E_{cathode} , $-0.818 \pm 0.006 \text{ V vs SHE}$). A polarization curve (Fig. 1) was performed, and higher current densities could be achieved compared to the membrane-containing MEC. Especially the cathode overpotentials in the high current range did not increase as fast as in the case of a membrane-containing MEC. An overpotential at an electrode is defined as the voltage difference between the electrode potential and the equilibrium potentials, which is the equilibrated electrode potential in the absence of current production.

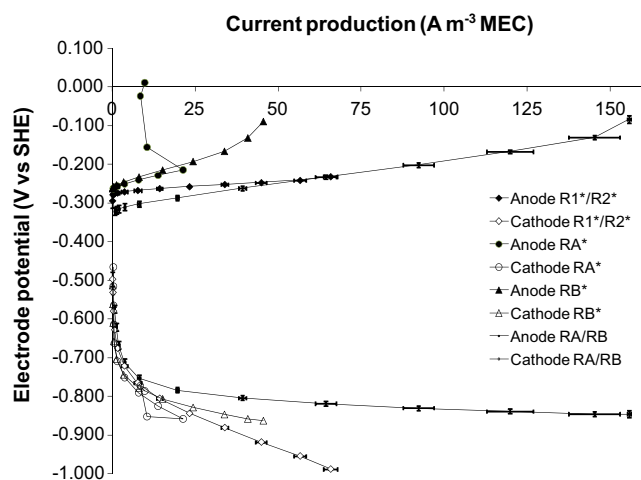


Fig. 1 The anode and cathode potential in function of the current density in the presence (*asterisk*) or absence of a cation exchange membrane during polarization. In the case of RA*, the polarization was performed 3 days after autoclaving the anodic graphite granules, while in the case of RB*, the polarization was performed 3 days after autoclaving the cathodic graphite granules. The *error bars* indicate the minimum and maximum of a duplicate scan for R1*, R2*, RA, and RB

The applied cell voltage of the membraneless MEC RA was increased to approximately -0.8 V . After an acclimatization period of 10 days, cell RA was fed with four subsequent spike doses of 12.2 mmol sodium acetate (Na-Ac) upon depletion (Table 1, test 1), four spike doses of 12.2 mmol acetic acid (H-Ac; Table 1, test 2), and then, as a control experiment with no applied voltage, two times with 12.2 mmol acetic acid (Table 1, test 3). The medium was renewed and sparged for 30 min with nitrogen gas when the substrate was changed from sodium acetate to acetic acid and before the control experiment. An intermediate feeding cycle (data not shown) was applied each time to remove most of the nitrogen gas from the headspace after sparging. The maximum current density was higher in the case of sodium acetate, and the average time per cycle was lower. The addition of HCl to compensate the pH increase, resulting in a higher conductivity, could have accounted for the higher current production. However, there was a higher rate of carbon dioxide production for the feeding cycles with acetic acid (Table 1). When sodium acetate was supplied to the MEC, the pH needed to be corrected from $7.4\text{--}7.6$ to $7.0\text{--}7.2$ with 1 M HCl after every feeding cycle. For acetic acid, there was also an increase in pH during every cycle, which compensated for the initial decrease in pH to 6.4 ± 0.2 after the spiking with acetic acid. This lower pH might explain the higher CO_2 content in the case of acetic acid.

Continuous operated microbial electrolysis cell

Cell RB^c was first operated at a volumetric COD loading rate of $1.38 \text{ kg COD m}^{-3} \text{ MEC day}^{-1}$ with continuous recirculation, and various parameters were monitored (Figure S4 of the Electronic Supplementary Material and Table 2, test 1). When there was no recirculation, the current production, coulombic efficiency, and methane production decreased (Table 2, test 2). An increase in the loading rate resulted in a higher current and methane production, while the acetate removal efficiency was much lower (Table 2, tests 3 and 4). As a control, no voltage was applied in a subsequent test. Methane production continued but at a lower rate (Table 2, test 5). In test 6, the carbonate buffer was omitted from the influent media, resulting in an influent pH decrease from 7.2 to 7.0 . This had no clear impact on the current production nor methane production rate compared with test 1. The phosphate buffer strength was lowered by a factor of 10 in test 7. As a result, the effluent pH decreased to $6.1\text{--}6.2$. This lower pH lowered the cathodic overpotentials, but the anodic overpotentials increased even stronger (Fig. 2). In test 8, the pH of the concentrated solution of acetic acid was adjusted from 2.4 to 6.9 with 1 N NaOH, which lowered the anodic overpotentials compared to test 7. The production of current remained lower than in test 1 (Table 1), which was likely

Table 1 Summary of three membraneless microbial electrolysis tests in cell RA, where every feeding cycle, 12.2 mmol of sodium acetate or acetic acid was batch-fed as a substrate

Test number		1	2	3
Test duration	(h cycle ⁻¹)	49±2	66±8	96±1
Applied cell voltage	(V)	-0.807±0.005	-0.816±0.002	-
Substrate	(-)	Na-Ac	H-Ac	H-Ac
Total number of feeding cycles	(-)	4	4	2
V_{CH_4}	(L CH ₄ cycle ⁻¹)	0.23±0.02	0.26±0.02	0.20±0.04
CH ₄ ^{biogas}	(% vol.)	71±3	58±2	37±15
CO ₂ ^{biogas}	(% vol.)	8±3	24±11	13±9
Acetate removal	(%)	100	100	100
η_{Ac-CH_4}	(%)	79±7	87±7	69±13
$\eta_{Ac-H_2Th.MAX}$	(%)	6±3	6±3	23±3
I_{max}	(A m ⁻³ MEC)	223	190	-
CE	(%)	87±5	83±2	-
E^{anode}	(V vs SHE)	-0.148	-0.052	-0.316
$E^{cathode}$	(V vs SHE)	-0.896±0.012	-0.820±0.021	-0.308±0.018
$\Delta pH \text{ day}^{-1}$	(-)	0.2±0.1	0.2±0.1	0.2±0.1

The test duration was defined as the time between substrate feeding and a complete removal of the substrate.

η_{Ac-CH_4} percentage of methane recovered in the biogas over acetate removed on COD basis ×100%, $\eta_{Ac-H_2Th.MAX}$ percentage of theoretical maximum hydrogen recovered in the biogas over moles acetate removed on COD basis ×100%, I_{max} the maximum hourly averaged current production, CE the coulombic efficiency, E^{anode} and $E^{cathode}$ the electrode potential of anode and cathode, $\Delta pH \text{ day}^{-1}$ the daily increase in pH in the recirculation vessel

Table 2 Summary of the continuous membraneless microbial electrolysis tests in RB^c

Test number		T1	T2 ^a	T3	T4	T5	T6 ^b	T7 ^{b,c}	T8 ^{b,c,d}
Test duration	(h)	165	167	166	102	147	169	166	164
Applied cell voltage	(V)	-0.812±0.004	-0.831±0.009	-0.818±0.009	-0.813±0.014	-	-0.827±0.007	-0.844±0.004	-0.833±0.002
B_v	(kg COD m ⁻³ MEC day ⁻¹)	1.38	1.38	2.41	4.13	1.38	1.38	1.38	1.38
r_{CH_4}	(L CH ₄ L ⁻¹ MEC day ⁻¹)	0.33±0.07	0.28±0.02	0.35±0.06	0.75±0.12	0.17±0.06	0.30±0.03	0.29±0.02	0.34±0.03
CH ₄ ^{biogas}	(% vol.)	80±5	77±5	78±5	79±8	69±17	79±7	72±3	90±8
CO ₂ ^{biogas}	(% vol.)	5±1	4±1	8±5	11±1	6±1	4±0	9±1	2±1
Acetate removal	(%)	98±1	93±3	58±23	56±2	69±5	99±1	96±1	96±2
η_{Ac-CH_4}	(%)	65±13	57±4	66±11	86±14	47±17	58±7	59±4	67±6
I	(A m ⁻³ MEC)	121±5	72±6	198±6	211±10	-	116±3	56±3	74±2
CE	(%)	63±3	37±3	59±2	36±2	-	60±1	29±2	39±1
E^{anode}	(V vs SHE)	-0.094	-0.065	-0.100	-0.096	-0.358	-0.085	+0.016	-0.143
$E^{cathode}$	(V vs SHE)	-0.859±0.006	-0.856±0.008	-0.842±0.004	-0.828±0.004	-0.363±0.012	-0.840±0.012	-0.694±0.010	-0.886±0.025
Ammonium removal	(%)	6±4	20±11	8±7	17±2	4±4	20±4	6±3	18±7
Sulfate removal	(%)	66±5	52±12	56±8	68±10	28±14	63±4	70±6	82±7
pH _{in} -pH _{out}	(-)	0.2±0.0	0.2±0.0	0.3±0.1	0.5±0.0	0.2±0.0	0.2±0.0	0.8±0.1	-0.5±0.1

B_v volumetric COD loading rate, r_{CH_4} the specific methane production rate, η_{Ac-CH_4} percentage of methane recovered over acetate removed on COD basis ×100%, I the average current production, CE the coulombic efficiency, E^{anode} and $E^{cathode}$ the electrode potential of anode and cathode

^aNo recirculation

^bNo carbonate buffer used

^c5.6 mM phosphate buffer used instead of 56 mM

^dpH in syringe adjusted to 6.9 with 1 N NaOH

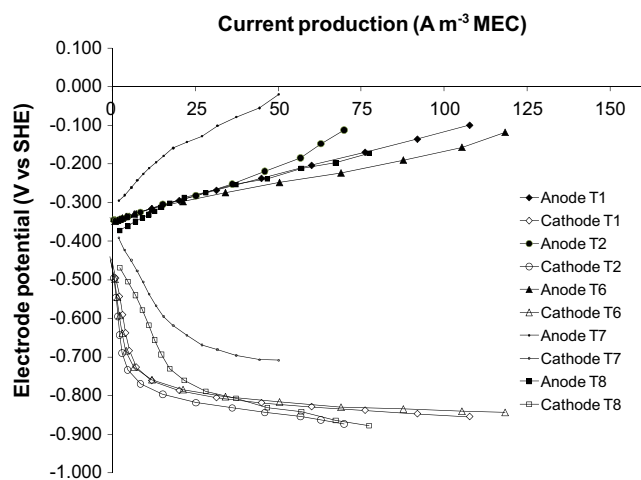


Fig. 2 The anode and cathode potential in function of the current density of cell RB^C during polarization. The cells were fed in a continuous way, and there was no recirculation during test T2, no carbonate buffer during tests T6, T7, and T8, and a 5.6 mM phosphate buffer instead of 56 mM during tests T7 and T8. The pH in the feeding syringe during T7 was 2.4 and was adjusted to 6.9 with 1 N NaOH during T8 (more details in Table 2)

due to the lower pH buffer capacity. The average ohmic cell resistance was $1.6 \pm 0.3 \Omega$ ($N=3$) during tests 1, 3, and 4. During test 2, the ohmic cell resistance was 2.2Ω , and removing the carbonate buffer (test 6) resulted in an increase of the ohmic cell resistance to 2.5Ω . Lowering the phosphate buffer strength resulted in an increase of the ohmic cell resistance to 8.9Ω (test 7), and during test 8, the ohmic cell resistance decreased to 4.8Ω , most likely due to the continuous addition of NaOH that increased the ionic strength.

The acetate that was removed in the reactor and that was not recovered as methane in the biogas ($100\% - \eta_{\text{Ac-CH}_4}$) was either converted into hydrogen gas ($\eta_{\text{Ac-H}_2}^{\text{Th,max}}$), soluble methane, and hydrogen in the effluent, reduced sulfur species, or biomass. The theoretical maximal hydrogen fraction in the biogas was calculated by excluding the measured volume of methane and carbon dioxide (Eq. 3). The theoretical percentage of acetate removed that was recovered as hydrogen ($\eta_{\text{Ac-H}_2}^{\text{Th,max}}$) was calculated to be between 2% and 5% for tests 1 to 8. The dissolved fraction of the biogas produced in the effluent could not be recovered. For a volumetric loading rate of $1.38 \text{ kg COD m}^{-3} \text{ MEC day}^{-1}$, a maximum percentage of 14% and 2% of the COD could be lost to dissolved methane and hydrogen, respectively, in the effluent (assumed solubility at 22°C , $20 \text{ mg CH}_4 \text{ L}^{-1}$ and $1.6 \text{ mg H}_2 \text{ L}^{-1}$). Sulfate reduction, when assumed to sulfide (8 mol electrons per mol sulfate), could on average have contributed to a COD removal rate of $0.08 \pm 0.02 \text{ kg COD m}^{-3} \text{ MEC day}^{-1}$ for all tests, except for test 5 where the calculated COD removal rate based on the sulfate removal was only

$0.04 \pm 0.1 \text{ kg COD m}^{-3} \text{ MEC day}^{-1}$ (Table 2). If sulfide was oxidized at the anode to elemental sulfur, the fraction of the COD loading rate that was not recovered as current was 25% lower. Polarization curves were performed during tests 1, 2, 6, 7, and 8 (Fig. 2).

Discussion

Ion exchange membranes in BESs are generally considered necessary to avoid short-circuit conversion of the reagents. They however cause higher ohmic cell resistance and the buildup of a pH gradient across the membrane (Clauwaert et al. 2008a; Rozendal et al. 2006a). Here, we have found that removing the cation exchange membrane lowered the ohmic cell resistance by approximately 50% (2.2 vs 1.1Ω or 0.56 vs $0.28 \text{ m}\Omega \cdot \text{m}^3 \text{ MEC}$), while alleviating the need to adjust the pH with acid or base. As previously described for MFCs, the membrane avoids the direct crossover conversion of the organic substrate by aerobic microorganisms, which lowers the coulombic efficiency (Liu and Logan 2004). MECs with an ion exchange membrane resulted in production of relative pure hydrogen gas in the cathode (Rozendal et al. 2007). However, when this membrane is omitted from the MEC, hydrogenotrophic methanogens can combine anodically produced carbon dioxide with cathodically produced hydrogen gas (Call and Logan 2008; Clauwaert et al. 2008b). In this research, we have demonstrated that methanogenesis can easily become dominant in membraneless MECs that are not exposed to air. Of an acetate-containing solution ($0.554 \text{ g COD L}^{-1}$), $98 \pm 1\%$ was removed in a membraneless MEC. Of the acetate removed, $65 \pm 13\%$ could be recovered as methane at a rate of $0.33 \pm 0.07 \text{ L CH}_4 \text{ L}^{-1} \text{ MEC day}^{-1}$ (Table 2, test 1). Methanogenesis was persistent throughout the experiments despite short hydraulic retention times of 5.3 h and slightly acidic conditions (pH 6.1–6.2). Operation under more acidified conditions is not desired as it would result in a further increase of anodic overpotentials during anodic substrate oxidation, especially since the pH in the proximity of the anode is even lower than in the bulk solution (Clauwaert et al. 2008a). Also, the operation without carbonate in the medium (Table 2, test 6) did not result in a lower methane production rate. Further research is necessary to reveal the proportion, localization, and activity of hydrogenotrophic and acetoclastic methanogens in the anode and cathode compartment. Moreover, other organic substrates and their effect on the microbial community are of specific interest for further research.

Our findings suggest that methane production is most likely to occur in membraneless MECs, even in acidified, carbonate-limited continuous systems. If, however, hydrogen production would be preferred over methane produc-

tion, alternative strategies have to be brought forward. Draining the reactor and exposure to air between every feeding cycle lowered the methane production rate (Call and Logan 2008). Another strategy could be the stimulation of nitrifying microorganisms on internally aerated hollow fiber membranes inside the cathode compartment (Downing and Nerenberg 2007). The presence of low levels of oxygen, nitrate, and denitrification intermediates could inhibit the methane production (Roy and Conrad 1999). The combined nitrification and denitrification in the cathode can be considered as an environmental benefit in the context of wastewater purification, however, rendering the produced hydrogen gas impure with nitrogen gas, the end product of autotrophic or heterotrophic denitrification. The impure hydrogen gas produced might than be of interest for the production of biopolymers like polyhydroxybutyrate (Ishizaki et al. 1993). Another strategy to minimize methanogenesis in situ could be a regular short-time polarity reversal at a higher applied cell voltage to produce oxygen and free radicals.

The maximum reported current productions described for MECs where the anode and cathode were in the same electrolyte were 292 A m^{-3} MEC for 28 mL MECs (Call and Logan 2008) and 145 A m^{-3} MEC for 225 mL MECs (Clauwaert et al. 2008b). In both cases, platinum was used as a catalyst in the cathode. In our research, no chemical catalysts were used in the cathode, and the maximum current production was 223 A m^{-3} MEC (or 446 A m^{-3} total cathodic compartment (TCC)) with graphite granules. The continuous removal of hydrogen by hydrogenotrophic methanogens lowering the hydrogen partial pressure might have contributed to lower cathodic overpotentials. Current densities from hydrogen producing cathodes were reported in the order of 2 A m^{-2} (approximately 121 A m^{-3} TCC, calculated value for 413 mL TCC) for a noncatalyzed graphite felt as a cathode, while a biological catalyzed graphite felt as cathode could produce up to 3.7 A m^{-2} (approximately 225 A m^{-3} TCC, calculated value) when the cathode potential was potentiostatically poised at -0.8 V vs SHE (Rozendal et al. 2008). In our research, no biological involvement in the cathodic reaction could be determined by autoclaving the cathodic graphite granules. This indicates that it may be necessary to acclimate an anode with hydrogen as the electron donor to establish an electrochemically active biofilm that can later be used for biocathodic catalysis of hydrogen production.

In this research, we demonstrated that no pH correction was needed when acetic acid was used as the electron donor. Besides acetate removal up to 99% during continuous MEC operation, 52% to 82% of the 0.81 mM sulfate and between 4% and 20% of the 1.87 mM ammonium in the influent were removed when a voltage was applied to the MEC. Since anaerobic ammonium oxidation has not

been demonstrated yet in BESs (Kim et al. 2008), ammonium was most likely used for biomass buildup. The use of pH buffers in BESs is popular because it masks strong pH increases or decreases in cathodes or anodes, respectively. When sodium acetate is the substrate and completely oxidized, only seven protons are delivered per eight electrons, and this alkalization effect becomes more dominant in poorly buffered solutions. The disadvantage of acetic acid as the substrate is that, especially in poorly buffered solutions, pH control might be necessary in order to avoid pH shocks.

If the substrate would be completely converted into hydrogen gas, the theoretical energy yield would be 5 kWh kg^{-1} COD converted, while this would only be 3.5 kWh kg^{-1} COD converted for methane ($0.079 \text{ kWh mol}^{-1} \text{ H}_2$; $0.225 \text{ kWh mol}^{-1} \text{ CH}_4$). During the continuous operation, the energy recovery, based on the measured methane production and calculated hydrogen production, for tests 1 to 8 was between 2.15 and 3.13 kWh kg^{-1} COD substrate converted, except for test 5 where the recovery was 1.87 kWh kg^{-1} COD substrate converted. The efficiency from biogas combustion for electricity production is around 35% (Zeeman et al. 2008). The overall energetic efficiency can be much higher, since the heat that is liberated through combustion is not used for heating the methane producing reactor but can be used for other processes. When no voltage was applied, the methane production was not completely hampered (Tables 1 and 2), which indicates that also acetoclastic methanogens were present and/or that syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis occurred in the absence of a voltage applied. The energy input under the form of electrical energy was between 2.72 and 2.83 kWh kg^{-1} COD converted into current for tests 1 to 8, except for test 5 where no voltage was applied (Table 2). This means that the energy invested was not always completely recovered under the form of an energy-rich biogas. Improvement in the reactor configuration and biofilm structure might enhance the conversion efficiency at a lower applied cell voltage.

Further research might demonstrate that a hydrogen-producing cathode at the effluent of an anaerobic digester is able to attract or retain the slow-growing, nongranular methanogens that would otherwise be flushed out of the reactor. Chemical reduction of polar compounds like humic acids in the proximity of a hydrogen producing cathode might also be used to make them apolar in order to remove them more easily by absorption or flotation (Satyawali et al. 2007).

We have demonstrated that methane production can easily be established in a membraneless MEC at ambient temperatures. However, the energy balance remains less favorable than anaerobic digestion where no energy

(tropical climates) or low caloric energy is needed to obtain methane production. The methane production rate in conventional anaerobic reactors fed with concentrated organic substrates ($> 1 \text{ g COD L}^{-1}$) can be in the order of several liters methane per liter reactor per day, even at moderate temperatures, while methane production rates in our lower-loaded MECs ($< 1 \text{ g COD L}^{-1}$) were below 0.7 L methane per liter reactor per day. Methane-producing MECs could be used in combination with conventional anaerobic digestion as a way to remove residual fatty acid and sulfides, for instance from anaerobically, low-temperature-treated domestic wastewater (Zeeman et al. 2008).

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